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Peeking into the future:
Fungi in the greening Arctic

PROEFSCHRIFT

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aan de Universititeit Leiden,
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door
Luis Miguel das Neves Morgado

Geboren te Lisboa, Portugal
in 1982

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No matter how long is the journey,
without love and passion it is nothing more than a drifting raft.

To my mother and my beautiful nieces

for their endless love, support and inspiration

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Toolik Lake field station, Alaska, with the Brooks Range on the horizon

Chapter 1

General introduction and thesis outline

Luis N. Morgado

General introduction

For more than 50 years there has been a scientific consensus regarding the fragility of the arctic tundra, either due to its biological simplicity when compared with the complex structure and biological diversity of temperate and tropical grasslands and forests, or due to the time-lag required by the biome to return to a steady state following perturbations, or fluctuations in biological populations (Bliss *et al*., 1973). Currently, the arctic tundra is on the brink of significant changes and there are serious concerns related to the future of arctic biodiversity due to the threats represented by climate change. Additionally, climate-induced changes in the Arctic will affect other ecosystems at lower latitudes via climate feedback loops (Kug *et al*., 2015). The soils of the planet store more Carbon (C) than the plants and atmosphere combined and a large fraction of this C is located in the soils of high-latitude ecosystems (Lal, 2008; Tarnocai *et al*., 2009). As climate changes have the potential to alter many processes that are interconnected with C and Nitrogen (N) cycles, the consequences of these alterations will have an impact not just on local but also on global scale. Fungi are a major component of arctic tundra soils and play important roles in ecosystem functioning as decomposers and symbionts. Therefore, it is expected that the effects of climate change in the fungal community of the arctic tundra will influence the ecological interactions and nutrient cycling in this biome. Even though this is generally recognized by the scientific community, not many studies addressed how climate changes will affect soil fungal communities in the Arctic, perhaps due to the cryptic nature of fungi and the former lack of adequate tools to assess the community structure. The work presented here is integrated in a larger project that aims to study and understand how arctic fungal community composition correlates with vegetation and what fungal taxa and ecological groups are expected to play roles in vegetation change in response to climatic stress. To better understand the following chapters of this thesis, it is necessary to draw a framework regarding current knowledge on the effects of climate change in the arctic tundra.

Climate change

Since 1884, the Earth's surface has warmed a total of 0.68 °C (http://climate.nasa.gov/) (Fig. 1.1). Additionally, proxies of global mean surface temperatures derived from tree rings, sediment layers, and ice cores revealed that temperatures during the past few decades exceeded those over the past four millennia (Mann *et al*., 1999; Mann & Jones, 2003; Salzer *et al*., 2014). Climate warming has accelerated since 1970 and the ten warmest years on record (131 years) occurred since the year 2000 (Post, 2013; http://climate.nasa.gov/) (Fig. 1.2). The global warming is largely due to increased concentration in atmospheric greenhouse gases. For example, according to comparisons of ice cores and detailed records, atmospheric concentration of CO_2 in 2015 is the highest in record of the last 800,000 years (Lüthi *et al*., 2008) and reached

levels where the risk of irreversible climate change is extremely high, such as the loss of major ice sheets, accelerated sea-level rise and abrupt changes in ecosystems (Rockström *et al*., 2009, http://co2now.org/; http://climate.nasa.gov/).

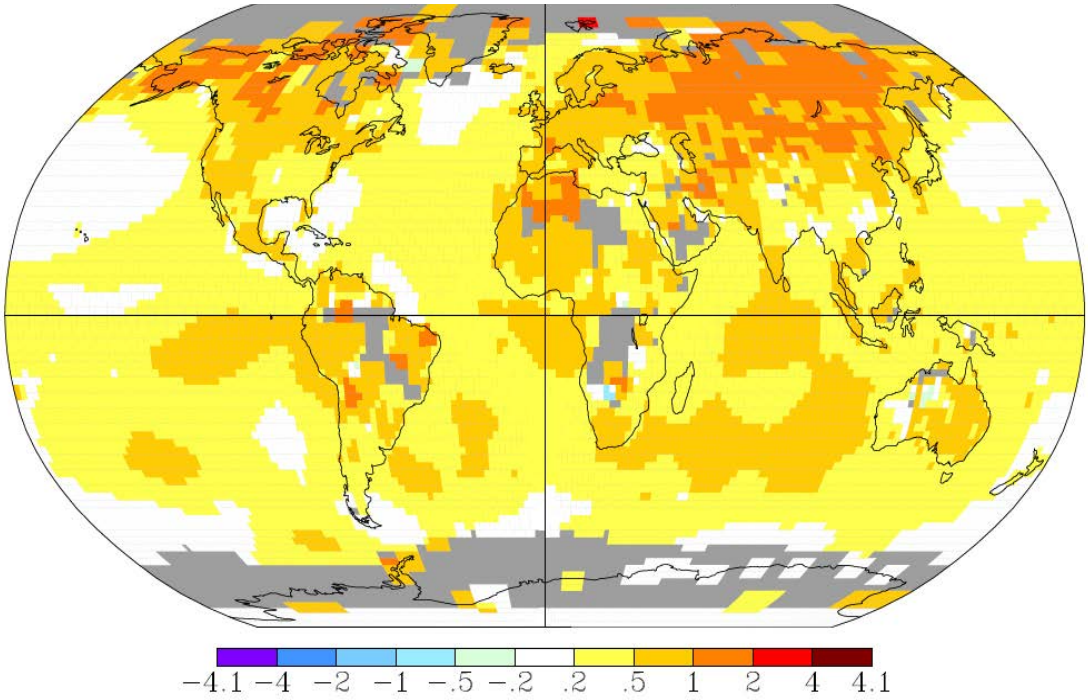


Figure 1.1. Map of annual average global temperature (°C) anomaly between 1981 and 2014 compared in relation to the period between 1950 and 1980. Adapted from http://www.giss.nasa.gov/.

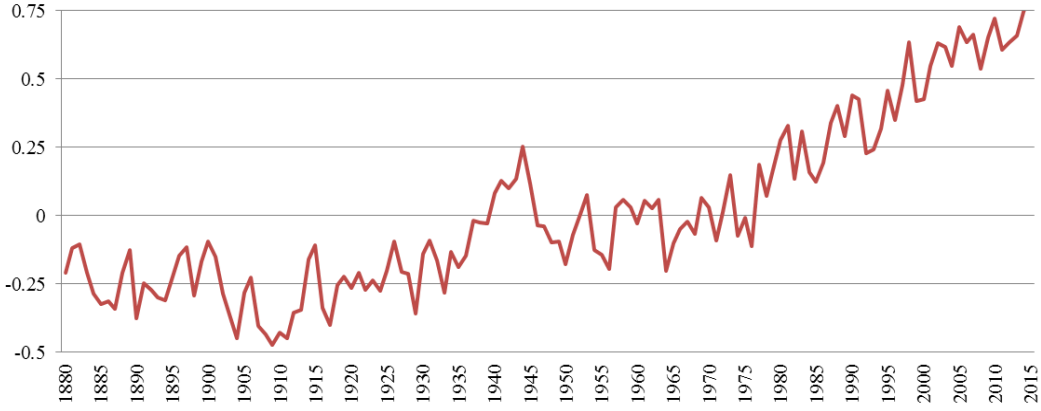


Figure 1.2. Change in global surface temperature relative to 1951-1980 average temperatures (°C). Data source NASA's Goddard Institute for Space Studies (GISS).

There are accumulating evidences that ecological responses to climate change are already occurring from polar to tropical environments. Many taxa show a consistent trend of polward expansion of species ranges and/or altitudinal shifts (Parmesan *et al.*, 1999; Thomas *et al.*, 2001; Walther *et al.*, 2002; Walther, 2010). Much progress has been made at the species level. However, scaling from individual species (populations) to communities and ecosystems is a great challenge. All species are embedded in complex networks of interaction that shape their existence and affect their viability. It is unlikely that the communities and ecosystems responses will be simply additive and their combinatorial dynamics linear. Present assemblages of interacting populations will not simply move polwards or to higher latitudes. Some species will move faster and further than others and spatial dislocation may occur (Walther, 2010). Species with short life span and high dispersal ability will reassemble differently than long-lived species with low dispersal potentials. Future communities will thus likely undergo reorganization and will function differently than those today (Montoya & Raffaelli, 2010).

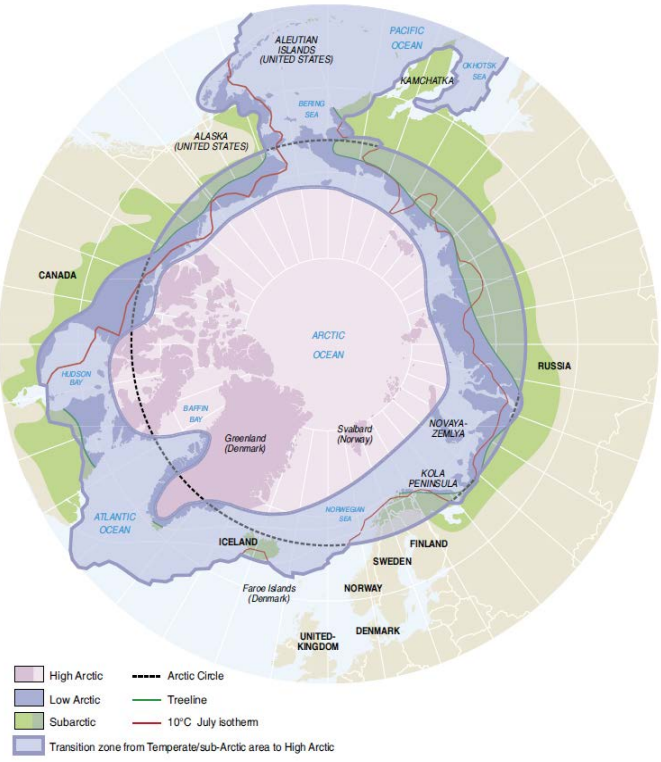


Figure 1.3. Boundaries of the Arctic. Picture adapted from http://www.grida.no/graphicslib/detail/definitions-of-the-arctic_12ba#. Sources: AMAP, 1998. AMAP Assessment Report: Arctic Pollution Issues. AMAP, 1997. Arctic Pollution Issues: A State of the Arctic Environment Report. CAFF, 2001. Arctic Flora and Fauna: Status and Conservation.

The ongoing climate change is expected to be a major threat to biodiversity in the coming decades (Schröter *et al.*, 2005, Pimm 2009; Montoya & Raffaelli, 2010). The remaining and pertinent questions are concerned with the extent and spatial variation of these changes. A related challenge is identifying which species are most sensitive to these changes and, through their biotic interactions, impart the largest effect on their communities, ecosystem services and how will these changes feedback to climate. Therefore, there is the need to move towards a predictive ecology in order to anticipate ecosystems changes with the final goal to understand and ameliorate the effects of climate changes.

Arctic tundra and climate change

The arctic tundra occur in the northern most regions (Fig. 1.3) where cold temperatures prohibit tree growth, spanning a total area of ca. 7,567,000 km^2 (appr. 5% of Earth's land surface), spread over Russia, Norway, Iceland, Greenland, Canada and the U.S.A. (Callaghan *et al*., 2005). The climate of the Arctic is largely driven by the relatively low solar angles relative to the Earth surface. Additionally, most arctic tundra surface is located near the Arctic Ocean and, the energetic balances between land and atmosphere are also greatly influenced by sea ice cover dynamics. Climatically, the arctic tundra is often defined as the area where the average temperature for the warmest month is below 10 °C (Köppen, 1931), however, mean annual air a temperature varies greatly according to location, even at the same latitude. The growing season is short, varying between 3.5 to 1.5 months from the southern to the northern boundaries. The cool summers and prolonged and cold winters produce a continuous permafrost soil layer, and a snow cover that lasts for two thirds of the year (Sturm *et al*., 2005).

High latitude permafrost regions are estimated to hold approximately 50% of Earth's reactive carbon (Tarnocai *et al*., 2009). Because these regions have been experiencing some of the highest rates of warming, varying between 0.06 and 0.1 °C per year over the past 40 years, a large fraction of this C is increasingly vulnerable to mobilization due to warming-induced melting of permafrost and higher microbial decomposition rates (Anisimov *et al.*, 2007; Hansen *et al.*, 2010; Comiso & Hall, 2014). This warming is resulting in a suite of climate feedbacks, including changes in sea ice cover and the length of ice-free periods (Arrigo & van Dijken, 2011; Post *et al.*, 2013), a greening of the surrounding land surface, and tree line advancement (Kharuk *et al.* 2013; Zhang *et al.*, 2013). All of these reduce the surface albedo, resulting in positive feedbacks to warming (Chapin *et al.*, 2005; Post *et al.*, 2009). For example, a greening of the Arctic driven by increases in shrub density (Sturm *et al.*, 2005; Loranty & Goetz, 2012; Tape *et al.*, 2012) could result in increased C sequestration (Welker *et al.*, 1997; Sistla *et al.*, 2013; Pattison & Welker, 2014). Increases in shrub density and canopy growth can further alter the tundra by local snow-trapping in winter, increasing soil insulation, causing higher winter and spring-time soil temperatures, and alter the rates of N and C turnover.

Another consequence of warming is the increase in arctic precipitation (Fig. 1.4) that greatly exceeds the global average, especially during the cold season, when most precipitation falls as snow (Kattsov & Walsh, 2000; Screen & Simmonds, 2012). State-of-the-art models predict further increases, possibly by more than 50% of the current precipitation, leading to deeper snow cover (Collins *et al.*, 2013; Bintanja & Selten, 2014). Deeper snow would have multiple consequences for tundra ecosystems, including providing protection from the abrasive winds (Liston *et al*., 2002; Sturm *et al*., 2005;

Blok *et al.*, 2015) as well as warmer winter soil temperatures and subsequent effects on nutrients cycling, plant mineral nutrition and vegetation composition (Schimel *et al.*, 2004; Welker *et al.*, 2005; Pattison & Welker, 2014).

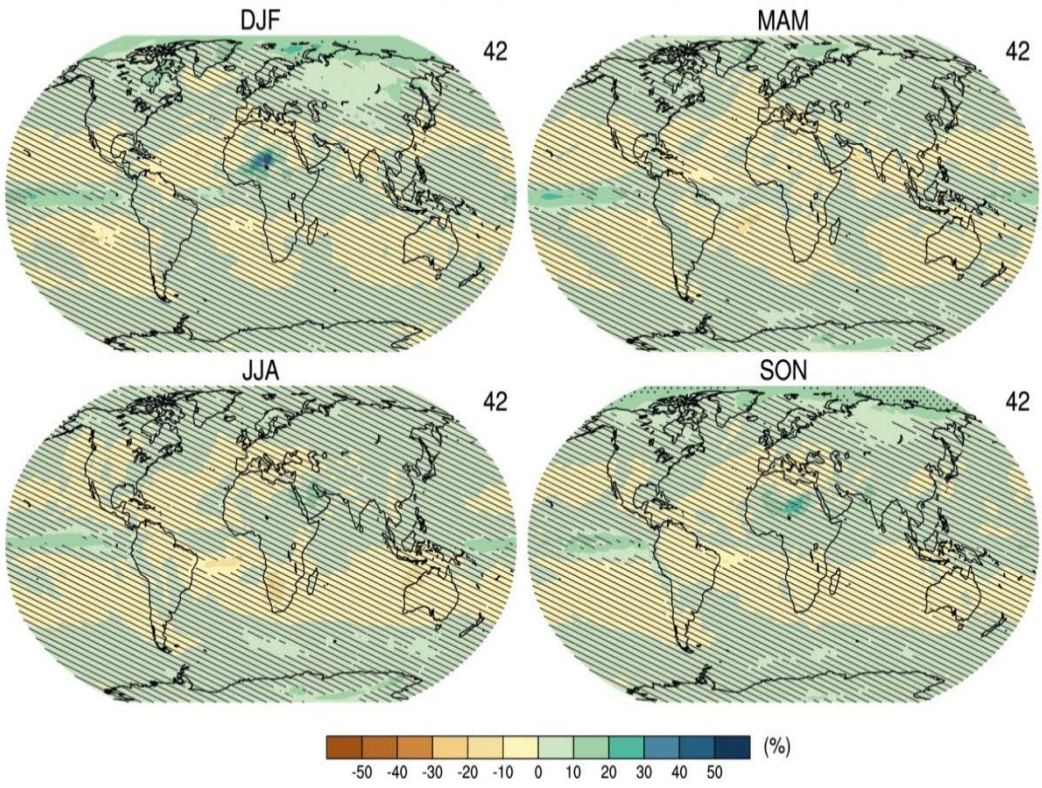


Figure 1.4. CMIP5 multi-model ensemble mean of projected changes (%) in precipitation for 2016–2035 relative to 1986–2005 under RCP4.5 for the four seasons. The number of CMIP5 models used is indicated in the upper right corner. Hatching indicates areas where projected changes are smaller than one standard deviation of estimated internal variability and stipping indicates regions where the multi-model mean projections deviate by at least two standard deviations of internal stability compared with the simulated period and where at least 90% of the models agree on the sign of change. The number of models considered are listed in the top-right portion of the panels. Legend: DJF: Deccember – January – February; MAM: March – April – May; JJA: June – July – August; SON: September – October – November. Figure and legend from Stocker *et al.* (2013). Technical details are in Annex I (Stocker *et al.*, 2013).

Arctic tundra plant communities respond to increased winter snow depth and summer warming both at local and circumpolar scales (Sturm *et al.*, 2001; Wahren *et al.*, 2005; Walker *et al.*, 2006; Welker *et al.*, 2014). The general trends include increase in litter layer, graminoid and shrub coverage, and decrease in coverage of lichens, bryophytes, and in leaf C:N ratio (Sturm *et al.*, 2001; Wahren *et al.*, 2005; Welker *et al.*

2005; Walker *et al*., 2006; Mercado-Díaz, 2011; Pattison & Welker, 2014). Naturally, this trend varies and responses differ according to tundra type, plant functional groups and species. These aboveground vegetation changes are likely accompanied by changes belowground, such as soil moisture, soil nutrient pools, fine-root abundance and turnover dynamics, which interplay with the fungal community dynamics (e.g., Read *et al.*, 2004; Dickie & Reich, 2005; Dickie *et al.*, 2005; Strand *et al.*, 2008; Toljander *et al.*, 2006; Twieg *et al.*, 2009; Peay *et al.*, 2011). In the arctic tundra, fungi are the major component of the soil microorganisms biomass and play a critical role in ecosystem functioning (Callaghan *et al*., 2010). Despite their recognized importance and the recent advances regarding belowground processes related with fungal dynamics such as C and N cycling (e.g., Schimel *et al.*, 2004; Borner *et al.*, 2008; Schaeffer *et al.*, 2013; Wieder *et al*., 2013) as well as microbial community responses to environmental changes (e.g., Clemmenson *et al.*, 2006; Campbell *et al.*, 2010; Deslippe *et al.*, 2011; Deslippe *et al.*, 2012), our knowledge about the compositional and functional changes of arctic fungal communities in response to climate change remains rudimentary at best.

Arctic fungi and climate changes

Fungi play a central role in the functioning of terrestrial arctic ecosystems as symbionts (e.g. mycorrhizae, endophytes, lichens) and decomposers. Almost all arctic plants are highly dependent on mutualistic relationships with mycorrhizal fungi for survival in these nutrient-poor environments (Hobbie *et al*., 2009; Gardes & Dahlberg, 1996; Bjorbækmo *et al*., 2010). Such associations include ectomycorrhizal (ECM), arbuscular mycorrhizal, ericoid and arbutoid mycorrhizal fungi (Väre *et al*., 1992; Newsham *et al*., 2009). It has been estimated that between 61 and 86% of N in Arctic tundra plants is obtained through mycorrhizal fungi (Hobbie & Hobbie, 2006). In addition, dark septate endophytic (DSE) fungi appear to be ubiquitous in the roots of arctic-alpine plants (Väre *et al*., 1992; Newsham *et al*., 2009), but almost nothing is known about their diversity, identity and ecological role. Similarly, there are highly diverse fungal endophytic communities living in above-ground plant parts that remain poorly known from arctic regions (Arnold *et al*., 2000; Higgins *et al*., 2007). Given their intimate relationships with plants in a wide range of symbioses, fungi are expected to play an important role in arctic vegetation change.

Currently, our ability to predict the response of fungal communities to climate change factors is hampered both by the few detailed descriptions of the members of these communities as well as our limited understanding of the ecology of many fungal species. Globally, approximately 100,000 species of fungi have been described, but their true diversity may be as high as 6 million species (Blackwell, 2011; Taylor *et al*., 2014). The Arctic in particular has been an understudied region, as the first works for molecular fungal diversity assessments in selected arctic sites have been initiated in the last 4-5

years (Bjorbækmo *et al*., 2010; Geml *et* al., 2008; Geml *et al*., 2012). Traditionally, fungal biodiversity studies have been based almost entirely on collection and taxonomic study of sporocarps. These studies assess only a fraction of the diversity of the fungal community because of their cryptic life style and the sporadic nature of the fructification process. However, in recent years an increasing number of molecular studies have been devoted to studying arctic fungi. The vast majority of these focused on root-associated, particularly ECM fungi, amassing valuable information on their diversity and biogeographic patterns (Bjorbaekmo *et al*., 2010; Blaalid *et al.*, 2012; Geml *et al*., 2012; Timling *et al*., 2012) and their responses to experimental warming (Clemmensen *et al*., 2006; Deslippe *et al*., 2011). ECM species are among the most ecologically important taxa, and seemingly represent one the most diverse fungal guilds but they represent only a fraction of the whole taxonomic and functional diversity of arctic fungi. With the exception of the work of Timling *et al*. (2014) who characterized arctic soil fungal communities in zonal tundra vegetation types along a latitudinal transect spanning the low and high arctic bioclimatic subzones of North America, most other groups of arctic fungi have received little attention. Despite these important advances, the effects of long-term climate changes on soil fungal communities remain largely unknown in terms of possible changes in ecological functions as well as in taxonomic diversity.

Fungi functional diversity

Functional diversity is based on the functional traits of the organismal assembly in the community. In community ecology, functional traits can be defined as biological features that play a role in the ecology of the community (Diaz & Cabido, 2001). Therefore, community composition is intrinsically linked with organismal functional traits. These traits are influenced by environment and biotic interactions, and determine suitability of the organism in a habitat and in a community. In turn, these traits can influence ecosystem functions. Traits that influence the organism's response to the environment are considered response traits, while those that influence ecosystem function are known as effect traits (Lavorel & Garnier, 2002). Importantly, these may be linked and function simultaneously as response and effect traits (Koide *et al*., 2014). Below, two selected examples that are used throughout this dissertation (melanized fungi and ECM extramatrical exploration types) are summarized.

Melanins are dark macromolecules composed of various types of indolic and phenolic monomers, usually complexed with proteins and/or carbohydrates (Butler & Day, 1998). When present, they are located in the cell wall or extracellular matrix of fungi, and constitute a considerable portion of total fungal cell weight and likely require a considerable energetic investment (Rast & Hollenstein, 1977; Butler and Day, 1998). This feature has been extensively argued and was recently shown in physiological experiments (Fernandez & Koide, 2013) to increase individual tolerance to several

environmental stressors, such as freezing (Robinson, 2001) and hydric stress (Fernandez & Koide, 2013). Indeed, the fungal communities of arid and seasonally water-stress environments, as well as communities with extreme environments, such as Antarctic have a high proportion of melanized fungi (Onofri *et al.*, 2007; Querejeta *et al.*, 2009; Sterflinger *et al.*, 2012). In turn melanins are resistant to decomposition and usually considered recalcitrant. Because fungi are an important component of total soil biomass the abundance of melanized mycelia in the habitat are likely to be an important component of C soil pools (Malik & Haider, 1982; Butler *et al.*, 2005).

Belowground ECM fungal mycelium morphology can be divided in two parts, the ectomycorrhizae, a morphological structure composed of fungal hyphae and plant roots, and the extramatrical mycelium (EMM), i.e. the mycelium external to the ectomycorrhizae that grows into the surrounding soil with the crucial functions of foraging the litter and/or mineral layers for nutrients and of seeking new roots for colonization (Martin *et al.*, 2001; Anderson & Cairney, 2007). The EMM may form an intricate hyphal network interconnecting plant roots that pave the way for inter-plant C and nutrient movements (Selosse *et al.*, 2006). EMM of different taxa are known to have distinct anatomical and physiological features that are attributable to various foraging strategies (Colpaert *et al.*, 1992; Agerer, 2001; Hobbie & Agerer, 2010). The main characteristics to classify EMM are the mycelium exploration types (ET), presence/absence of rhizomorphs (vessel-like structures) and hyphae hydrophobicity (Fig. 1.5) (Agerer, 2001; Hobbie & Agerer, 2010; Peay *et al.*, 2011; Lilleskov *et al.*, 2011; Cairney, 2012). Several studies linked the EMM characteristics with the type of N pools they explore in the soil, and with their roles in soil-plant interaction, taking into account energetic cost-benefit for both fungi and plant host (e.g., Agerer, 2001; Lilleskov *et al.*, 2002; Hobbie & Agerer, 2010; Lilleskov *et al.*, 2011; Cairney, 2012). Additionally, species with abundant EMM generally showed stronger

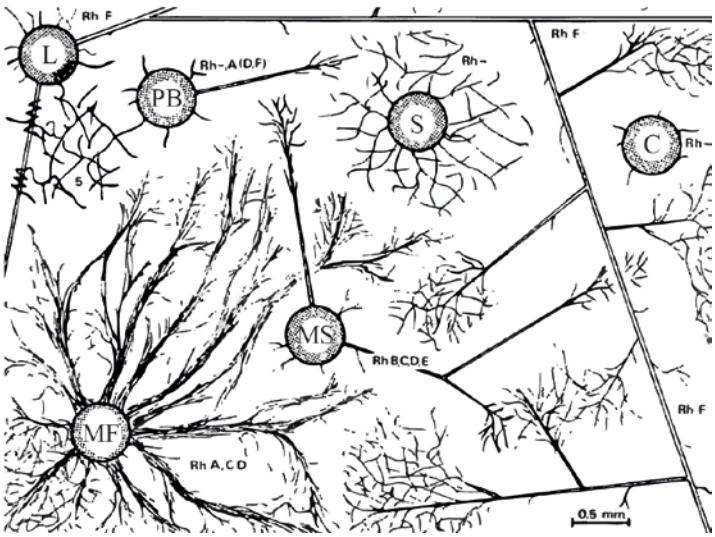


Figure 1.5. Schematic drawings of different extramatrical mycelia exploration types. Legend: L, long-distance; PB, pick-a-back; S, short-distance; C, contact; MS, medium-distance smooth; MF, medium-distance fringe; Rh, rhizomorphs (with classification according to Agerer 1987-2004); Rh-, lack of rhizomorphs. Adapted from Agerer, 2001.

potential to produce extracellular enzymes than species with scarce EMM (Tedersoo *et al*., 2012), an essential feature to acquire organically bounded N. It has been hypothesized that species with EMM of the medium-distance fringe, and long-distance exploration types might have the potential to explore recalcitrant nutrient-pools through extracellular enzyme activity, and that species with contact, short, and medium-distance smooth exploration types might be associated with labile nutrient soil-pools (e.g. Lilleskov *et al.*, 2002; Hobbie & Agerer, 2010; Lilleskov *et al.*, 2011). Therefore, the exploration type strategy is connected with soil N turnover ratio, plant mineral nutrition and inter-plant nutrient transfer. In exchange plants may allocate more or less C derived photosynthates to the symbiotic fungi. The fate of the allocated C will greatly depend on life span and turnover ratio associated with the EMM.

Aims, thesis outline and methodological overview

The main goal of this thesis is to understand how the arctic fungal community responds to long-term changes in climatic conditions. Specifically, this work focused on the effects of summer warming and increased winter snow depth on belowground fungal community composition, richness and functional traits. In chapter 2 of this thesis, the effects of 18 years of summer temperature increases in the ECM basidiomycete community in dry and moist tussock tundra in Northern Alaska are addressed. The increase in temperature was passively achieved using open top chambers (OTC). It has been repeatedly shown that OTCs provide a reasonable approximation to the predicted climatic changes in the Arctic (e.g. Marion *et al.,* 1997; Sharkhuu *et al.,* 2013; Bokhorst *et al.,* 2013). Chapter 3 focuses on the effects of long-term summer warming on the whole fungal community in dry heath and moist tussock tundra in Northern Alaska. Chapter 4 aims to provide insight into the changes in the ECM basidiomycete community induced by long-term increased snow depth. To achieve long-term increased snow depth, snow fences (in dry heath and moist tussock tundra) were set up every winter during 18 years previous to this work. The snow fences are 2.8 m high and 60 m long, and constitute a partial barrier to airflow that carries snow, inducing leeward snow drifts of ca. 60 m long (Walker *et al.*, 1999; Pattison & Welker, 2014).

Both the OTCs and the snow fence experiments were set up at Toolik Lake Long-Term Ecological Research site (LTER), and are part of the International Tundra Experiment (ITEX) (Henry & Molau, 1997, Welker *et al*., 1997). This site is located on the northern foothills of the Brooks Range (68°38'N, 149°36'W, 670m asl) (Fig. 1.6). The area lies in the Arctic tundra biome within the bioclimatic subzone E. The mean air annual temperature is -7 °C and annual precipitation ranges between 200 and 400 mm with approximately 50% falling as snow. The average snow depth is 50 cm (DeMarco *et al.*, 2011). The distribution of vegetation depends on edaphic factors determined by topography and geological history. The oldest soils developed on glacial till from the

Sagavanirktok glacial advance (> 300,000 years ago), the next oldest soils on till from the Itkillik I advance (ca. 60,000 years ago), and the youngest soils on till from the Itkillik II advance (ca. 10,000 years ago) (Hobbie *et al*., 2014). The availability of N limits primary productivity and net ecosystem productivity is approximately 10-20 g C m-2yr-1 (McGuire *et al*., 2000).

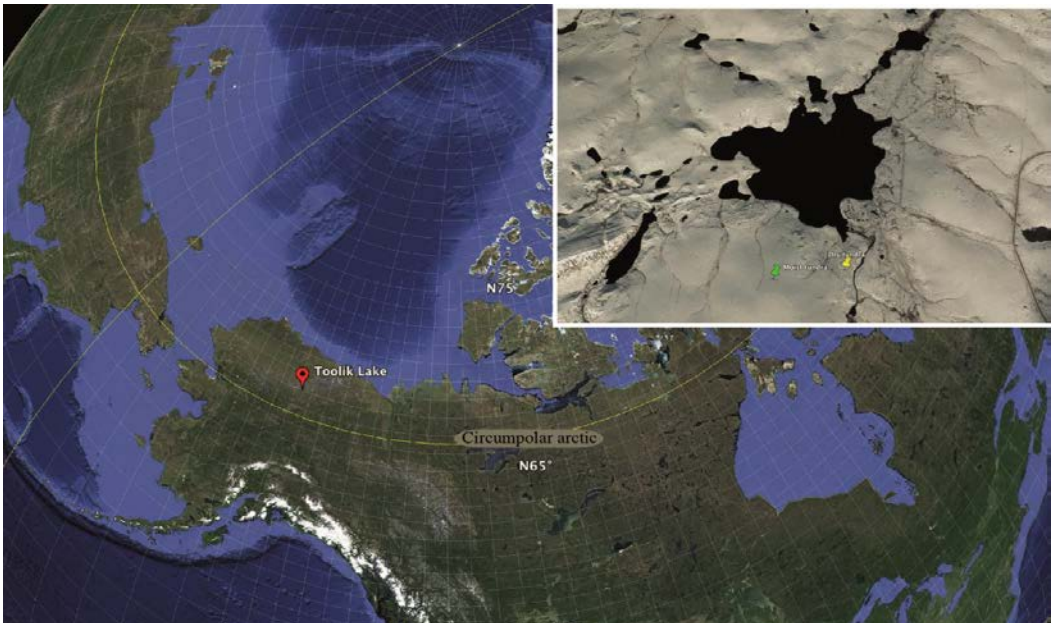
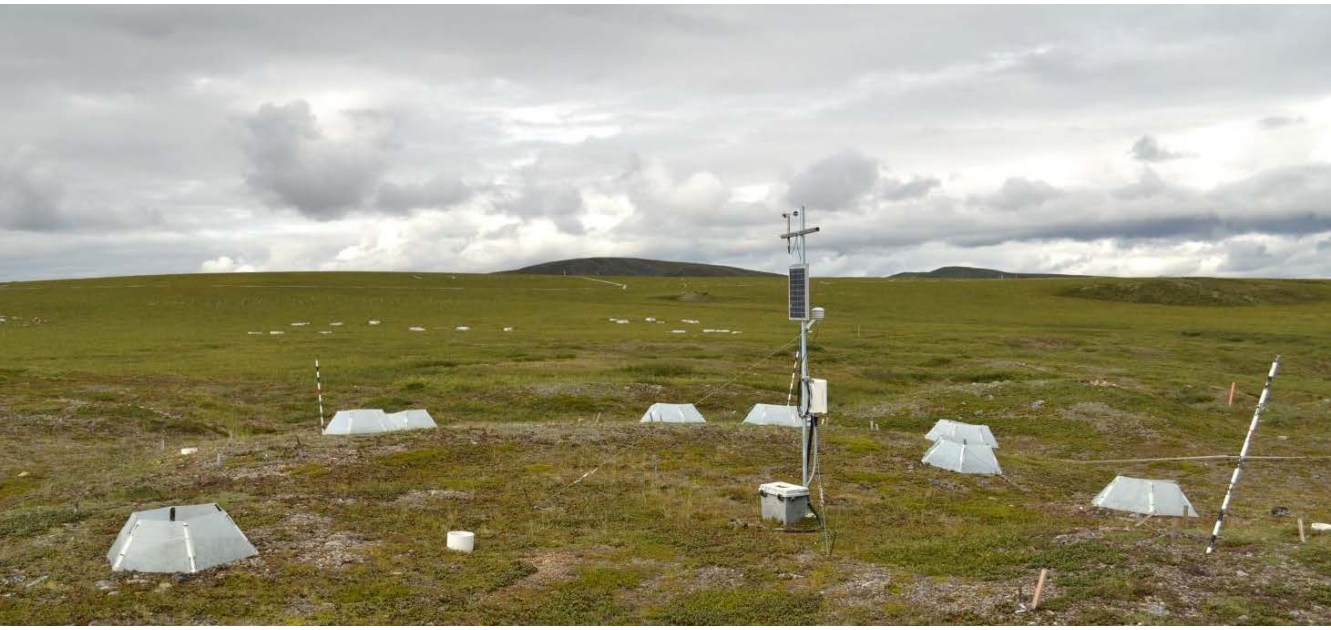


Figure 1.6. Toolik Lake location with close up (inset) with sampling localities, dry tundra with yellow pin and moist tundra with green pin. Source: Google Earth accessed 16 September 2015.

In this work, belowground soil fungal community composition was assessed through massive parallel sequencing. The soil samples were performed with a soil corer in experimental and control plots. Soil samples were frozen until lyophilization. Afterwards, soil DNA was extracted, PCR targeted fungal DNA, and Ion Torrent sequencing was performed. For the bioinformatics analysis, generally accepted filters and thresholds of datasets clean up were used. The statistical analysis utilized aimed to compare the community composition and its relation with the different taxonomic and ecological groups. All methods were standardized across the different chapters, and the results are fully comparable.



Open top chambers on the dry tundra at Toolik Lake research field station, Alaska.

Chapter 2

Summer temperature increase has distinct effects on the ectomycorrhizal fungal communities of moist tussock and dry tundra in Arctic Alaska

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Published in: Global Change Biology 2015, 21(2): 959-972

Abstract

Arctic regions are experiencing the greatest rates of climate warming on the planet and marked changes have already been observed in terrestrial arctic ecosystems. While most studies have focused on the effects of warming on arctic vegetation and nutrient cycling, little is known about how belowground communities, such as root-associated fungi, respond to warming. Here we investigate how long-term summer warming affects ectomycorrhizal (ECM) fungal communities. We used Ion Torrent sequencing of the rDNA internal transcribed spacer 2 (ITS2) region to compare ECM fungal communities in plots with and without long-term experimental warming in both dry and moist tussock tundra. *Cortinarius* was the most OTU-rich genus in the moist tundra, while the most diverse genus in the dry tundra was *Tomentella*. On the diversity level, in the moist tundra we found significant differences in community composition, and a sharp decrease in the richness of ECM fungi due to warming. On the functional level, our results indicate that warming induces shifts in the extramatrical properties of the communities, where the species with medium-distance exploration type seem to be favoured with potential implications for the mobilization of different nutrient pools in the soil. In the dry tundra, neither community richness nor community composition was significantly altered by warming, similar to what had been observed in ECM host plants. There was, however, a marginally significant increase of OTUs identified as ECM fungi with the medium-distance exploration type in the warmed plots. Linking our findings of decreasing richness with previous results of increasing ECM fungal biomass suggests that certain ECM species are favoured by warming and may become more abundant, while many other species may go locally extinct due to direct or indirect effects of warming. Such compositional shifts in the community might affect nutrient cycling and soil organic C storage.

Introduction

Soils of the northern circumpolar region cover approximately 16% of the global soil surface and contain an estimated 50% of all soil organic carbon (C) pool (Tarnocai *et al.*, 2009). Because these regions have been experiencing some of the highest rates of warming (0.06 to 0.1°C per year over the past 40 years), a large proportion of this C is increasingly vulnerable to mobilization due to warming-induced melting of permafrost and higher microbial decomposition rates (Anisimov *et al.*, 2007; Hansen *et al.*, 2010; Comiso & Hall, 2014). This warming is resulting in a suite of climate feedbacks, including changes in sea ice cover and the length of ice-free periods (Arrigo & van Dijken, 2011; Post *et al.*, 2013;), a greening of the surrounding land surface, and tree line advancement (Kharuk *et al.* 2013; Zhang *et al.*, 2013). All of these are altering the albedo of the Arctic (Chapin *et al.*, 2005; Post *et al.*, 2009). Although many of these feedbacks are positive, some could potentially be negative. For example, a greening of the Arctic driven by increases in shrub density (Sturm *et al.*, 2005; Loranty & Goetz, 2012; Tape *et al.*, 2012) could result in greater degrees of C sequestration (Welker *et al.*, 1997; Anderson-Smith 2013; Sistla *et al.*, 2013; Pattison & Welker, 2014), but see Hartley *et al.* (2012) for counter-argument. Increases in shrub density and canopy growth can further alter the tundra by local snow-trapping in winter, increasing soil insulation, causing higher winter and spring-time soil temperatures, and increasing the rates of nitrogen (N) and C mineralization. Greater rates of winter CO_2 emissions, in turn, may enhance the potential for shrub growth and further expansion (Sturm *et al.*, 2001; Schimel *et al.*, 2004; Sturm *et al.*, 2005; Weintraub & Schimel, 2005, Tape *et al.*, 2006). However, whether these changes are accompanied by a simultaneous reorganization of the soil fungal community and whether these responses differ in moist tussock and dry tundra have not been resolved.

Arctic soils have limited availably of nutrients and arctic plants are highly dependent on mutualistic relationships with mycorrhizal fungi for survival (Gardes & Dahlberg, 1996; Hobbie *et al.*, 2009; Bjorbækmo *et al.*, 2010). It has been estimated that 61 to 86% of the N in Arctic tundra plants is obtained through mycorrhizal fungi (Hobbie & Hobbie 2006). Ectomycorrhizal (ECM) fungi are the predominant fungal guild in the Arctic (Gardes & Dahlberg, 1996; Clemmenson *et al.*, 2006; Bjorbækmo *et al.*, 2010). Recent studies of belowground Arctic ECM fungal communities, revealed higher species richness than what had previously been known from above-ground surveys (Ryberg *et al.*, 2009; Bjorbækmo *et al.*, 2010; Geml *et al.*, 2012; Timling *et al.*, 2012; Timling & Taylor, 2012). These studies indicated that the most diverse arctic ECM genera are *Tomentella* (here interpreted as including *Thelephora)*, *Inocybe*, *Cortinarius*, *Sebacina*, *Russula* and *Hebeloma*.

ECM fungal community composition in the Arctic is generally correlated with soil properties, geology, plant productivity and climate (Timling *et al.*, 2012; Timling *et al.*, 2014). There is also evidence to suggest that ECM plant-host identity is not a main driver of ECM fungal community composition in the Arctic (Ryberg *et al.*, 2009; Timling *et al.*, 2012). Although there are a few studies focused on the molecular diversity of below-ground ECM fungal communities in the Arctic (Ryberg *et al.*, 2009; Bjorbaekmo *et al.*, 2010; Geml *et al.*, 2012 Timling *et al.*, 2012; Timling *et al.*, 2014), the main drivers at the landscape scale remain largely unresolved, and this hampers our current in-depth comprehension of arctic soil ecology.

Recent evidences, reported from other biomes than the Arctic, suggest that the extramatrical mycelium (EMM) morphology and ECM fungi extracellular enzyme activity are of great relevance to understand the nutrient dynamics of the ECM symbiosis (Carney & Burke, 1996; Agerer, 2001; Anderson & Cairney, 2007; Hobbie & Agerer, 2010; Peay *et al.*, 2011; Tedersoo *et al.*, 2012; Talbot *et al.*, 2013; Bodeker *et al.*, 2014) that is crucial to understand soil ecology. ECM fungi produce EMM that grows from the ectomycorrhizae into the surrounding soil with the crucial functions of foraging the litter and/or mineral layers for nutrients, and of seeking new root tips for colonization (Martin *et al.*, 2001; Anderson & Cairney, 2007). The EMM forms an intricate hyphal network that interconnects plant roots, and paves the way for inter-plant C and nutrient movements (Selosse *et al.*, 2006). EMM of different taxa are known to have distinct anatomical and physiological features that are attributable to various strategies of foraging (Colpaert *et al.*, 1992; Agerer, 2001; Hobbie & Agerer, 2010). Several studies linked the EMM characteristics with the pools of nutrients they explore in the soil, and with their roles in soil-plant interaction, taking into account energetic cost-benefit for both fungi and plant host (e.g., Agerer, 2001; Lilleskov *et al.*, 2002; Hobbie & Agerer, 2010; Lilleskov *et al.*, 2011; Cairney, 2012). The main characteristics to classify the EMM are the mycelium exploration type, presence/absence of rhizomorphs and hydrophobicity of the hyphae (Agerer, 2001; Hobbie & Agerer, 2010; Peay *et al.*, 2011; Lilleskov *et al.*, 2011; Cairney, 2012). Moreover, besides EMM characteristics *per se*, species with abundant EMM generally showed stronger potential to produce extracellular enzymes than species with scarce EMM (Tedersoo *et al.*, 2012), even though multiple exceptions exist. It has been hypothesized that species with EMM of the medium-distance fringe, and long-distance exploration types might have the potential to explore recalcitrant nutrient-pools through extracellular enzyme activity, and that species with contact, short, and medium-distance smooth exploration types might be associated with labile nutrient soil-pools (e.g. Lilleskov *et al.*, 2002; Hobbie & Agerer, 2010; Lilleskov *et al.*, 2011). Such functional information is still under investigation, and therefore, currently only available for a limited number of taxa. Nevertheless, this framework constitutes a valuable insight into the ecological functions of ECM fungal community.

The long-term effects of climate change on arctic tundra function and structure have primarily been investigated with respect to aboveground growth, phenology, vegetation composition, and plant and ecosystem C exchange (e.g., Chapin & Shaver, 1985; Arft *et al.*, 1998; Welker *et al.*, 1997; Welker *et al.*, 2000; Welker *et al.*, 2004; Elmendorf *et al.*, 2012; Tape *et al.*, 2012; Cahoon *et al.* 2012; Sharp *et al.*, 2013; Pattison & Welker 2014). Vegetation studies, in the moist tussock tundra at Toolik Lake, Alaska, indicated that long-term experimental summer warming induced significant increases in the abundance and height of *Betula nana*, *Salix pulchra*, and graminoids, and in the accumulation of the litter layer (Wahren et al., 2005; Mercado-Díaz, 2011). Conversely, the bryophytes decreased significantly (Mercado-Díaz, 2011), most likely due to competitive exclusion by shrubs (Cornelissen *et al.*, 2001; Jägerbrand *et al.*, 2009). These aboveground vegetation changes are likely correlated with changes below ground, such as soil moisture, soil nutrient pools, fine-root abundance, and root turn-over dynamics, which interplay with ECM fungal community dynamics (e.g., Read *et al.*, 2004; Dickie & Reich, 2005; Dickie *et al.*, 2005; Strand *et al.*, 2008; Toljander *et al.*, 2006; Twieg *et al.*, 2009; Peay *et al.* 2011). Even though some studies addressed belowground processes, such as N cycling (e.g., Schimel *et al.*, 2004; Borner *et al.*, 2008; Schaeffer *et al.*, 2013) and microbial community change (e.g., Clemmenson *et al.*, 2006; Campbell *et al.*, 2010; Deslippe *et al.*, 2011; Deslippe *et al.*, 2012), our knowledge about the compositional and functional changes of arctic communities in response to long-term warming remains rudimentary.

In this study, we use high-throughput sequencing techniques to study the long-term effects of experimental warming on the ECM basidiomycete community in dry and moist tussock tundra in Northern Alaska. Our hypotheses were two-fold. First, we hypothesize that long-term warming induces changes in the ECM fungal community composition, because above-ground changes in the vegetation, including several ECM host plants, have already been documented (Wahren *et al.*, 2005; Mercado-Díaz, 2011) and this is suggestive of changes in below-ground processes (Sullivan & Welker, 2005; Sullivan et al. 2007). Secondly, based on the results from the above vegetation studies and reported warming-induced increases in ECM fungal and fine-root biomass (Clemmensen *et al*., 2006), we expect that the ECM fungal community of the moist tussock tundra will show a stronger response to warming than the dry tundra. Furthermore, we expect to find a more diverse ECM community in the warmed moist tussock tundra plots, because Deslippe *et al*., (2011) reported significant increases in the diversity of arctic ECM fungi associated with root tips of *Betula nana* as a response to warming. *Betula nana* is a dominant in our sampling plots and has shown strong, positive response to experimental warming (Wahren *et al.*, 2005; Mercado-Díaz, 2011).

Material and Methods

Sampling location

The sampling area is located at the Arctic Long Term Ecological Research site in the Toolik Lake region in the northern foothills of the Brooks Range, Alaska, USA (68°37'N, 149°32'W; 760 m above sea level). The region lies within the bioclimatic subzone E that is the warmest subzone of the arctic tundra with mean July temperatures ranging from 9 to 12°C (Walker *et al.*, 2005). The two main vegetation types of the region are: the dry heath tundra, characterized by *Dryas octopetala*, *Salix polaris*, *Vaccinium* spp. and fruticose-lichens, and the moist tussock tundra, dominated by *Betula nana*, *Salix pulchra* and the sedge *Eriophorum vaginatum.* Detailed descriptions of the plant communities can be found in Walker *et al.* (1999) and Kade *et al.* (2005).

Experimental design

Between July 23 and 25, 2012, we sampled soil from 20 plots representing the dry and the moist tussock tundra. In each tundra type, we sampled five plots that were subjected to passively increased summer air temperature by hexagonal open top chambers (OTCs), subsequently referred to as "treatment", and five adjacent areas with unaltered conditions ("control"). The sampling was performed with a soil corer of approximately 2 cm × 20 cm (diameter x depth). In each of the 20 plots, five soil cores were taken, thoroughly mixed and kept frozen until lyophilization.

The OTCs used are 1 m^2, 0.4 m high, and constructed of translucent fiberglass (Marion *et al.*, 1997; Walker et al., 1999). Within the OTCs the summer air temperature increases by a mean daily average of 1.5 °C, while soil temperatures remain the same as in the control plots (Walker *et al.*, 1999). Every year, since 1994, the OTCs are set up as soon as 50% of the ground area of a given plot was snow-free (usually early June) and are removed at the end of August or early September, following the International Tundra Experiment (ITEX) protocol (Welker *et al.*, 1997). It has been repeatedly shown that OTCs provide a reasonable approximation to the predicted climatic changes in the Arctic as they alter daytime temperature significantly and minimize unwanted ecological effects, such as changes in soil moisture, the influence of wind speed on air temperature (Marion *et al.*, 1997; Sharkhuu *et al.*, 2013; Bokhorst *et al.*, 2013 and references therein). Therefore, OTCs have been recommended to study the response of high-latitude ecosystems to warming (Marion *et al.*, 1997).

Molecular work

Genomic DNA was extracted from 1ml (0.4-1 g) of lyophilized soil from each of the twenty samples using NucleoSpin® soil kit (Macherey-Nagel Gmbh & Co., Düren,

Germany), according to manufacturer's protocol. For each sample, two independent DNA extractions were carried out and pooled in order to optimize the homogenization of the extraction. The extracted DNA was eluted with 30µl of SE buffer. PCR amplification and Ion Torrent sequencing of the ITS2 region (ca. 250 bp) of the nuclear ribosomal rDNA repeat were carried out as described by Geml *et al.* (2014b) using primers fITS7 (Ihrmark *et al.*, 2012) and ITS4 (White *et al.*, 1990). The ITS4 primer was labeled with sample-specific Multiplex Identification DNA-tags (MIDs). The amplicon library was sequenced using an Ion 318^{TM} Chip by an Ion Torrent Personal Genome Machine (PGM; Life Technologies, Guilford, CT, U.S.A.) at the Naturalis Biodiversity Center.

The initial clean-up of the raw sequence data was carried out using the online platform Galaxy (https://main.g2.bx.psu.edu/root), in which the sequences were sorted according to samples and sequence regions of primers and adapters (identification tags) were removed. We used a parallel version of MOTHUR v. 1.32.1 (Schloss *et al.* 2009) for subsequent sequence analyses following the protocol described in detail in Geml *et al.* (2014b). The quality-filtered sequences were normalized following Gihring *et al.* (2012) by random subsampling so that each sample contained 56,483 reads (the lowest number of sequences obtained for a sample). The resulting sequences were clustered into operational taxonomic units (OTUs) using OTUPIPE (Edgar, 2010) with the simultaneous removal of putatively chimeric sequences using *de novo* and reference-based filtering using curated dataset of fungal ITS sequences of Nilsson *et al.* (2011) as reference. We used a 97% sequence similarity clustering threshold as has been routinely done in fungal ecology studies (e.g. O'Brien *et al.*, 2005; Higgins *et al.*, 2007; Geml *et al.*, 2008; Geml *et al.*, 2009; Amend *et al.*, 2010; Tedersoo *et al.*, 2010; Geml *et al.*, 2012; Kauserud *et al.*, 2012; Brown *et al.*, 2013; Blaalid *et al.*, 2013; Geml *et al.*, 2014a). Global singletons were discarded from further analysis. The reference database published by Kõljalg *et al.* (2013) was used to determine the taxonomic affinity of the OTUs using USEARCH v7 (Edgar, 2010). OTUs with less than 80% similarity to any identified fungal sequence were also excluded from the final analysis due to unreliable classification, and therefore, uncertainty regarding their ecological role. A representative sequence of each OTU was deposited in GenBank under the accession numbers KJ792472 - KJ792742.

ECM fungal database and EMM determination

For in-depth analyses related to the research hypotheses stated above, we selected all OTUs that showed affinity with ECM basidiomycete genera based on Tedersoo & Smith (2013). However, in Sebacinales, we used phylogenetic analyses to select the OTUs representing the ECM lineages, because many sebacinoid taxa are not ECM. In the Sebacinales, ECM OTUs were selected based on their supported phylogenetic placement (with ≥70% bootstrap and/or ≥0.95 posterior probability) among sequences of known

ECM taxa published by Urban *et al.* (2003), Ryberg *et al.* (2009) and Tedersoo & Smith (2013). We followed the work of Agerer (2006) and consulted the DEEMY database (http://deemy.de), an information system for the characterization and determination of ECM fungi (accessed in January and February of 2014), in order to determine the EMM characteristics per species. In the genus *Russula*, if no EMM information was available for the species of interest, we assumed the EMM characteristics based on the closest species with known characteristics. To determine the closest species we followed the phylogenetic study by Miller & Buyck (2002). Similarly, for OTUs of the genus *Hebeloma*, we followed the phylogenetic study by Boyle *et al.* (2006).

Statistical analysis

For each sample, we calculated rarefied OTU accumulation curves using the R package Vegan (Oksanen *et al.*, 2012) and determined the Good's coverage (complement of the ratio between the number of local singletons and the total sequence counts). Because of demonstrated uncertainties regarding the reliability of read abundance as indicators of species abundance in the samples (Amend *et al.* 2010), we carried out the further analyses with two types of data transformations. First, we transformed the data into presence-absence matrix, where OTU presence was defined as 5 or more sequences on a per sample basis following the suggestion of Lindahl *et al.* (2013) to minimize false positives (e.g., OTUs that are common in one sample, but may be low-abundant contaminants in others). In addition, we used square-root transformed read abundance to moderate the influence of OTUs with high sequence counts, while maintaining some approximation of template abundance that may reflect ecological significance. We used PC-Ord v. 5.32 (McCune & Grace, 2002) to run non-metric multidimensional scaling (NMDS) on a primary matrix of experimental plots by OTUs and a secondary matrix of plots by OTU richness per taxon (this analysis was also performed with root-square abundance of sequence counts as a surrogate to species abundance). The dataset was subjected to 500 iterations per run using the Sørensen similarity (Bray-Curtis index) and a random starting number. We also calculated the Pearson's correlation coefficient (r) values between relative OTU richness, OTU diversity per taxon, and axes 1 and 2. We tested whether fungal communities were statistically different across the treatments using a multi-response permutation procedure (MRPP) and determined any preferences of individual OTUs for either control or treatment plots in moist tussock and dry tundra using Indicator Species Analyses (Dufrêne & Legendre, 1997) as implemented in PC-Ord v. 5.32. We also tested for significant differences in OTU richness between moist tussock and dry tundra, control and treatment plots, genera, and EMM characteristics using Student's t-test. We determined the Venn diagram for the genera with higher OTU richness, using the online version of the publication by Oliveros (2007).

Results

Taxonomic composition and OTU richness

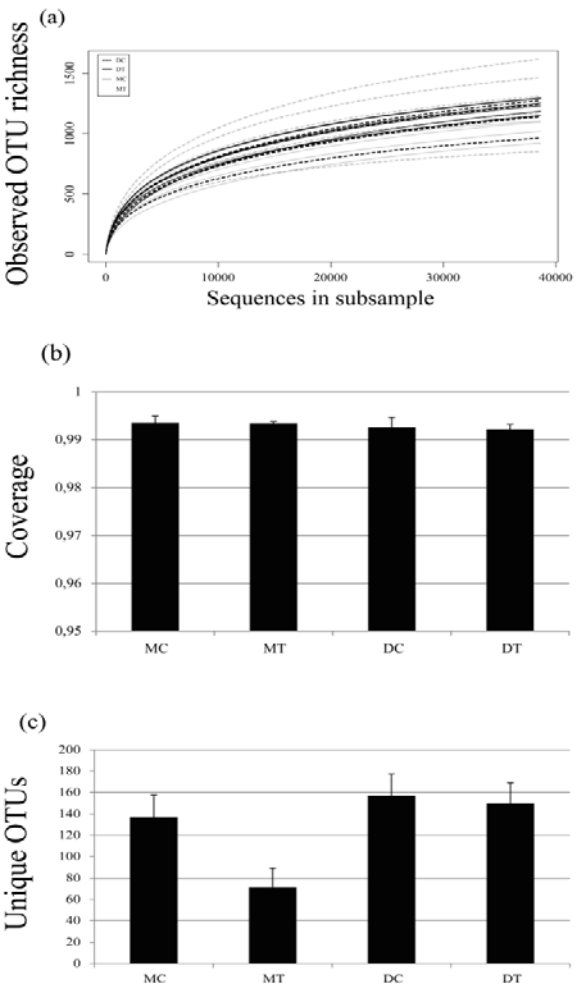


Figure 2.1. – a) Rarefaction curves of each plot for both tundra types. b) Good's coverage, average of the plot per site with standard deviation. c) Total OTUs per site and tundra type with standard deviation. Legend: DC – dry control, DT – dry warming treatment, MC – moist tussock control, MT – moist tussock warming treatment.

We obtained 4,046,811 reads with an average length of 211.6 ± 111 bp (SD). From this, approximately 87% of the data had a mean Phred ≥ 20. After quality control, 2,068,216 reads (51%) were kept, and after random subsampling we retained 1,129,660 reads with an average length of 254.9 ± 56 bp (SD). After clustering at 97% sequence similarity, 10,035 OTUs were generated. From this dataset, we removed 3,148 putative chimeras and 1,249 singletons. The asymptotic rarefaction curves (Fig. 2.1a) and Good's coverage (Fig. 2.1b) suggest that the deep sequencing allowed for a very high OTU coverage and that likely all fungi present in the samples were sequenced. The final dataset included 343 ECM basidiomycete OTUs (110,665 reads).

We detected 20 ECM basidiomycete genera (Table 2.1). Four of these dominated the communities, accounting for approximately 82% of all OTU richness: *Tomentella* (106 OTUs, 31%), *Cortinarius* (77, 22%), *Inocybe* (63, 18%) and *Russula* (34, 10%). OTU richness in the control plots was not significantly different ($p = 0.296$, $t_8 = 1.119$) between the dry and moist tundra types, although the plot-based richness values were somewhat higher in the dry than in the moist tussock tundra (Fig. 2.1c). The NMDS analysis of the full dataset indicated that species assemblages in the dry and moist tundra types are highly dissimilar (Fig. 2.2a). Therefore,

we analyzed the results for the two types of tundra separately to focus on the effect of warming on ECM community composition.

Table 2.1. Number of mean OTUs per plot in the control and warming treatment plots in the dry and moist tussock tundra. Significance of treatment effects were determined by comparing the control and treatment plots using Students *t*-test. * Significant treatment effect (α = 0.05).

	Moist tussock tundra			Dry heath tundra		
	Control	Treatment	*p*	Control	Treatment	*p*
Tomentella	14.6 ± 6.23	3.8 ± 7.40	0.04 *	18.4 ± 12.74	20.8 ± 6.30	0.75
Cortinarius	16.6 ± 7.95	7.6 ± 10.33	0.16	8.2 ± 3.49	7.8 ± 7.86	0.92
Inocybe	8.2 ± 3.12	1.4 ± 1.14	0.05 *	7.4 ± 5.23	5.2 ± 6.76	0.16
Russula	6.4 ± 4.16	1.6 ± 2.07	0.002 *	3.4 ± 2.88	8.8 ± 7.29	0.62
Sistotrema	3 ± 4.12	0.2 ± 0.45	0.17	0.6 ± 0.55	0.6 ± 0.89	1.0
Tremellodendron	2.4 ± 2.51	0.2 ± 0.45	0.09	1.4 ± 2.19	0.4 ± 0.55	0.35
Hebeloma	2 ± 0	1.2 ± 0.45	0.004 *	0.4 ± 0.89	1.4 ± 1.95	0.33
Leccinum	2.8 ± 1.64	0.4 ± 0.89	0.021 *	0.4 ± 0.55	0.6 ± 0.89	0.68
Laccaria	1.4 ± 0.89	1.4 ± 0.55	1.0	0 ± 0	0.8 ± 1.30	0.21
Clavulina	0.4 ± 0.55	0.6 ± 0.89	0.68	0.8 ± 0.84	0.8 ± 0.84	1.0
Alnicola	0.8 ± 0.45	1 ± 1	0.69	-	-	-
Pseudotomentella	-	-	-	0.6 ± 0.89	0.8 ± 0.45	0.67
Sebacina	-	0.2 ± 0.45	0.35	0.2 ± 0.45	-	0.35
Tulasnella	-	-	-	1.2 ± 1.10	0.8 ± 1.10	0.14
Clavicorona	-	-	-	0.2 ± 0.45	0.4 ± 0.55	0.55
Boletus	-	-	-	0.6 ± 0.55	0.6 ± 0.55	1.0
Ceratobasidium	-	-	-	0.6 ± 0.55	0.2 ± 0.45	0.24
Lactarius	0.2 ± 0.45	-	0.35	-	-	-
Piloderma	-	-	-	0.2 ± 0.45	-	0.35
Tomentellopsis	-	-	-	0	0.4 ± 0.55	0.14
Total community	59 ± 21	20 ± 18	0.013 *	45 ± 20	50 ± 19.28	0.66

Moist tussock tundra

The total ECM OTU richness in the warmed plots was approximately half of that in the control plots, 71 and 138, respectively. Similarly, OTU richness per plot was significantly greater in the control, 59 ± 21 (mean ± SD) OTUs per plot, than in the treatment (20 ± 18) (t_8 = 3.19, p = 0.013) (Table 2.1). NMDS analyses of the presence-absence matrix resulted in a 2-dimensional solution with a final stress of 0.0395 and a final instability < 0.00001. The two axes explained the majority of variability in the sampled fungal communities (axis 1: r^2 = 0.816; axis 2: r^2 = 0.085; total r^2 = 0.901;

orthogonality = 88.5%). The NMDS ordination plot was orthogonally rotated by the treatment to visualize correlations between warming and fungal community composition in general, and the taxonomic groups in particular. The MRPP analysis suggested a significant correlation between community composition and the warming treatment (A = 0.12345835, p = 0.0066) that was visually depicted on the NMDS ordination plot (Fig. 2.2b). The NMDS and MRPP results obtained from the square-root abundance were very similar to the presence-absence based results (appendix S2.1a).

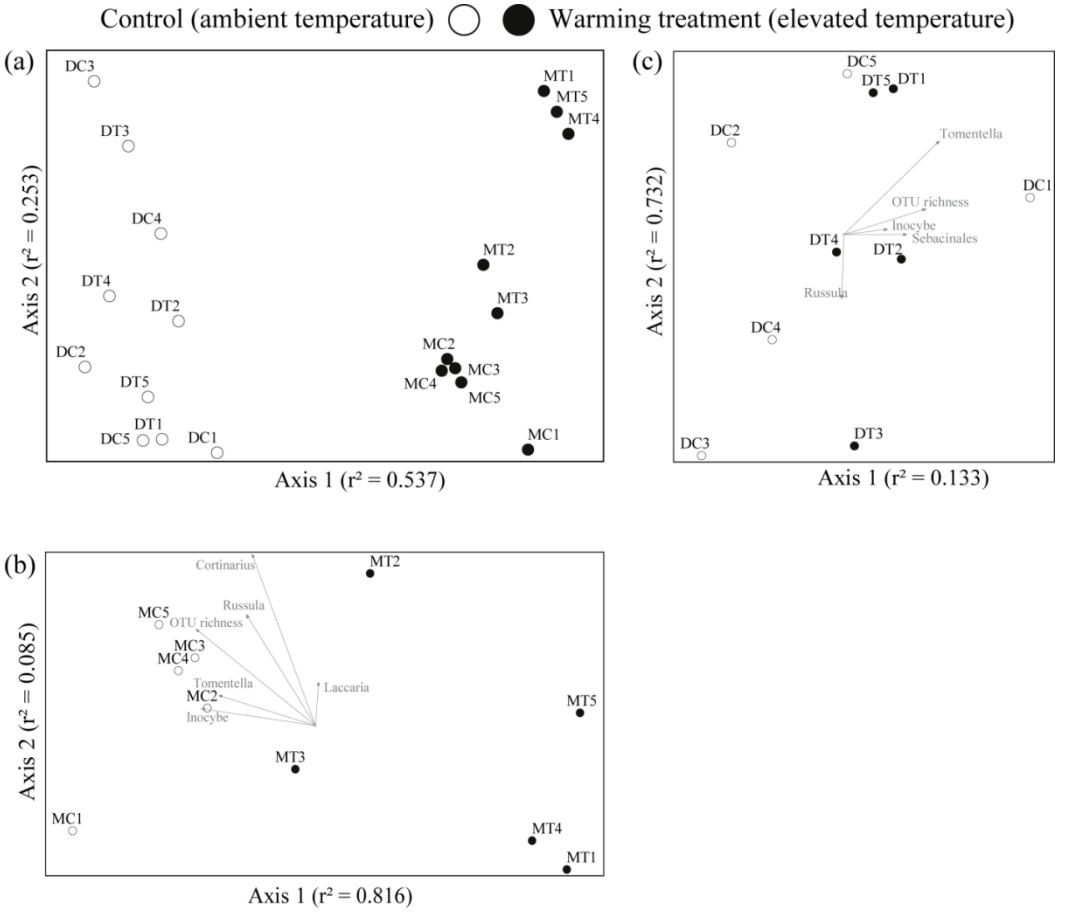
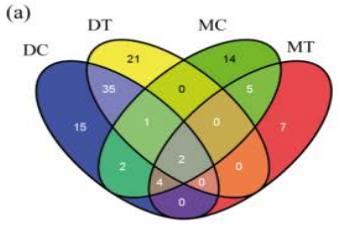
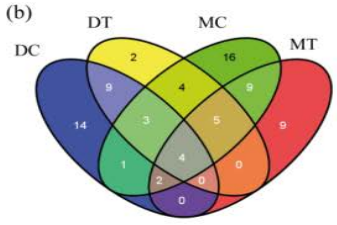


Figure 2.2. a) NMDS analysis of the dry and moist tussock tundra with control and warming treatment sites using the presence-absence dataset. b) NMDS analysis of the ECM fungal communities of the moist tussock tundra replicates. c) NMDS analysis of the ECM fungal communities of the dry tundra replicates. Legend: DC – dry control, DT – dry warming treatment, MC – moist tussock control, MT – moist tussock warming treatment.

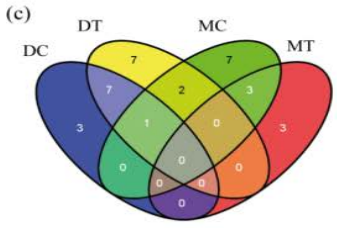
C*ortinarius* was the genus with the highest richness, followed by *Tomentella*, *Russula*, and *Inocybe* (Table 2.1). Several groups that were present in dry tundra were not detected in moist tussock tundra: *Boletus*, *Ceratobasidium*, *Piloderma*, *Pseudotomentella*, *Tomentellopsis* and *Tulasnella*. On the other hand, *Alnicola* and *Lactarius* were only



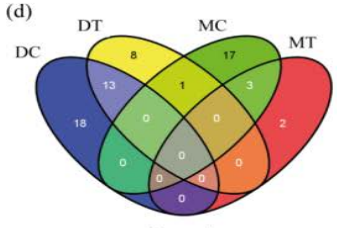
Tomentella



Cortinarius



Inocybe



Russula

Figure 2.3. Venn diagrams of the 4 most diverse genera. Legend: DC – dry control, DT – dry warming treatment, MC – moist tussock control, MT – moist tussock warming treatment.

found in the moist tussock tundra and were generally rare there as well (Table 2.1). In the four dominant genera, only 26% of the OTUs were present in both the control and the treatment plots, and most of the OTUs were only found in the control plots (Fig. 2.3). OTU richness values were significantly lower in the treatment plots, except in *Cortinarius* where the decrease in per-plot OTU richness between control and treatment was not significant.

OTU richness values in most genera were negatively correlated with the warming, with *Hebeloma* (r = -0.909), *Inocybe* (r = -0.751), and *Tomentella* (r = -0.691) as well as total OTU richness (r = -0.768) showing the strongest correlation. On the other hand, *Laccaria* and *Alnicola* did not seem to be influenced by the treatment (r = 0.126 and r = 0.108, respectively), perhaps due, in part, to their rarity. The indicator species analysis revealed 14 OTUs significantly associated with the control plots, while none of the OTUs were found to be indicators of the treatment plots (Table 2.2).

Two EMM types dominated the community, the medium-distance fringe and the contact/short-distance type, in both the control and the treatment plots (Fig. 2.4a). There was a significant decrease in the number of OTUs of most of the EMM types in the treatment plots. However, the effects in the medium-distance fringe types were not statistically significant.

Dry tundra

OTU richness in the control and treatment plots did not differ significantly (t_8 = 0.46, p = 0.66) (Table 2.1) with 45 ± 20 and 50 ± 19 OTUs per plot, respectively. T*omentella* was the most OTU-rich genus (having nearly double the amount of total number of OTUs, than the second most diverse taxonomic group), followed by *Cortinarius*, *Russula*, and *Inocybe* (Table 2.1). Approximately 40% of the OTUs were present in both the control and the

treatment plots (Fig. 2.3). In the dominant genera, the relative frequency of OTUs present in both the control and treatment plots was relatively high (compared with the values obtained for the moist tundra), varying from 33% in *Russula* to 48% in *Tomentella* (Fig. 2.3).

Table 2.2. Indicator species analysis of OTUs with significant correlation (α = 0.05) with the site, their taxonomic affinity and similarity with referenced species hypothesis (SH) and/or known sequences from UNITE database or GenBank.

OTU	Correlated site	Kõljalg *et al.* (2013) and UNITE classification	Similarity (%)
1281	DC	SH112690.05FU - *Tomentella coerulea* (UDB016493)	97.9
3369	MC	SH115895.05FU - *Leccinum holopus* (UDB001378)	99.6
484	MC	- *Tomentella fuscocinerea* (UDB016484)	99.6
3351	MC	SH108145.05FU - *Tomentella lateritia* (UDB016439)	97.8
181	MC	SH112435.05FU - *Tomentella coerula* (UDB018451)	98.1
4645	MC	SH108158.05FU - *Tomentella* sp. (UDB017832)	98.9
6618	MC	- *Hebeloma collariatum* (UDB17969)	96.2
1120	MC	SH102330.05FU - *Russula renidens* (UDB015975)	100
4313	MC	- *Tomentella fuscocinerea* (UDB016188)	95.9
1124	MC	- *Tomentella fuscocinerea* (UDB016188)	99.6
1625	MC	SH166458.05FU - *Cortinarius croceus* (UDB011339)	99.7
801	MC	SH111588.05FU - *Inocybe nitidiuscula* (HQ604382)	96.6
3413	MC	SH111588.05FU - *Inocybe nitidiuscula* (HQ604382)	95.9
5841	MC	SH099601.05FU - *Inocybe leiocephala* (AM882793)	96.7
219	MC	SH099601.05FU - *Inocybe leiocephala* (AM882793)	99

The MRPP analysis suggested no significant correlation between community composition and treatment (A = -0.00147, p = 0.4288), which was confirmed by the NMDS analysis (Fig. 2.2c). Again, the NMDS and MRPP results obtained from the square-root abundance matrix were very similar to the presence-absence based results (Supporting information, appendix S2.1b). However, Pearson's correlation values suggested that OTU richness in *Tomentella* (r = 0.789), Sebacinales (*Sebacina* and *Tremellodendron*) (r = 0.640) and *Inocybe* (r = 0.535), as well as the total OTU richness (r = 0.730) were positively correlated with the treatment. Even though the remaining groups did not show strong correlation with warming, the genera *Russula* and *Laccaria* exhibited an interesting pattern. Although the mean richness of *Russula* did not differ significantly (t_8 = 1.5397, p = 0.1622) in the control and treatment plots (3 ± 3 and 9 ± 7 OTUs per plot, respectively), the total number of *Russula* OTUs with EMM medium-distance smooth type in the treatment plots was considerably higher than in the control plots (17 and 7, respectively). Also, *Laccaria* OTUs were only found in the treatment

plots. Species from this genus have been argued to (1) possess an EMM of the medium-distance smooth exploration type with hydrophilic hyphae (Unestan & Sun, 1995; Agerer, 2001) and (2) to be nitrophilic with positive response to disturbance (Dickie & Moyersoen, 2008). The indicator species analysis (Table 2.2) revealed that OTU 1281, identified as *Tomentella atramentaria* (SH112690.05FU), was negatively correlated with warming.

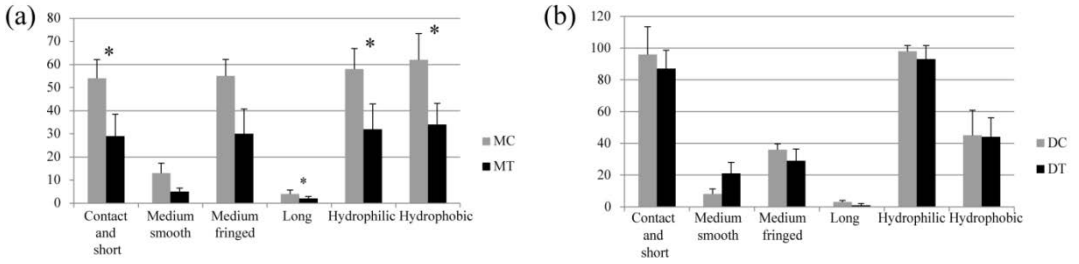


Figure 2.4. a) Number of unique OTUs per extramatrical mycelium characteristics in the moist tussock control *vs* moist tussock warming treatment plots with standard deviation of the 5 replicates. b) Number of unique OTUs per extramatrical mycelium characteristics in the dry control plots *vs* dry warming treatment plots with standard deviation of the 5 replicates.

We found that the majority of the OTUs were of the contact and short distance EMM type with hydrophilic hyphae, in both the control and the treatment plots. There was a non-significant decrease in the number of OTUs of most of the EMM types in the treatment plots. However, the medium-distance smooth type showed an opposite pattern, having an increment in the number of OTUs in the treatment plots and this difference was marginally significant ($t_8 = 2.28$, $p = 0.0567$).

Discussion

Diversity

We found 343 OTUs of ECM basidiomycetes in the sampled moist tussock and dry tundra in Alaskan Arctic. These OTUs were spread across 20 genera. This richness is the highest ever reported for arctic ECM fungi. Previous studies on below-ground diversity of arctic ECM fungi that used similar methods, reported between 73 and 202 OTUs of ca. 12 genera (Bjorbaekmo *et al.*, 2010; Geml *et al.*, 2012; Timling & Taylor, 2012; Timling *et al.*, 2012; Timling *et al*., 2014). Moreover, several genera remain undersampled in our dataset (e.g. *Lactarius*, *Amanita*), possibly due to their small genet size and relative rarity at the landscape-scale. Because observed fruitbodies of several *Amanita* and *Lactarius* species near the sampled plots, in identical vegetation types, it is likely that the real diversity of ECM fungi in the sampled region is even higher than our estimates.

In general, the dominant taxonomic groups that we uncovered, *Tomentella*, *Cortinarius*, *Inocybe*, and *Russula*, are agreement with the findings of previous studies that used molecular techniques to study below-ground diversity in arctic tundra communities (Bjorbaekmo *et al.*, 2010; Geml *et al.*, 2012; Timling & Taylor 2012; Timling *et al.* 2012). On the other hand, the dry and moist tundra types were dominated by distinct taxonomic groups, namely *Tomentella* and *Cortinarius*, respectively (Table 2.1). Such a difference was also apparent in the EMM types that were found more prevalent in the different tundra types. While there seems to be a co-dominance of two EMM types (medium-distance and contact/short-distance) in the moist tussock tundra with equal richness of OTUs with hydrophobic and hydrophilic hyphae; in the dry tundra, only the contact/short-distance EMM type with hydrophobic hyphae were dominant (Fig. 2.4b).

Warming-induced changes in the moist tussock tundra

Our results show a clear decrease in ECM fungal richness in response to warming in the moist tussock tundra, which is in clear contradiction with the single previous study addressing the effects of long-term warming on ECM fungal diversity in the Arctic (Deslippe *et al*., 2011). Deslippe *et al*. (2011) reported a warming-induced increase in diversity of ECM fungi associated with *Betula nana*. The contradiction might be due to methodological differences and the sampling depth of the ECM communities. While our data are derived from soil associated with the whole plant community, and comprised 110,684 sequences that were clustered into 343 OTUs, the observations of Deslippe *et al.* (2011) were based on 1,060 non-clustered sequences (ca. 70 OTUs) derived from cloning of root-tips of a single ECM host, *B. nana*. The steep OTU rarefaction curves generated by Deslippe *et al.* (2011) suggest that only a fraction of all ECM taxa at the sites were sequenced. Therefore, their sampling intensity likely was inadequate to obtain near-complete coverage to capture changes in richness. Due to our deep sequencing efforts, our rarefaction curves indicate that the vast majority of fungal taxa in the sampling sites have been sequenced that, in turn, provides a more solid base for among-site comparisons.

Cortinarius was the only dominant genus with non-significant decrease in per-plot OTU richness. *Cortinarius* also stands out by having most OTUs present in both control and treatment plots, a pattern that is distinct from the other three dominant genera (Fig. 2.3). This suggests that most *Cortinarius* spp in the moist tundra may be resilient and/or adapted to the conditions induced by warming. In light of the EMM characteristics, it is interesting to note that, contrary to the other three dominant genera, *Cortinarius* has an EMM with medium-distance fringed exploration type and hydrophobic rhizomorphs (Agerer, 2001, 2006). Taking into account that prior evidence point to a lack of effect of warming on ECM colonization ratios and that ECM fungal

biomass increases with warming (Clemmenson *et al.*, 2006), it seems reasonable to hypothesize that *Cortinarius* spp. might have an advantage over other ECM taxa under long-term summer temperature increase.

Lilleskov *et al.* (2011) suggested that fungi with medium-distance fringed exploration type and hydrophobic rhizomorphs, such as *Cortinarius*, are likely to have hydrolytic capabilities that would facilitate acquisition and translocation of insoluble proteins. It has been postulated for an extended period that most extracellular enzyme secretion is likely to be confined to the hyphae tip close to the apex, where the wall pore size is large enough to be permeable to enzymes of relatively large molecular weight (e.g., Chang & Trevithick, 1974; Unestam & Sun, 1995; Lindahl *et al.*, 2005). Indeed, the functional compartmentalization in gene expression between root-tips and foraging mycelium (Wright *et al.*, 2005), coupled with the very low activity of enzymes correlated with ECM fungi measured on ECM root-tips compared to levels of activity measured in bulk soils (Talbot *et al.*, 2013) support the hypothesis that the EMM have a crucial role in nutrient acquisition. Recently, Bödeker and colleagues, (2014) provided compelling evidence supporting the hypothesis that at least, some *Cortinarius* spp. are directly involved in soil organic matter degradation through extracellular enzymatic activity. This capability coupled with a diffuse mycelium may be advantageous for *Cortinarius* spp. to colonize recalcitrant and unevenly distributed nutrient soil-pools, nutrients uptake, and to translocate them from the soil to the host roots while promoting root connectivity (Hobbie & Agerer, 2010; Lilleskov *et al.*, 2011). Such functional roles can facilitate plant mineral nutrition resulting in higher plant and leaf N, as well as greater growth and greater leaf photosynthesis as observed in the warmed plots by Welker *et al.*, (2005) and by Pattison & Welker (2014). In exchange the host plant might increase carbohydrates allocation to the root system and EMM as a tradeoff, justifying the high energetic investment by the ECM fungi. Such mechanisms of soil C accumulation derived from roots and root-associated fungi have been shown to contribute 50 to 70% of stored C in ECM-dominated boreal forests (Clemmensen *et al.*, 2013).

The genus *Tomentella* shows a similar pattern of OTU distribution as *Cortinarius* – high relative frequency (31% and 38%, respectively) of OTUs that are present in both control and treatment plots (Fig. 2.3). This pattern is distinct from that observed in *Russula* and *Inocybe*, both of which have a low relative frequency (13% and 19%, respectively) of OTUs present in both the control and the treatment plots. A couple of hypotheses can be raised to interpret this trend. First, the EMM characteristics of *Tomentella* spp. are still largely unknown and there is some evidence that EMM morphology varies considerably within this diverse genus. There are species with EMM with variable hydrophobicity and exploration types, such as contact, short-distance and medium-distance (Agerer, 2001; Agerer, 2006; Hobbie & Agerer, 2010). Therefore, it is possible that the EMM characteristics of the OTUs affiliated with *Tomentella* are more

varied than initially assumed and distinct from the OTUs affiliated with *Russula* and *Inocybe*.

OTU richness of taxa having an EMM with the long-distance type was very low when compared to the medium-distance fringe type (Fig. 2.4a). In the moist tussock tundra, all OTUs with EMM of the long-distance type, that has been hypothesized to play an important role in nutrient translocation and new root-colonization (Hobbie & Agerer, 2010, Weigt *et al.*, 2011), were affiliated with *Leccinum* spp. that is specific to *Betula nana* in the Arctic. It has been hypothesized that *Leccinum* species have proteolyctic capabilities (Nygren *et al.*, 2008; Eaton & Ayres, 2002). Therefore, it is surprising that the OTU richness with this EMM type decreased significantly in the treatment plots, since we expected a similar pattern as in the species with medium-distance fringe exploration type (for in-depth discussion for potential similar patterns between these two groups consult Hobbie & Agerer, (2010)). However, the significance of this decrease might be influenced by the overall low diversity of taxa with long-distance EMM type in the arctic tundra. It is possible that these changes in diversity might not relate to actual differences in overall ecological function, since 2 out of 4 OTUs that represented this EMM type are present in the treatment plots.

It is important to understand that we do not claim that temperature increase affects the fungi directly. Instead, we argue that the warming induces changes in the entire biotic community and, via the numerous intimate interactions fungi have with other living organisms in these plots, changes are apparent in the ECM fungal community as well, regardless if they are directly or indirectly caused by warming. Because OTCs minimize unwanted ecological effects while effectively elevating temperature (Marion *et al*., 1997), it is reasonable to assume that most of the changes in the fungal community composition are induced by increased air and surface temperature. However, it is unclear if shifts in ECM fungal communities are due to direct effects of temperature on fungal metabolism or caused indirectly, e.g., via changes in plant-fungus interactions, rather than by the direct effect of temperature on fungal metabolism (or, likely, are combinations of the two). On the other hand, it is conceivable that other factors (e.g., leaf temperature increase on sunny days) may or may not have some indirect influence on the ECM fungi associated with these plants. Due to the lack of information and relevant empirical data, it is difficult to even speculate about possible mechanisms and disentangling such causal relationships are beyond the scope of this paper.

Resilience in the dry tundra

Our results do not show significant differences in either ECM fungal community composition or richness in the dry tundra. Former vegetation studies on dry tundra community also indicated no effect of warming on plant species richness, but there were significant increases in shrub canopy height and cover, mainly due to increase in evergreen shrubs (Mercado-Díaz, 2011). These changes in plant community only occurred more than 8 years after initiating the experiment (Walker *et al.*, 1999, Wahren *et al.*, 2005, Mercado-Díaz, 2011) reflecting a less pronounced effect of the warming treatment on the dry tundra than what is reported for the moist tussock tundra (see above). Contrary to the patterns observed in the moist tundra, the changes in the dry tundra vegetation were mainly in non-ECM plants. Therefore, the absence of significant changes in the ECM fungal community of the dry tundra is in agreement with the lack of compositional changes in the ECM plant hosts. However, since the significant shifts in the vegetation are in non-ECM hosts (Mercado-Díaz, 2011), we hypothesize that other root-associated fungi, particularly ericoid mycorrhizal fungi, may exhibit stronger response to warming.

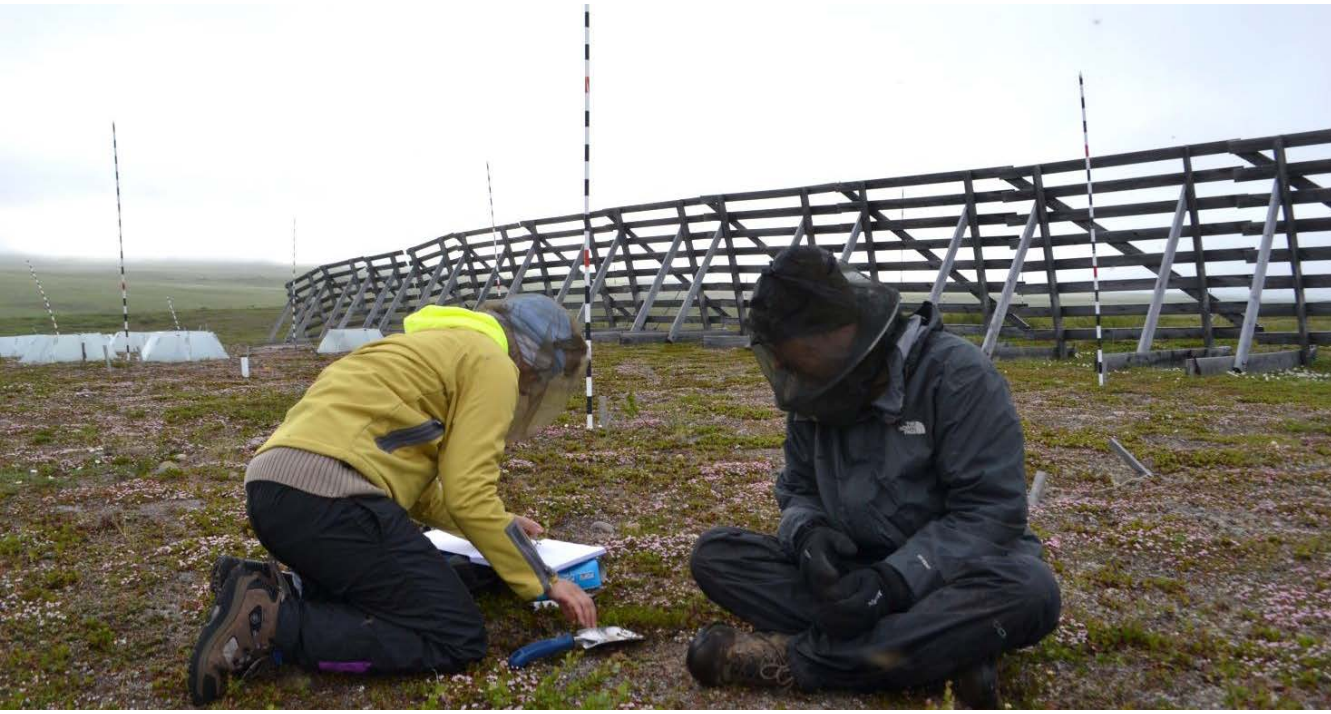
Although the NMDS analysis indicated no significant changes in the ECM fungal community composition, we found an interesting pattern in two taxonomic groups at the functional level, i.e. in the EMM types. For example, in the control plots, the genus *Russula* was represented by two EMM types: the contact with hydrophilic hyphae and the medium-distance smooth type with varying hydrophobicity. On the other hand, only OTUs with medium-distance type with mainly hydrophilic hyphae were detected in the warmed plots. Similarly, the genus *Laccaria* that has EMM of the medium-distance smooth type with hydrophilic hyphae, was only found in the treatment plots. Even though this pattern might result from chance alone, it can also be indicative of slow and/or minute shifts in the community.

When analyzing the EMM of the OTUs that compose the dry tundra community, it is interesting to note that the contact and short-distance exploration types with hydrophilic hyphae were represented by more than twice as many OTUs as the medium-distance exploration type with hydrophobic rhizomorphs. The majority of ECM fungi with contact and short-distance exploration types with hydrophilic hyphae have been hypothesized to explore the pools of labile nutrients in the soil, since most of them showed reduced proteolytic capabilities in laboratory experiments (Lilleskov *et al.*, 2002, Nygren *et al.*, 2007). Recently, Hobbie *et al.* (2013), using radiocarbon data, provided evidence from natural ecosystems that also support this hypothesis. Lilleskov *et al.* (2011) hypothesized that taxa with this EMM type are predominant in conditions of low below ground C allocation, because they probably have a low C cost to the host. This is agreement with previous findings that suggest that the dry tundra has far less productivity

than the moist tundra (Gough *et al*., 2007). Unfortunately, the scarcity of knowledge on the nutrient acquisition strategies of most ECM fungi prevent us from further speculations about possible warming-induced functional changes in the community and their effects on nutrient cycling.

High contrast in ECM communities' responses to warming at the low Arctic

This paper is the first to study the effects of long-term experimental warming in the ECM fungal community at a habitat-scale of the low Arctic tundra using high-throughput sequencing techniques. We provide evidence that (1) long-term summer temperature increases have contrasting effects on various ECM fungal genera, and (2) these effects are habitat-dependent. These patterns might be indicative of ecological strategies of various ECM fungi as well as of differences in patterns of C storage and N cycling in these two tundra types (Welker *et al.*, 2000; Schimel *et al.*, 2004; Welker *et al.*, 2005; Pattison & Welker, 2014). The non-significant changes in richness and composition of the ECM fungal community in the dry tundra (this study) coupled with evidence of non-significant changes in the ECM fungal biomass in a subarctic heath tundra, near Abisko, Sweden, (Clemmensen *et al*., 2006) might indicate that the biogeochemical processes in the dry tundra remain largely unaltered with moderate warming. Also, environmental changes other than air and surface temperature, might play a more important role in this tundra type, e.g. changes in snow depth have been reported to influence microbial activity and N cycle (Schimel *et al*., 2004), which in turn might also affect the ECM fungal community. In the moist tundra, the observed changes in the ECM fungal community (this study) coupled with the increase of ECM fungal biomass (Clemmensen *et al*., 2006) suggest increased capability of N mobilization, which might be derived from recalcitrant soil pools. This may favor enhanced C allocation from the plants to the ECM fungi and the rhizosphere, and, therefore, enhanced C storage in the soil biotic community. Even though these processes might surpass soil organic matter decomposition and microbial respiration rates, and therefore, contribute to positive feedbacks in climate change, this still remains an open question that needs further investigations.



Tatiana and József sampling in the dry tundra with the open top chambers and the snow fence experiments in the background.

Chapter 3

Long-term warming alters richness and composition of taxonomic and functional groups of arctic fungi

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Abstract

Fungi, including symbionts, pathogens and decomposers, play crucial roles in community dynamics and nutrient cycling in terrestrial ecosystems. Despite their ecological importance, the response of most arctic fungi to climate warming is unknown, so are their potential roles in driving the observed and predicted changes in tundra communities. We carried out deep DNA sequencing of soil samples to study the long-term effects of experimental warming on fungal communities in dry heath and moist tussock tundra in Arctic Alaska. The data presented here indicate that fungal community composition responds strongly to warming in the moist tundra, but not in the dry tundra. While total fungal richness were not significantly affected by warming, there were clear correlations among OTU richness of various ecological and taxonomic groups and long-term warming. Richness of ectomycorrhizal, ericoid mycorrhizal and lichenized fungi generally decreased with warming, while richness of saprotrophic, plant and animal pathogenic, and root endophytic fungi tended to increase in the warmed plots. More importantly, various taxa within these functional guilds followed opposing trends that highlight the importance of species-specific responses to warming. We recommend that species-level ecological differences are taken into account in climate change and nutrient cycling studies that involve arctic fungi.

Introduction

The arctic tundra occupies an area of 8 million km^2 and is on the threshold of significant structural and functional changes (Tape *et al*., 2012). Because arctic soils store a great portion of the Earth's reactive carbon (C), nutrient cycling in the Arctic has major consequences for global change (Welker *et al*., 2000, 2004, 2005; Callaghan *et al*., 2004; Oechel *et al*., 2014). Regional rates of warming in several arctic areas, e.g. in the region that is the focus of this paper, are among the highest globally, reaching a mean temperature increase of 0.1 °C per year over the past 35 years and further increases in temperature are predicted (Anisimov *et al*., 2007).

Marked changes have already been observed in terrestrial arctic ecosystems, including increased microbial activity leading to increased plant nitrogen (N) availability (Chapin, 1983; Nadelhoffer *et al*., 1992; Aerts, 2006), faster C turnover in soils (Hobbie & Chapin, 1998; Shaver *et al*., 2006), and shifts in land surface vegetation (Bret-Harte *et al*., 2002; Chapin *et al*., 2005; Stow *et al*., 2004) in response to warming. For example, increases in the abundance and extent of arctic shrubs have been reported (Sturm *et al*., 2001, 2005; Stow *et al*., 2004; Tape *et al*., 2006, 2012). In addition, vegetation studies carried out in plots of long-term experimental warming featured in this study indicated significant increases in the cover and height of shrubs (e.g., *Betula nana* and *Salix pulchra*) (Walker *et al*., 2006; Pattison & Welker, 2014). Conversely, the bryophytes and lichens decreased significantly (Mercado-Díaz, 2011), most likely due to competitive exclusion by shrubs (Cornelissen *et al*., 2001; Jägerbrand *et al*., 2009).

Fungi are central to the functioning of terrestrial arctic ecosystems due to their roles as symbionts (e.g. mycorrhizae, endophytes, lichens) and decomposers. Almost all arctic plants are highly dependent on mutualistic relationships with mycorrhizal fungi for survival in these nutrient-poor environments (Gardes and Dahlberg, 1996; Hobbie *et al*., 2009; Bjorbaekmo *et al*., 2010). Such associations include ectomycorrhizal (ECM), arbuscular mycorrhizal (AM), ericoid mycorrhizal (ERM) fungi (Väre *et al*., 1992; Michaelson *et al*., 2008; Newsham *et al*., 2009). It has been estimated that 61–86% of N in Arctic tundra plants is obtained through mycorrhizal fungi (Hobbie & Hobbie, 2006). In addition, endophytic fungi appear to be ubiquitous in the roots and above-ground parts of arctic-alpine plants (Väre *et al*., 1992; Higgins *et al*., 2007; Newsham *et al*., 2009), but little is known about their diversity, identity and ecological role in the Arctic. Given their intimate relationships with plants in a wide range of symbioses and importance in nutrient cycling, fungi are expected to play an important role in arctic vegetation change.

Currently, our ability to predict the response of fungal and plant communities to climate change factors is hampered both by the few detailed descriptions of the members of these communities as well as our limited understanding of the ecological role of many fungal species. Globally, approximately 100 000 species of fungi have been described,

but their true diversity may be as high as 5 million species (Blackwell, 2011). In recent years, an increasing number of molecular studies have been devoted to studying arctic fungi. The vast majority of these focused on root-associated, particularly ECM fungi, amassing valuable information on their diversity and biogeographic patterns (Bjorbaekmo *et al.*, 2010; Blaalid *et al.*, 2012; Geml *et al.*, 2012; Timling *et al.*, 2012) and their responses to experimental warming (Clemmensen *et al.*, 2006; Deslippe *et al.*, 2011; Morgado *et al.*, 2015). While ECM species are among the most ecologically important taxa, they represent a small fraction of the taxonomic and functional diversity of fungi. Yet, most other groups of arctic fungi have received little attention with the exceptions of the work of Semenova *et al.* (2015) on arctic ascomycetes and that of Timling *et al.* (2014) on soil fungal communities in zonal tundra vegetation types along a latitudinal transect spanning the low and high arctic bioclimatic subzones of North America.

Despite these important advances in our knowledge, the effect of long-term experimental warming on the taxonomic and functional diversity of a broad range of arctic soil fungal communities remains largely unknown. This knowledge is important to understand what roles fungi may have in the observed and predicted changes in tundra communities. In this study, we use deep DNA sequencing of soil samples to study the long-term effects of experimental warming on total fungal community in dry heath and moist tussock tundra in Northern Alaska. Specifically, we aimed to examine: 1) how total fungal richness changes after 18 years of summer warming; 2) which ecological and taxonomic groups are favoured or hindered by warming; and 3) how the observed changes in fungal community composition relate to formerly reported vegetation trends.

Material and methods

Experimental design and soil sampling

The study was undertaken at the International Tundra Experiment (ITEX) long-term research site in the Toolik Lake region in the northern foothills of the Brooks Range, Alaska, USA (68°37’ N, 149°32’ W; 760 m above sea level) (Walker *et al.* 1999; Welker *et al.* 2000; Pattison & Welker, 2104). The region lies within the bioclimatic subzone E that is the warmest subzone of the arctic tundra with mean July temperatures ranging from 9 to 12° C (Walker *et al.*, 2005). The two main vegetation types of the region are: dry acidic heath tundra, characterized by *Dryas octopetala*, *Salix polaris*, *Vaccinium* species and fruticose lichens, and moist acidic tussock tundra, dominated by *Betula nana*, *Salix pulchra* species, the sedge *Eriophorum vaginatum*, and several peat moss species (*Sphagnum* spp.). Detailed descriptions of the plant communities can be found in Walker *et al.* (1999) and Kade *et al.* (2005).

We sampled soil from 20 plots representing the dry and the moist tussock tundra in the last week of July, 2012. In each tundra type, we sampled five plots that were

subjected to passively increased summer air and upper soil temperature by hexagonal open top chambers (OTCs), subsequently referred to as "treatment", and five adjacent areas with unaltered conditions ("control"). The OTCs have a 1 m^2 surface area, are 0.4 m high, and are made of translucent fiberglass (Marion *et al.*, 1997; Walker *et al.*, 1999); they increase the summer air and upper soil temperature by a mean daily average of 1.5 to 2 °C measured at 15 cm height and 5 cm depth, respectively, within the OTCs (Jones *et al.*, 1998; Walker *et al.*, 1999). Every year since 1994, the OTCs were set up as soon as 50% of the ground area of a given plot was snow-free (late May or early June) and were removed at the end of August or early September, following the International Tundra Experiment (ITEX) protocol (Welker *et al.*, 1997). It has been repeatedly shown that OTCs provide a reasonable approximation to the predicted climatic changes in the Arctic as they alter daytime temperature significantly and minimize unwanted ecological effects, such as changes in soil moisture, the influence of wind speed on air temperature etc. (Marion *et al.*, 1997; Bokhorst *et al.*, 2013 and references therein). Therefore, OTCs have been recommended to study the response of high-latitude ecosystems to warming (Marion *et al.*, 1997). The soil sampling was performed with a soil corer of 2 cm × 20 cm (diameter × depth). In each of the 20 plots, five randomly chosen soil cores were taken, thoroughly mixed and kept frozen until lyophilisation.

Molecular work

Genomic DNA was extracted from 1ml (0.4-1 g) of lyophilized soil from each of the twenty samples using NucleoSpin® soil kit (Macherey-Nagel Gmbh & Co., Düren, Germany), according to manufacturer's protocol. For each sample, two independent DNA extractions were carried out and pooled in order to optimize the homogenization of the extraction. PCR amplification and Ion Torrent sequencing of the ITS2 region (ca. 250 bp) of the nuclear ribosomal rDNA repeat were carried out as described in detail by Geml *et al*. (2014b) using primers fITS7 (Ihrmark *et al.*, 2012) and ITS4 (White *et al.*, 1990). The ITS4 primer was labelled with sample-specific Multiplex Identification DNA-tags (MIDs). The amplicon library was sequenced at the Naturalis Biodiversity Center using an Ion 318™ Chip and an Ion Torrent Personal Genome Machine (PGM; Life Technologies, Guilford, CT, U.S.A.).

Sequence data analysis

The raw sequence data contained 4 046 811 reads with an average length of 212 ± 111 bp (SD). The initial clean-up of the raw sequence data was carried out using the online platform Galaxy (https://main.g2.bx.psu.edu/root), in which the sequences were sorted according to samples and sequence regions of primers and adapters (identification tags) were removed. We used a parallel version of MOTHUR v. 1.32.1 (Schloss *et al.*, 2009) for subsequent sequence analyses following the protocol described in detail in Geml *et al.* (2014ab). A total of 2 068 216 sequences remained after quality filtering and

trimming with an average read length of 255 ± 56 (mean ± SD). The quality-filtered sequences were normalized following Gihring *et al.* (2012) by rarefying to the lowest number of sequences obtained for a sample (56 483 reads). The resulting 1 129 660 sequences were clustered into operational taxonomic units (OTUs) using OTUPIPE (Edgar, 2010) with the simultaneous removal of putatively chimeric sequences with both *de novo* and reference-based filtering using curated dataset of fungal ITS sequences with representative of known taxonomic groups across the kingdom of fungi (Nilsson *et al.*, 2011) as reference. We used a 97% sequence similarity clustering threshold as has been routinely done in fungal ecology studies (e.g., O'Brien *et al.*, 2005; Geml *et al.*, 2008, 2009, 2010; Kauserud *et al.*, 2012; Brown *et al.*, 2013). Global singletons were discarded from further analysis. Because of the very high number of sequences generated per sample and because most singletons in next-generation sequencing datasets tend to be artifactual and can overestimate the diversity of 'rare taxa' (e.g., see Kunin *et al*,. 2010; Tedersoo *et al.*, 2010b), we opted to be conservative and excluded all global singletons (OTUs that were found only once across all samples despite the deep sequencing effort) from further analyses. The reference database published by Kõljalg *et al.* (2013) was used to determine the taxonomic affinity of the OTUs using USEARCH v7 (Edgar, 2010). OTUs with less than 80% similarity to any identified fungal sequence were also excluded from the final analysis due to unreliable classification. The raw sequence data for all samples have been deposited to Dryad (doi:10.5061/dryad.2fc32), while the OTU distribution matrices for the moist and dry tundra types and the representative sequences of OTUs are provided as supporting information in Geml *et al*. (2015).

Statistical analysis

We calculated Good's coverage (complement of the ratio between local singletons and the total sequence count) for each sample to estimate the exhaustiveness of our deep sequencing efforts. The effect of tundra and treatment type on observed richness of OTUs (S), Good's coverage estimators, Simpson's diversity (D = 1 – sum $[p_i^2]$, where p_i is the importance probability in element *i*), and Shannon's diversity (H = sum$[p_i \times \ln p_i]$) were tested across all sites using analysis of variance in R (Faraway, 2002). In addition, we calculated the species-area curve of all fungal OTUs vs. the number of sampled sites with first- and second-order jackknife to estimate the total fungal diversity in the moist and dry tundra types. Beta diversity was calculated for each tundra type and treatment combination following Whitaker (1972), i.e., $\beta = S_c / S - 1$, where S_c is the total number of OTUs in all plots in a certain tundra and treatment type and S is the average number of OTUs per plot.

Because preliminary analyses using one-way clustering based on an OTU vs. plot matrix revealed substantial differences in the community composition of the dry and moist tundra plots (appendix S3.1), we carried out the ordinations separately for the two

tundra types in order to eliminate the influence of habitat and to focus only on the effects of warming. We used PC-Ord v. 5.32 (McCune and Grace, 2002) to run non-metric multidimensional scaling (NMDS) on a primary matrix of experimental plots by OTUs and a secondary matrix of plots by OTU richness in various taxonomic and ecological groups. Because of demonstrated uncertainties regarding the reliability of read abundance as indicator of taxon abundance or biomass in the samples (Amend *et al.* 2010; Baldrian *et al*., 2013), we carried out the ordination analyses based on presence-absence values as well as with square-root transformed read abundance to moderate the influence of OTUs with high sequence counts, while maintaining some approximation of template abundance. Given the very high sequencing coverage we achieved, 'presence' was defined as ≥5 sequences on a per sample basis following the recommendations of Lindahl *et al.* (2013) to minimize false positives (e.g., OTUs that are common in one sample, but may be low-abundant contaminants in others). The dataset was subjected to 500 iterations per run using the Sørensen similarity (Bray-Curtis index) and a random starting number. Orthogonal rotation of the resulting NMDS solution with the lowest stress was used to maximize correlation between the warming treatment and the major axes. Pearson's correlation coefficient (*r*) values between relative OTU richness, OTU diversity per taxon, and axes 1 and 2 were calculated. OTU richness values were calculated for fungal taxonomic groups based on the current classification in Index Fungorum as implemented in UNITE, while we assigned OTUs to ecological groups based on the identities of the most similar sequences coupled with the source information of the reference sequence, when available, or based on published ecological information of the taxon in question (Kirk *et al.*, 2008; Tedersoo *et al.*, 2010a; Tedersoo and Smith, 2013; Grünig *et al.*, 2011). For this latter, only OTUs with ≥ 95% similarity to a reference sequence identified to genus were used. We tested whether fungal communities were statistically different across the replicates using two different methods: multiresponse permutation procedure (MRPP) and permutation-based nonparametric MANOVA (Anderson, 2001). We determined any preferences of individual OTUs for either control or treatment plots in moist tussock and dry tundra using indicator species analyses (Dufrêne and Legendre, 1997) as implemented in PC-Ord v. 5.32. Finally, we compared the OTU richness values of functional groups among the control and warmed plots using Student´s *t*-test.

Results

Fungal diversity

Out of the 4 046 811 original sequences, 2 068 216 passed the series of quality-filtering steps. After normalizing the library size across all samples by rarefying, 1 129 660 sequences were assembled into 6887 non-singleton OTUs, while 1249 singletons and 3148 putatively chimeric clusters were removed. After excluding OTUs with < 80% similarity or < 150 bp pairwise alignment length to a fungal sequence as performed by

USEARCH, 5438 OTUs were retained for further analyses. The observed number of OTUs did not differ significantly among the tundra and treatment types (F = 1.22, p = 0.33) (Fig. 3.1a). Good's coverage estimators (0.993 ± 0.001, mean ± SD across all sites) indicate that the deep sequencing allowed for a very high OTU coverage. The coverage estimators did not differ among tundra or treatment types (F = 1.02, p = 0.41), suggesting that our sequencing effort were similarly deep across all plots (Fig. 3.1b). Similarly, there was no significant difference among Shannon's diversity index (F = 0.42, p = 0.74) and Simpson's diversity index (F = 0.04, p = 0.99) values among the tundra and treatment types (Fig. 3.1cd). The species-area curves generated based on the accumulating number of OTUs with increased number of sites indicated that the majority of the soil fungi that occur in the moist tussock and dry heath tundra types in the Toolik Lake region likely have been sampled in our study. In the moist tundra, there were 3534 observed OTUs (Sobs), with first and second order Jackknife estimates of 4429 and 4725, respectively (Fig. 3.1e), while the values were somewhat higher in the dry tundra, with 3543 observed OTUs and first and second order Jackknife estimates of 4503 and 4894, respectively (Fig. 3.1f). Beta diversity values were relatively low within the tundra and treatment types, although in both the dry and the moist tundra, warmed plots had slightly decreased beta diversity values among the replicates (dry control: 1.389, dry warmed: 1.147, moist control: 1.143, moist warmed: 1.123).

Of the 5438 fungal OTUs, Ascomycota was by far the most OTU-rich phylum and accounted for 2189 OTUs (40.25%), followed by Basidiomycota with 1206 (22.18%). Glomeromycota was represented by 9 OTUs (0.17%), while basal lineages formerly classified in Zygomycota accounted for 145 OTUs (2.66%) and Chytridiomycota for 5 OTUs (0.09%). In addition, there were 1884 (34.65%) unidentified fungal OTUs with most similar sequences to other environmental sequences without assignment to a phylum. In Ascomycota, there were several taxonomic orders with a high number of OTUs, such as Helotiales, Chaetothyriales, and Lecanorales, while in Basidiomycota, the order Agaricales was the most diverse, followed by Sebacinales and Thelephorales. The proportional distribution of OTUs representing the taxonomic phyla and orders are shown in appendix S3.2.

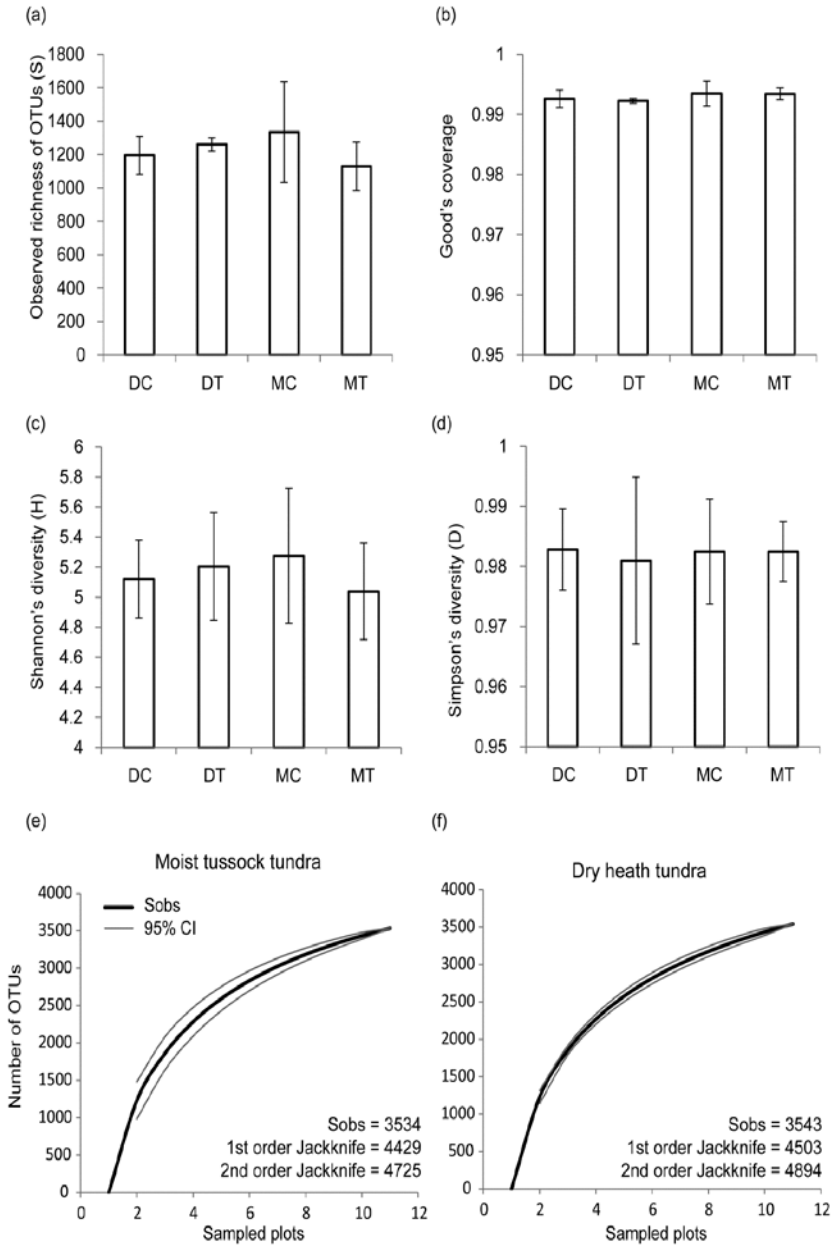

Figure 3.1. Community richness and coverage estimators in the warmed and control plots in the dry and moist tundra types with standard deviations: a) observed number of OTUs (S), b) Good's coverage, c) Shannon's diversity index (H), d) Simpson's diversity index (D), and e-f) rarefaction curves of the total number of fungal OTUs with 95% confidence interval and with first and second order Jackknife estimates of OTU richness in the dry and moist tundra types. Abbreviations are M = moist tundra, D = dry tundra, C = control, T = warming.

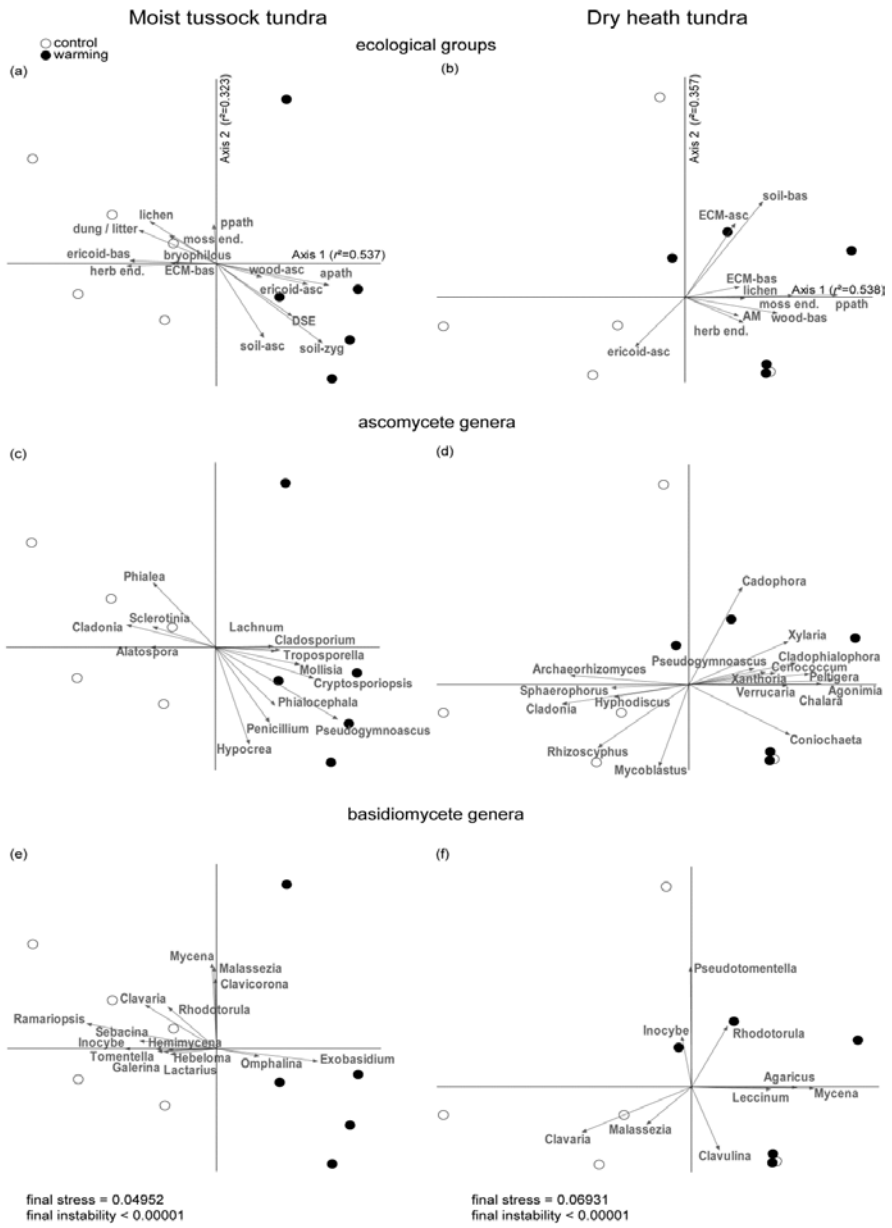

Figure 3.2. Non-metric multidimensional scaling (NMDS) ordination plots for fungal communities from the warmed and control plots in the dry and moist tundra types based square-root abundance. Because of the high number of variables tested, vectors of variables representing OTU richness in ecological groups and in genera of Ascomycota and Basidiomycota are distributed over six identical ordination plots. Only variables that correlated with any ordination axis at $|r| \geq 0.5$ are displayed. Abbreviations are apath = animal pathogen, bryophilous = fungi living on mosses, DSE = dark-septate endophyte, dung / litter = secondary decomposer fungi that live on litter and/or dung; ECM-asc = ectomycorrhizal ascomycete, ECM-bas = ectomycorrhizal basidiomycete, ericoid-asc. = ericoid mycorrhizal ascomycete, ericoid-bas. = ericoid mycorrhizal basidiomycete, herb end = endophyte of herbs, lichen = lichenized fungi, moss end = endophyte of mosses, ppath = plant pathogen, soil-asc = soil saprotrophic ascomycete, soil-bas = soil saprotrophic basidiomycete, soil-zyg = soil saprotrophic zygomycete, wood-asc = wood-rotting ascomycete, wood-bas = wood-rotting basidiomycete

The effect of warming on fungal community composition

For the moist tussock and dry heath tundra types, NMDS analyses resulted in 2-dimensional solutions with final stress values of 0.04952 (square-root abundance) and 0.05131 (presence-absence) in the moist and 0.06931 (square-root abundance) and 0.04641 (presence-absence) in the dry tundra, with final instability values < 0.00001 (Fig. 3.2 and appendix S3.3). The Monte Carlo test results indicated that all 2-dimensional solutions using the real data were significantly better than chance occurrences ($p < 0.01$). In the moist tundra, coefficients of determination for the correlations between ordination distances and distances in the original n-dimensional space were axis 1: $r^2 = 0.537$, axis 2: $r^2 = 0.323$, total $r^2 = 0.861$, with orthogonality = 84.9% (square-root abundance), axis 1: $r^2 = 0.550$, axis 2: $r^2 = 0.377$, total $r^2 = 0.927$, with orthogonality = 88.1% (presence-absence). For dry tundra, these values were axis 1: $r^2 = 0.538$, axis 2: $r^2 = 0.357$, total $r^2 = 0.895$, with orthogonality = 99.9% (square-root abundance), axis 1: $r^2 = 0.618$, axis 2: $r^2 = 0.334$, total $r^2 = 0.952$, with orthogonality = 100% (presence-absence). The NMDS ordination plots were orthogonally rotated by treatment. Because the ordinations plots based on square-root abundance and presence-absence were almost identical (Fig. 3.2 and appendix S3.3, respectively), only the square-root abundance NMDS plots were used to calculate the Pearson correlation coefficient (r) values between all environmental and fungal community variables and the ordination axes. Following Rogers *et al.* (2009), variables with $|r| \geq 0.5$ values for either axis were considered important for characterizing changes in fungal community structure and were superimposed on the ordination plot as direction and strength vectors to best illustrate differences among ecological groups (Fig. 3.2ab), ascomycete (Fig. 3.2cd) and basidiomycete (Fig. 3.2ef) genera.

The NMDS plot revealed a strong structuring of fungal communities according to the warming treatment in the moist tundra, while in the dry tundra the compositional difference between the warmed and the control plots were less pronounced (Fig. 3.2). MRPP confirmed that the total fungal community composition was significantly altered by long-term warming in the moist tundra (effect size $A = 0.13293$, probability $p = 0.00379$), while the changes were not significant in the dry tundra ($A = 0.01546$, $p = 0.12576$). Similarly, permutation-based MANOVA indicated that fungal community structure in the warmed and control plots differed significantly in the moist tundra ($p = 0.0064$), where the treatment explained 36.26% of the variation. In contrast, the warming treatment did not significantly alter fungal community composition in the dry tundra ($p = 0.1224$) and accounted for only 5.54% of the variation.

With respect to functional groups, ECM basidiomycetes represented the most OTU-rich ecological guild in both tundra types, followed by lichenized fungi in the dry tundra and saprotrophic zygomycetes in the moist tundra. In the moist tussock tundra, OTU richness in the following groups was negatively correlated with axis 1 (warming

treatment): lichens, ERM basidiomycetes (in Sebacinales), endophytes of herbs, dung/litter fungi, moss endophytes, and ECM basidiomycetes. In contrast, some groups were more diverse in the moist warmed plots, such as predominantly saprotrophic (and opportunistically pathogenic) soil asco- and zygomycetes, DSE fungi, ERM ascomycetes, animal pathogens, and wood-rotting ascomycetes (Fig. 3.2a). The patterns were different in the dry heath tundra, where only ERM ascomycetes showed strong negative correlation with warming, while ECM asco- and basidiomycetes, plant pathogens, wood-rotting basidiomycetes, herb endophytes, moss endophytes, lichens, saprotrophic soil basidiomycetes, and AM fungi were represented by more OTUs in the warmed plots (Fig. 3.2b). These patterns were confirmed by statistical comparisons of the ecological groups using *t*-tests that the majority of the above differences between control and warmed plots were significant ($p < 0.05$) or marginally significant ($p < 0.1$) (Fig. 3.3).

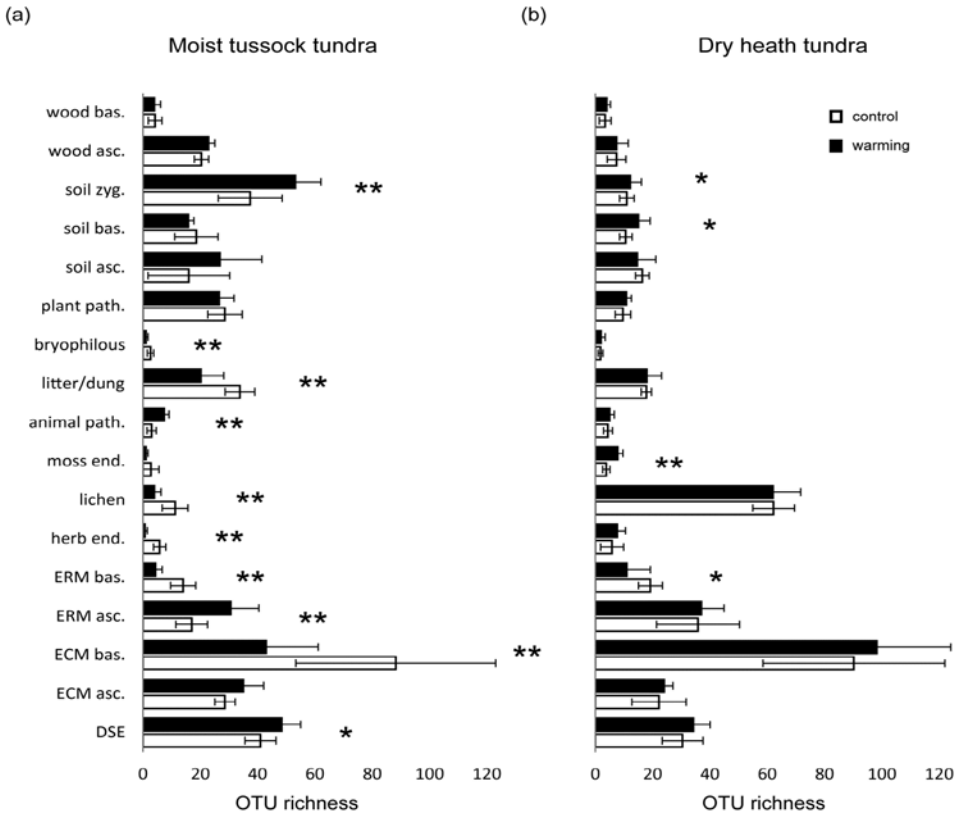

Figure 3.3. OTU richness of ecological groups of fungi represented by at least 5 OTUs with standard deviations. Significance values of Student's *t*-tests are indicated as follows: * = marginally significant ($p<0.1$), ** = significant ($p<0.05$). Abbreviations are as explained in Table 3.1.

In the moist tundra, the following genera had higher OTU richness in the control plots: *Alatospora*, *Cladonia*, *Phialea*, and *Sclerotinia* in Ascomycota, and *Clavaria*, *Galerina*, *Hebeloma*, *Hemimycena*, *Inocybe*, *Lactarius*, *Ramariopsis*, *Rhodotorula*, *Sebacina*, and *Tomentella* in Basidiomycota. Genera with strong positive correlation with warming included *Cladosporium*, *Cryptosporiopsis*, *Hypocrea*, *Lachnum*, *Mollisia*, *Penicillium*, *Phialocephala*, *Pseudogymnoascus*, and *Troposporella* in Ascomycota and *Exobasidium* and *Omphalina* in Basidiomycota (Fig. 3.2ce). In the dry tundra, OTU richness in *Archaeorhizomyces*, *Cladonia*, *Hyphodiscus*, *Mycoblastus*, *Rhizoscyphus*, and *Sphaerophorus* (Ascomycota) and in *Clavaria* and *Malassezia* (Basidiomycota) were strongly negatively correlated with warming. In contrast, OTU richness in the following genera was higher in the warmed plots: *Agonimia*, *Cadophora*, *Cenococcum*, *Chalara*, *Cladophialophora*, *Coniochaeta*, *Peltigera*, *Pseudogymnoascus*, *Verrucaria*, *Xanthoria*, and *Xylaria* in Ascomycota and *Agaricus*, *Mycena*, and *Leccinum* in Basidiomycota (Fig. 3.2df). Pearson correlation values of all ecological groups and genera are displayed in Table 3.1.

Indicator species

In the moist tundra, 365 OTUs had significant (< 0.05) *p*-values in the indicator species analyses. Of these, 212 were indicators for the control and 153 for the warming treatment. In contrast, there were only 83 OTUs in the dry tundra with significant indicator values, of which 25 were indicators of the control and 58 of the warmed plots. We conservatively identified 130 indicator OTUs to species or species complexes based on ≥ 97% sequence similarity to a reference sequence (appendix S3.4). Among plant-associated fungi in the moist tundra, ECM basidiomycetes were generally negatively affected by the warming and all indicator species representing this ecological group were associated with the control treatment. In the moist tundra, ECM basidiomycetes negatively affected by warming included *Hebeloma radicosum* SH039561.06FU, *Russula renidens* SH025103.06FU, *Sebacina* sp. SH231852.06FU, and *Tomentella* spp. SH220174.06FU, SH202711.06FU, SH202533.06FU, and SH220001.06FU. In the dry tundra, *Tomentella* sp. SH112690.05FU and the unidentified ECM agaric fungus SH220993.06FU were indicators of the control treatment (Appendix S4.1).

Similarly, ERM and root endophytic fungi belonging to the Sebacinales appeared to be negatively affected by warming. In the moist tundra, the unidentified sebacinoid ERM species SH113701.05FU, SH101765.05FU, and SH265789.06FU, and root endophytic SH265824.06FU, SH167198.06FU, and SH285151.06FU, as well as other members of the Sebacinales previously sequenced from soils (SH214727.06FU and SH265810.06FU) were all indicators of the control plots.

In contrast, trends in ascomycetes capable of forming both ECM and DSE symbioses were more complex and appeared to be taxon-specific. ECM/DSE ascomycete indicators of the moist control treatment were *Cladophialophora* sp. SH228315.06FU, *Meliniomyces bicolor* SH161266.06FU, *Meliniomyces* sp. SH207177.06FU, and unidentified Helotiales SH209187.06FU and SH208255.06FU. ECM/DSE ascomycetes that were indicators of the moist warmed plots included *Cadophora melinii* SH267533.06FU, *Cladophialophora* sp. SH228303.06FU, *Lachnum* spp. SH189825.06FU and SH189775.06FU, *Meliniomyces bicolor* SH207165.06FU, *Phialocephala fortinii* SH113844.06FU, and unidentified ECM fungi in Chaetothyriales and Helotiales, SH220001.06FU and SH207207.06FU, respectively. In the dry tundra, the unidentified ECM Chaetothyriales SH305874.06FU was indicator of the control plots, while the unidentified ECM Helotiales SH209187.06FU, SH108616.06FU, and

Table 3.1. Pearson's correlation values (*r*) for variables in the NMDS ordination performed with the OTU vs. site matrix. Variables with $|r| \geq 0.5$ values are shown in bold and are displayed in the NMDS ordinations in Fig. 2, where Axis 1 represents the warming treatment. Pearson's correlation values were not calculated for taxonomic orders with less than 3 OTUs in any site. Abbreviations are apath = animal pathogen, bryophilous = fungi living on mosses, DSE = dark-septate endophyte, dung / litter = secondary decomposer fungi that live on litter and/or dung; ECM-asc = ectomycorrhizal ascomycete, ECM-bas = ectomycorrhizal basidiomycete, ericoid-asc. = ericoid mycorrhizal ascomycete, ericoid-bas. = ericoid mycorrhizal basidiomycete, herb end = endophyte of herbs, lichen = lichenized fungi, moss end = endophyte of mosses, ppath = plant pathogen, soil-asc = soil saprotrophic ascomycete, soil-bas = soil saprotrophic basidiomycete, soil-zyg = soil saprotrophic zygomycete, wood-asc = wood-rotting ascomycete, wood-bas = wood-rotting basidiomycete.

	Moist Axes		Dry Axes			Moist Axes		Dry Axes			Moist Axes		Dry Axes	
	1	2	1	2		1	2	1	2		1	2	1	2
AM			**0.500**	-0.312	*Agaricus*			**0.705**	-0.050	*Acremonium*			0.452	0.108
apath	**0.829**	-0.382	-0.107	0.075	*Alnicola*	0.002	-0.338			*Agonimia*			**0.826**	0.152
bryophilous	**-0.513**	0.089	0.264	0.056	*Arrhenia*			0.049	0.422	*Alatospora*	**-0.628**	0.024		
DSE	**0.680**	**-0.598**	0.420	0.095	*Clavaria*	**-0.662**	**0.545**	**-0.720**	-0.486	*Archaeorhizomyces*	-0.070	-0.278	**-0.750**	0.223
dung / litter	**-0.690**	0.474	0.390	-0.427	*Clavicorona*	-0.106	**0.687**			*Cadophora*	-0.237	-0.234	**0.500**	**0.713**
ECM-asc	0.445	-0.098	0.486	**0.620**	*Clavulina*	0.017	-0.145	0.360	**-0.574**	*Capronia*	0.230	0.492	0.405	0.164
ECM-bas	**-0.532**	0.067	**0.505**	0.239	*Corticium*			0.153	-0.423	*Cenococcum*	-0.343	0.154	**0.638**	0.246
ericoid-asc	**0.747**	-0.376	-0.486	**-0.506**	*Cortinarius*	-0.287	0.346	0.358	-0.108	*Chalara*	**-0.739**	0.390	**0.791**	0.076
ericoid-bas	**-0.730**	0.143	-0.304	-0.101	*Cryptococcus*	-0.106	-0.134	0.378	0.257	*Cladonia*	-0.075	0.185	**-0.775**	-0.316
herb end	**-0.744**	-0.125	**0.524**	-0.362	*Dioszegia*	-0.392	0.204			*Cladophialophora*	**0.630**	-0.132	**0.712**	0.336
lichen	**-0.640**	**0.535**	**0.531**	-0.067	*Entoloma*	0.027	0.129	-0.369	0.156	*Clathrosphaerina*	-0.190	0.291		
moss end	**-0.539**	0.435	**0.713**	0.102	*Exobasidium*	**0.785**	-0.286			*Coniochaeta*			**0.691**	**-0.512**
ppath	-0.126	**0.513**	**0.849**	0.114	*Fellomyces*	0.278	-0.122			*Cryptosporiopsis*	**0.775**	-0.458		
soil-asc	**0.538**	**-0.706**	-0.083	0.380	*Galerina*	**-0.570**	-0.144			*Hyaloscypha*	-0.063	-0.239		
soil-bas	-0.096	0.150	**0.606**	**0.706**	*Hebeloma*	**-0.544**	-0.071	0.140	0.225	*Hymenoscyphus*	-0.197	0.272		
soil-zyg	**0.805**	**-0.730**	0.247	0.232	*Hemimycena*	**-0.611**	-0.006			*Hyphodiscus*	-0.326	0.286	**-0.596**	-0.254
wood-asc	**0.524**	-0.297	0.215	0.199	*Inocybe*	**-0.748**	-0.014	-0.216	**0.511**	*Hypocrea*	0.455	**-0.810**		
wood-bas	0.072	0.271	**0.660**	-0.288	*Kockovaella*	0.079	0.042			*Hypogymnia*			-0.261	-0.185
					Lactarius	**-0.535**	-0.191			*Lachnum*	**0.595**	0.073	-0.009	-0.076
					Leccinum	-0.238	-0.285	**0.612**	-0.115	*Lecanora*	-0.165	0.186		
					Malassezia	-0.124	**0.743**	-0.460	-0.441	*Lecythophora*	0.055	-0.100	**0.566**	0.177
					Mrakia	0.390	0.350			*Meliniomyces*	0.462	-0.111	0.339	-0.222
					Mycena	-0.171	**0.758**	**0.761**	-0.081	*Mollisia*	**0.718**	-0.339		
					Omphalina	**0.511**	-0.225			*Mycoblastus*			-0.374	**-0.653**
					Psathyrella	-0.444	-0.023			*Neobulgaria*	-0.057	0.361		
					Pseudotomentella			-0.074	**0.789**	*Parmelia*			-0.060	-0.308
					Psilocybe	-0.041	-0.164			*Peltigera*			**0.755**	0.239
					Ramariopsis	**-0.893**	0.410			*Penicillium*	**0.572**	**-0.711**	0.352	-0.089
					Rhodocybe			0.412	0.173	*Pertusaria*			0.172	0.466
					Rhodotorula	**-0.546**	**0.531**	0.411	**0.562**	*Phaeomoniella*			-0.276	-0.248
					Russula	-0.488	0.143	0.021	0.272	*Phialea*	**-0.619**	**0.659**		
					Sebacina	**-0.686**	0.234	-0.021	0.381	*Phialocephala*	**0.601**	**-0.624**		
					Sistotrema			-0.278	-0.113	*Phoma*			0.343	0.453
					Sporobolomyces	-0.109	0.343			*Pseudogymoascus*	**0.864**	**-0.697**	**0.555**	0.289
					Tomentella	**-0.600**	-0.124	0.291	0.029	*Ramalina*			**0.554**	0.205

SH209187.06FU, *Meliniomyces bicolor* SH207295.06FU, and *Meliniomyces* sp. SH207172.06FU were significant indicators of warming treatment.

Discussion

Our results show that arctic tundra fungal communities respond strongly to long-term experimental summer warming, particularly in the moist tussock tundra. We found that while total fungal diversity and richness are not significantly affected by warming and are comparable across moist and dry tundra sites, there are clear patterns of correlations among OTU richness of various ecological and taxonomic groups and long-term warming. In general, the changes are more pronounced in the moist tundra, where the effect of warming on the total fungal community was strongly significant and there were more ecological and taxonomic groups with significant differences in OTU richness between the control and the warmed plots. The greater responsiveness of fungi in the moist tussock tundra may partly be explained by the fact that ambient moist tundra soils, being generally cool throughout the summer, tend to experience less fluctuations in temperature than dry tundra soils that are regularly exposed to higher temperatures as well as pronounced water stress in the upper layers (Jones *et al.*, 1998). Sedge-dominated moist tundra sites were also shown to be more responsive to warming than dry tundra sites in a study focusing on the richness and community composition of nitrogen-cycling bacterial communities in the Canadian High Arctic (Walker *et al*., 2008). These findings and the accumulating evidence from alpine and arctic dry tundra plant communities (Welker *et al*., 1993, 1997; Sharp *et al*., 2013; Lupascu *et al*., 2013, 3014) suggest that temperature responses of microbial and plant communities likely are predicated on soil water conditions and resulting differences in productivity among tundra types.

The warming-induced changes we report here are particularly notable when taking into account that warming-induced vegetation shifts at the same experimental site were caused by changes in the relative abundance of various plant functional groups rather than changes in richness or species identities (Wahren *et al*., 2005). In fungi, most of the differences in community composition among the control and warmed plots were caused by the presence of many OTUs in a particular treatment type and absence in the other, as shown by the ordinations and the indicator species analyses. While the currently prevailing view is that altered plant community composition drives fungal community change in the Arctic (Dahlberg *et al*., 2013), we conclude that fungal community composition may change independently and that fungi may be particularly well-suited to monitor early responses to environmental changes.

One finding in our paper is that OTU richness of ERM and ECM basidiomycetes was negatively affected by warming in the moist tundra. ECM basidiomycetes represent the most OTU-rich functional guild and the strong decrease in richness in the moist warmed plots in almost all ECM basidiomycete genera may have functional implications.

For example, in the moist tundra, warming appeared to favour taxa with medium-distance fringe mycelial exploration types (e.g., *Cortinarius*), that showed non-significant decrease in richness as opposed to the significant decrease seen in all other exploration types, potentially affecting the mobilization of different nutrient pools in the soil (Morgado *et al.*, 2015). In the dry tundra, although total ECM basidiomycete richness tended to increase slightly in response to the warming treatment in dry tundra, the difference was not significant. Similarly, Morgado et al. (2015) did not detect significant differences among the control and treatment plots in various genera of ECM basidiomycetes, although *Cortinarius*, *Hebeloma*, *Leccinum*, and *Tomentella* tended to be more diverse in the warmed plots, in agreement with our study. In ERM basidiomycetes (Sebacinales), the warming-induced decrease in OTU richness was evident in both tundra types, albeit with only marginally significant *p* values in the dry tundra. The decrease in the number of observed OTUs of bryophilous and moss endophytic fungi (many of them sebacinoid) in the moist tundra are in line with the previously reported decline of moss cover in the warmed plots (Wahren *et al.*, 2005; Walker *et al.*, 2006). However, moss endophytes were significantly more OTU-rich in the warmed dry tundra plots. A similar pattern was shared by endophytes of herbs that had significantly less OTUs in the warmed moist plots, but showed positive correlation with warming in the dry tundra.

The responses of root-associated ascomycetes to warming appear less clear, as different species within various groups of root-associated fungi respond differently to warming. For example, OTU richness of DSE and ECM ascomycetes (e.g., *Cadophora*, *Cenococcum*, *Cladophialophora*, *Cryptosporiopsis*, *Phialocephala*) tended to be higher in the warmed plots in both tundra types, although the difference was only significant for DSE fungi in the moist tundra. Because these fungi are able to mineralize organic nitrogen in the rhizosphere (Newsham *et al.*, 2009), they may contribute significantly to the growth of their hosts, possibly including increased shrub growth. However, it is worth to note that although several OTUs of DSE and ECM ascomycetes were indicators for the warmed plots, there were also some that showed significant association with the controls as shown in the Results. Similarly, we observed differential responses in ascomycete genera capable of forming ERM symbioses, e.g., *Pseudogymnoascus* and *Rhizoscyphus*. OTU richness in *Rhizoscyphus* declined in the warmed dry plots, but showed no difference in the moist plots, while *Pseudogymnoascus* clearly benefited from the warming in both tundra types. Because several *Pseudogymnoascus* species are also known to live as psychrotolerant saprotrophs (Rice and Currah, 2006), the increase in available nutrients resulting from the warming-induced thawing of permafrost (Schuur *et al.*, 2008) may contribute to the increase in their richness. This indicates that, to some extent, response to warming may be species-specific within these broadly defined ecological groups. In addition, it is likely that many binomials in our species list refer to morphological species and which, in reality, may harbour multiple phylogenetic species. For example, the DSE/ECM *Meliniomyces bicolor* was among the indicators of both the

moist control and warmed plots as well as the dry warmed plots. However, the matching sequences and the phylogeny-based Species Hypothesis numbers differed among the indicators of the distinct treatments, suggesting that *Meliniomyces bicolor* may in fact include multiple evolutionary lineages, each with a specific niche and distinct response to warming. These results further emphasize the need for ecological studies at fine taxonomic scales.

The significant decrease of OTU richness of litter/dung fungi (e.g., *Clavaria*, *Coprinus*, *Lycoperdon*, *Preussia* spp.) in the moist warmed plots is surprising, given the increased litter accumulation observed in the warmed plots (Walker *et al.*, 2006). OTU richness in diverse taxonomic groups of soil saprotrophic (e.g., *Cladosporium*, *Coniochaeta*, *Hypocrea*, *Mollisia*, *Penicillium*, *Troposporella*, and *Xylaria* in Ascomycota, *Rhodotorula* in Basidiomycota, and *Mortierella* and *Umbelopsis* in Zygonycota) and animal pathogenic fungi (*Isaria* sp., *Lecanicillium* sp., *Pochonia bulbillosa*) increased in or were indicators of the warmed plots, although less so in the dry tundra that may be explained by the general environmental conditions described above. These results are in agreement with previous findings on warming-induced increase in microbial decomposition of organic matter (Nadelhoffer *et al*., 1992; Sistla *et al*., 2013) and in insect abundance in the Arctic (Hasle, 2013). Similar patterns were observed in plant pathogens as well, e.g., *Chalara*, *Davidiella*, and *Exobasidium*. It is unclear whether these plant pathogens primarily benefit from the increased biomass of the deciduous and evergreen shrubs (Walker *et al.*, 2006) or from higher temperatures (or both). However, similar to other broader groups, distinct species-specific responses to warming were revealed by the indicator species analyses in saprotrophic and pathogenic taxa as well. For example, while most plant pathogen or saprotrophic indicators were associated with the moist or dry warmed plots (*Bullera* sp., *Capronia* sp., *Davidiella tassiana*, *Exobasidium arescens*, *E. woronichinii*, *Leptodontidium* sp., various *Mortierella* spp., *Phacidium lacerum*, *Rhodotorula* sp., *Pseudocercosporella* sp., *Trimmatostroma botulinum*, various *Umbelopsis* spp., and *Venturia alpina*), *Leptosphaeria doliolum* and the unidentified *Archaeorhizomyces* sp. and *Dothierella* sp. were indicators of the control plots.

Lichens showed a mixed response to warming both in terms of tundra type as well as lichenized fungal genera. The negative effect of warming on lichen richness in the moist plots may be partly explained by the observed decrease in cover of shade-intolerant lichens and plants in the moist warmed plots (Walker *et al.*, 2006), while the low abundance of erect shrubs results in little shading in the dry plots. However, strong taxon-specific responses were observed again, as OTU richness of *Cladonia* and *Shaerophorus* strongly negatively correlated with warming particularly in the dry plots, while *Agonimia*, *Peltigera*, *Verrucaria*, and *Xanthoria* benefited from the increased temperatures in the dry tundra. The reindeer lichens (*Cladonia* spp.) are considered keystone components in

arctic ecosystems and are the main winter food source for caribou (Dahlberg and Bültmann, 2013). *Cladonia* was one of the very few fungal genera that showed consistent and strong warming-induced decline in richness in both tundra types. Because the sampled tundra types represent communities with the greatest surface areas in Northern Alaska (Walker *et al*., 2005), the observed trend of declining lichen richness (Semenova *et al*. 2015; and this study) and cover (Walker *et al*., 2006; Joly *et al*., 2009) may have detrimental effects on Alaskan caribou populations, with potentially profound social implications for local people (Joly *et al*., 2009).

The low diversity of AM fungi (Glomeromycota) in our samples is somewhat surprising, given that, although AM fungi tend to be rare in high arctic regions (Kohn and Stasovski, 1990; Väre *et al*., 1992), they have nevertheless been reported from numerous sites and plant species, particularly in the Low Arctic (Strelkova, 1956; Miller and Laursen, 1978; Olsson *et al*, 2004). It seems unlikely that the low diversity of AM is a methodological artefact, although this possibility cannot be ruled out. Glomeromycota was represented by 409 OTUs (ca. 3% of all fungal OTUs) from diverse taxonomic orders in a Neotropical study that used the same primers and methodology as in the present study (Geml *et al*., 2014b). Also, Stockinger *et al*. (2010) showed that primers routinely used to amplify ITS2 rDNA (including primer ITS4 used in this study) show mismatches in only a small fraction of known glomeromycete sequence types and they considered the ITS2 fragment a good candidate for AM species identification by metabarcoding studies. It is possible that AM fungi are present in very low biomass in the sampled arctic soils and some species may have been missed by our sampling efforts. The positive correlation of AM fungi with warming are in agreement with their presumed preference for higher temperatures, although the low number of AM OTUs prevented their statistical comparison between the controls and the warming treatments. In addition, because AM fungi have been shown to prefer non-acidic soils in a wide range of ecosystems (Porter *et al*., 1987; Coughlan *et al*., 2000; Geml *et al*., 2014ab), it is possible that their richness in the acidic tundra sites, such as those sampled in this study, is unusually low. Future studies should assess the diversity of AM fungi in non-acidic arctic tundra habitats as well.

Conclusions

The data presented in this study describe changes within arctic soil fungal communities induced by long-term experimental warming. Many OTUs in this dataset could not be confidently assigned to species or genera due to limited fungal reference sequences. Nevertheless, we were able to show how the richness and composition of fungal functional groups may respond to decades of summer temperature increases. We were also able to present evidence that compositional changes in fungal communities in response to warming are species-specific, and may be masked when communities are

compared at higher taxonomic levels. Therefore, we recommend that studies of arctic fungal communities (for example, their roles in nutrient cycling) take into account species-level differences.

Snow fence experiment in the dry tundra at Toolik Lake, Alaska.

Chapter 4

Long-term increase in snow depth leads to compositional changes in arctic ectomycorrhizal fungal communities

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Abstract

Arctic ecological processes are mainly regulated by air and soil temperature, snow cover distribution and persistence. Recently, various climate-induced changes have been observed in arctic tundra ecosystems, e.g. shrub expansion, resulting in reduction in albedo and greater C fixation in aboveground vegetation as well as increased rates of soil C mobilization by microbes. Importantly, the net effects of these shifts are unknown, in part because our understanding of belowground processes is limited. Here, we focus on the effects of increased snow depth, and as a consequence, increased winter soil temperature on ectomycorrhizal (ECM) fungal communities in dry and moist tundra. We analyzed deep DNA sequence data from soil samples taken at a long-term snow fence experiment in Northern Alaska. Our results indicate that, in contrast to previously observed responses of plants to increased snow depth at the same experimental site, the ECM fungal community of the dry tundra was more affected by deeper snow than the moist tundra community. In the dry tundra, both community richness and composition were significantly altered while in the moist tundra, only community composition changed significantly while richness did not. We observed a decrease in richness of *Tomentella*, *Inocybe* and other taxa adapted to scavenge the soil for labile N forms. On the other hand, richness of *Cortinarius*, and species with the ability to scavenge the soil for recalcitrant N forms, did not change. We further link ECM traits with C soil pools. If future warmer atmospheric conditions lead to greater winter snow fall, changes in the ECM fungal functional ecology will likely influence C emissions and C fixation through altering N plant availability, fungal biomass and soil-plant C-N dynamics, ultimately determining important future interactions between the tundra biosphere and atmosphere.

Introduction

Artic ecosystems are beginning to exhibit significant shifts in ecosystem structure and function induced by changes in climatic conditions (Tape *et al*., 2012; Ellmendorff *et al*., 2012). Despite interannual and regional variability, global mean surface temperature have consistently increased since the late 19th century (Collins *et al.,* 2013). In the Arctic, temperatures have risen between 0.06 to 0.1 °C per year, while the global average increase has been ca. 0.017 °C per year during the past 30 years (Comiso & Hall, 2014). These temperature increases have already had major consequences, including accelerated summer ice loss, extended periods of open water in the Arctic Ocean and delayed autumn freeze up (Stroeve *et al.,* 2014). At the same time, precipitation in the Arctic has increased (greatly exceeding the global average increase), especially during the cold season, where most precipitation falls as snow (Kattsov & Walsh, 2000; Screen & Simmonds, 2012). Additionally, state of the art models predict further increases, possibly by more than 50% of the current precipitation, leading to thicker snow cover (Colins *et al*., 2013; Bintanja & Selten, 2014). Deeper snow would have a suite of consequences for tundra ecosystems. These include protection from the abrasive wind (Liston *et al.,* 2002; Sturm *et al.,* 2005; Blok *et al.,* 2015), warmer winter soil temperatures and increased soil moisture with subsequent effects on thaw depth and C storage (Natali *et al.,* 2012; 2014), N turnover (Schimel *et al.,* 2004; DeMarco *et al*., 2011), plant phenology and mineral nutrition (Borner *et al*., 2008; Leffler & Welker, 2013; Pattison & Welker 2014), vegetation composition (Wahren *et al*., 2005; Welker *et al.,* 2005) and soil microbial respiration (Aanderud *et al*., 2012*;* Natali *et al.,* 2014). However, with the exception of Buckeridge & Grogan (2008) that compared bacterial and fungal biomass growth responses, how arctic soil fungi communities may respond to changes in winter snow depth conditions is still largely unknown.

Microbial activity in the Arctic has been shown to increase due to higher winter soil temperatures inducing changes in the nitrogen (N) cycle dynamics, particularly in moist tussock tundra and less so in dry heath tundra in Arctic Alaska (Schimel *et al.,* 2004; DeMarco *et al.,* 2011; Natali *et al*., 2014; Pattison & Welker, 2014). In the Arctic, fungi are considered to constitute the bulk of soil microorganisms biomass (Callaghan *et al*., 2005) and Hobbie & Hobbie (2006) estimated that up to 86% of the N obtained by tundra plants is via mycorrhizal fungi. In exchange, plants can allocate between 10 to 20% of their photosynthate-derived C to their fungal partners (Harley, 1971; Hobbie, 2006), constituting an important pool of soil C. Additionally, these exchanges might be positively correlated, i.e., increased allocation of plant C to the mycorrhizal partner might lead to increased uptake of N from the soil pool and subsequent delivery to the plant host (Talbot & Treseder, 2010). The limiting step in soil N cycling is the breakdown of macromolecular organic compounds, particularly the depolymerization of proteins (Schimel & Bennet, 2004; Jones & Kielland, 2012) that has been correlated with fungal

biomass in high-latitude ecosystems (Wild *et al*., 2013), and is particularly attributed to ectomycorrhizal (ECM) and ericoid mycorrhizal fungi (Read & Perez-Moreno, 2003). Recently, several studies reported major changes in the arctic fungal mycorrhizal communities in response to summer warming (Deslippe *et al.*, 2011; Geml *et al*., 2015; Morgado *et al.*, 2015; Semenova *et al.*, 2015), with the fungal community of moist tussock tundra typically showing more pronounced response than the dry heath tundra, including potential shifts in functional traits and the subsequent ecosystem functional processes. However, possible effects of increased winter soil temperatures on the richness and compositional structure of soil fungal communities have not yet been investigated.

Tundra plant community responses to increased winter snow depth include a combination of shifts in community composition as well as increases in net plant productivity (Borner *et al.*, 2008; Natali *et al*., 2014) and plant N tissue concentrations (Leffler & Welker, 2013). At the community level, the general trends are increases in shrub coverage and litter layer, decrease in lichens, bryophytes, and in leaf C:N ratio (Welker *et al.*, 2005; Wipf & Rixen, 2010; Pattison & Welker, 2014). Wahren *et al.* (2005) and Mercado-Díaz (2011) reported (from the same experimental plots that we used in our study) an increased coverage of several species of deciduous and evergreen shrubs, and a sedge species. Although most of these plants are highly dependent on root-associated fungi, especially ECM fungi, to acquire soil nutrients, how soil fungi community changes in response to deeper snow remains uncertain. Here we focus on ECM fungal community responses to long-term increased snow depth and the associated warming soil temperatures across the dry heath and moist acidic tussock tundra.

ECM fungi represent the most taxonomically diverse fungal guild in the Arctic tundra (Gardes & Dahlberg, 1996; Geml *et al*., 2012; Timling & Taylor, 2012), and provide crucial roles in soil-root interaction, particularly in plant N uptake (Read *et al*., 2004; Ekblad *et al.*, 2013) and in soil C dynamics (Clemmensen *et al*., 2013; Averill *et al.*, 2014). Recently, an increasing amount of studies on fungal functional traits are amassing valuable insights into the potential in-depth role of the community structure in potential ecosystem functions (e.g. reviewed in Fernandez & Koide, 2014; Treseder & Lennon, 2015). For example, Hobbie & Agerer (2010), gathered evidences from $\delta^{15}N$ patterns and argued that ECM fungi have two main strategies for growth and nitrogen acquisition that match the extramatrical mycelium (EMM) characteristics of the ectomycorrhizae. ECM fungi with low abundance of EMM and hydrophilic mycorrhizae with contact, short-distance and medium-distance smooth hyphal exploration types (ETs) were argued to focus on uptake of labile nitrogen (N) forms, such as amino acids, ammonium and nitrate. Supporting this hypothesis, many taxa composing this group showed limited protein growth in culture conditions (Lilleskov *et al.*, 2011). On the other hand, the ECM fungi with higher abundance of EMM with medium-distance fringe, medium-distance mat and long-distance ETs with hydrophobic rhizomorphs (or mycelial

cords), likely focus on widely dispersed and spatially concentrated soil resources requiring efficient long-distance translocation. Such investment in exploratory hyphae should not rely on labile substrates under low nutrient availability; therefore, this group of taxa would require hydrolytic capabilities in order to access non-labile N forms, such as proteins (Hobbie & Agerer, 2010). Supporting this hypothesis some studies pointed to increased exoenzyme activity in ECM fungi with abundant EMM (Tedersoo *et al.*, 2012; Talbot *et al.*, 2013). Another example of a fungal trait and a potential ecosystem function is the presence of melanin in hyphal cell walls, which was thoroughly discussed by Koide *et al*. (2014).Given these evidences it seems reasonable to use certain fungal traits as a response to environmental conditions which in turn might also induce changes in the ecosystem.

This research focuses on the effects of long-term increased snow depth on ECM basidiomycete communities. Based on the evidence previously stated, we hypothesize that: 1) ECM fungal community composition is strongly affected by increased snow depth, and that the response will be more pronounced on the moist tundra ECM community; and 2) changes in ECM fungal community composition will reflect altered patterns in vegetation, soil nutrient pools and moisture, induced by the increased snow depth. Therefore, we expect altered patterns in the ECM fungal community functional traits.

Material and Methods

Study site and experimental design

This International Tundra Experiment (ITEX) (Henry & Molau, 1997, Welker *et al.*, 1997) study site is located on the northern foothills of the Brooks Range, at the Toolik Lake Field Station The area lies in the Arctic tundra biome within the bioclimatic subzone E, which covers approximately 36% of the Arctic dry land surface (Walker *et al.*, 2005). The mean air annual temperature is -7 °C and annual precipitation ranges between 200 mm and 400 mm with approximately 50% falling as snow. The average snow depth is 50 cm (DeMarco *et al.*, 2011). The snow fence experiment was established in the summer of 1994 in moist tussock and dry tundra (Jones *et al.*, 1998; Walker *et al.*, 1999; Welker *et al*., 2000). The snow fences are 2.8 m high and 60 m long, inducing leeward drifts of ca. 60 m long (Walker *et al.*, 1999; Pattison & Welker, 2014). Our sampling was focused on the intermediate zone near the center of the experimental setup, corresponding to ca. 1-1.5 m winter snow depth. Although the deeper snow slightly shortens the growing season by ca. 5-8 days, this does not affect plant phenology significantly (Borner *et al*., 2008). The average winter soil temperatures at 2 cm depth were -2.9 °C (±0.2) and -4.7 °C (±0.2) in the increased snow depth plots and in the control plots, respectively (Pattison & Welker, 2014).

The vegetation of the dry heath tundra is characterized by *Dryas octopetala*, *Salix polaris*, *Vaccinium* spp. and fruticose-lichens, while the moist tussock tundra is dominated by *Betula nana*, *Salix pulchra* and the sedge *Eriophorum vaginatum.* Detailed descriptions of the plant communities can be found in Walker *et al*. (1999) and Kade *et al.* (2005), and their detailed response to the altered snow depths in Walker *et al.* (1999), Wahren *et al.* (2005), Welker *et al.* (2005), Mercado-Díaz (2011), Pattison & Welker (2014).

We sampled soil at the end of July 2012 from two tundra types, the dry heath and moist tussock tundra experimental sites. In each tundra type, we sampled five plots/replicates with increased snow depth and five plots with ambient snow depth ("control plots"). Each replicate consisted of five soil cores of 2 cm diameter and 20 cm depth each. For each replicate the soil cores were thoroughly mixed and kept frozen until lyophilization. In total we sampled 100 soil cores across 20 plots of ca. 1 m^2 each.

Molecular work and sequence quality control

The DNA extraction, PCR protocol, Ion Torrent sequencing and data clean-up procedures were described in detail elsewhere (Geml *et al.*, 2014a). For each sample we carried out two independent DNA extractions, using ca. 1 ml of lyophilized soil and pooled them to optimize extraction homogenization. In the PCR we targeted the *ITS2* region of the nuclear ribosomal internal transcribed spacer that is currently accepted as the universal barcode marker for fungi (Schoch *et al.*, 2012). We used primers fITS7 (Ihrmark *et al.*, 2012) and ITS4 (White *et al.*, 1990). The ITS4 primer was labeled with sample-specific Multiplex Identification DNA-tags (MIDs). The amplicon library was sequenced using an Ion 318^{TM} Chip by an Ion Torrent Personal Genome Machine (PGM; Life Technologies, Guilford, CT, U.S.A.) at Naturalis Biodiversity Center. For the initial clean-up of the raw sequence data we used the online platform Galaxy (https://main.g2.bx.psu.edu/root), in which the sequences were sorted according to samples. Primers and adapters were removed. We used a parallel version of MOTHUR v. 1.32.1 (Schloss *et al.*, 2009) for subsequent sequence analyses. Sequences shorter than 150 bp and longer than 400 bp were removed following Blaalid *et al.* (2013), Geml *et al.* (2014ab), Morgado *et al.* (2015) and Semenova *et al.* (2015), with the goal to increase phylogenetic identification potential and quality control while preserving data coverage. The quality-filtered sequences were normalized following Gihring *et al.* (2012) by random subsampling so that each sample contained equal number of sequences. We then clustered the sequences into operational taxonomic units (OTUs) using OTUpipe (Edgar 2010) with the simultaneous removal of putatively chimeric sequences using *de novo* and reference-based filtering using the curated dataset of fungal *ITS* sequences of Nilsson *et al.* (2011), with the default settings. We used a 97% sequence similarity clustering threshold following many other fungal ecology studies (e.g. O'Brien *et al.*, 2005; Higgins

et al., 2007; Geml *et al.*, 2008; Geml *et al.*, 2009; Amend *et al.*, 2010; Tedersoo *et al.*, 2010; Geml *et al.*, 2012; Kauserud *et al.*, 2012; Brown *et al.*, 2013; Blaalid *et al.*, 2013; Geml *et al.*, 2014b, Davey *et al*., 2015). Global singletons were discarded from further analysis. The reference database published by Kõljalg *et al.* (2013) was used to determine the taxonomic affinity of the OTUs using USEARCH v7 (Edgar, 2010). OTUs with less than 80% similarity to any identified fungal sequence were excluded from the final analysis due to unreliable classification, and/ or uncertainty regarding their ecological role. A representative sequence of each OTU was deposited in GenBank under the accession numbers KP827673 - KP828017.

ECM fungal database and EMM determination

We followed the publication of Tedersoo & Smith (2013) to select the basidiomycete ECM OTUs. For most OTUs we used a ≥90% sequence similarity to determine genera. Because Sebacinales have a diverse ecology we selected ECM OTUs based on their supported phylogenetic placement (with ≥70% bootstrap and/or ≥0.95 posterior probability) among sequences of taxa that were morphologically confirmed as ECM published by Glen *et al.* (2002), Urban *et al.* (2003), Ryberg *et al.* (2009) and Tedersoo & Smith (2013).

To determine the EMM characteristics, we followed the work of Agerer (2006), Tedersoo & Smith (2013) and consulted the DEEMY database (http://deemy.de, accessed in November, 2014 - an information system for the characterization and determination of ectomycorrhizae). In the genus *Russula,* if no EMM information was available for the species of interest, we assumed the EMM characteristics based on the closest species with known characteristics. To determine the closest species, we followed the phylogenetic study by Miller & Buyck (2002). Similarly, for OTUs of the genus *Hebeloma,* we followed the phylogenetic study by Boyle *et al.* (2006).

Statistical analysis

For each replicate, we calculated rarefied OTU accumulation curves using the R package Vegan (Oksanen *et al.*, 2012) and determined the Good‘s coverage (complement of the ratio between the number of local singletons and the total sequence counts). OTU presence was defined as more than 4 sequences on a per sample basis following the suggestion of Lindahl *et al.* (2013) to minimize false positives (e.g. OTUs that are common in one sample, but may be low-abundant contaminants in others). Due to uncertainty of sequence abundance as indicator of species abundance in the samples (Amend *et al.*, 2010), we carried out analyses with two types of data transformations. First, we transformed the data into presence-absence matrix. Secondly, we used square-root transformed sequence abundance to moderate the influence of OTUs with high sequence counts, while maintaining some approximation of template abundance that may

reflect ecological significance. We used PC-Ord v. 5.32 (McCune & Grace, 2002) to run non-metric multidimensional scaling (NMDS) on a primary matrix of experimental plots by OTUs and a secondary matrix of plots by OTU richness per taxon, EMM characteristics and sequence counts. The dataset was subjected to 500 iterations per run using the Sørensen similarity (Bray-Curtis index) and a random starting number. We also calculated the Pearson's correlation coefficient (*r*) values between relative OTU richness per taxon and axes 1 and 2. We tested whether fungal communities were statistically different across the treatments using a multi-response permutation procedure (MRPP) and determined any preferences of individual OTUs for either control or increased snow depth plots in dry and moist tundra using Indicator Species Analyses (Dufrêne & Legendre, 1997) as implemented in PC-Ord v. 5.32. We also tested for significant differences in OTU richness across the dry and moist tundra control and deeper snow plots, per taxa (genera) and EMM characteristics using Student's *t*-test. Correlation coefficients were calculated as implemented in Microsoft Excel v. 2010 between the most OTU-rich genera and the hyphal exploration types (combined in two functional groups, I – contact, short-distance, medium-distance smooth, and II – medium-distance fringe and long-distance ETs) within plot type, across the dry and moist tundra for the presence-absence and sequence abundance datasets. The Venn diagram for the whole community and genera with higher OTU richness was also calculated, using the online version of the publication by Oliveros (2007).

Results

Through the pipeline: from raw data to taxonomic diversity

We obtained 3,960,925 sequences with a median length of 268 bp. After quality control and random subsampling we retained 1,161,160 sequences with a mean length and standard deviation of 255.1 ± 52.7 bp. Clustering the sequences at 97% similarity generated 7015 OTUs, excluding global singletons and putative chimeric OTUs, of which 459 ECM basidiomycete OTUs were retained for further analyses. Across all treatments, ECM fungi were represented by 23 genera classified in 7 orders (Table 4.1, Fig. 4.1). Overall, *Cortinarius* and *Tomentella* were the most OTU-rich genera, with 125 (ca. 27%) and 124 OTUs (ca. 27%), respectively, followed by *Inocybe* (79 OTUs, 17%) and *Russula* (40 OTUs, 9%), with the remaining genera having less than 5% of the OTUs per genus. The order Agaricales had by far the highest OTU richness (224 OTUs, ca. 49%), followed by Thelephorales (128 OTUs, ca. 28%), Russulales (57 OTUs, ca. 12%), Cantharellales (33 OTUs, ca. 7%), Sebacinales (11 OTUs, ca. 2%), Boletales (4 OTUs, ca. 1%) and Atheliales (2 OTUs, ca. 1%). The analysis with sequence abundance resulted in similar patterns as the OTU richness analysis (appendix S4.1). The recovered OTU richness was higher than in previous publications that used similar methods to investigate arctic ECM fungal communities, but genera diversity and patterns of genera richness

were in general agreement (Bjorbaekmo *et al.*, 2010; Timling *et. al.* 2012; Geml *et al.*, 2012; Morgado *et al.*, 2015). The asymptotic rarefaction curves (Fig. 4.2a) and estimated Good's coverage (Fig. 4.2b) indicate that the deep sequencing allowed a very high OTU coverage and that most fungi in the samples were sequenced.

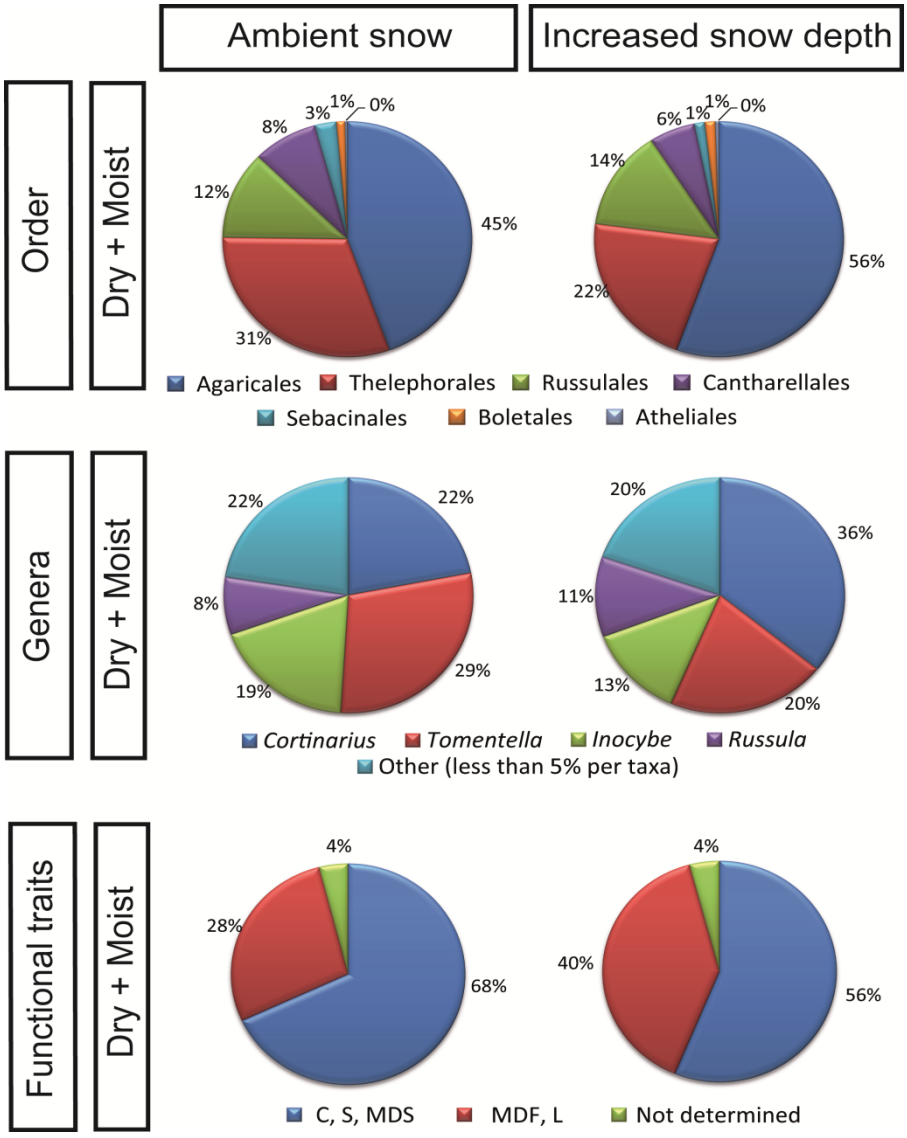

Figure 4.1. Total ECM fungal OTUs, classified by taxonomic and functional traits, comparing ambient snow with increased snow depth plots. The legend for each pair of graphics is organized by colors and in a clock-wise manner. Abbreviations are C: contact, S: short-distance, MDS: medium-distance smooth, MDF: medium-distance fringe, L: long-distance.

Table 4.1. Average and standard deviation OTU richness per genus and extramatrical mycelium features. Legend – DC: dry heath tundra with ambient snow, DS: dry heath tundra with increased snow depth, MC: moist acidic tussock tundra with ambient snow, MS: moist acidic tundra with increased snow depth, MDS: medium-distance smooth, MDF: medium-distance fringe, C: contact ET, S: short-distance ET; MDS: medium-distance smooth ET, MDF: medium-distance fringe ET, L: long-distance ET, Hi: hydrophilic, Ho: hydrophobic. *Significant treatment effect (α = 0.05).

	Dry heath tundra			Moist acidic tussock tundra		
	DC	DS	*p*	MC	MS	*p*
Tomentella	21 ± 13.55	3.4 ± 2.07	0.02*	20.6 ± 7.40	12.6 ± 8.33	0.07
Cortinarius	9 ± 4.12	9.4 ± 6.80	0.46	16.6 ± 7.13	17.2 ± 16.44	0.47
Inocybe	10.4 ± 5.32	5.2 ± 1.92	0.05*	8.6 ± 4.28	3.2 ± 1.92	0.02*
Russula	1.8 ± 2.48	2.8 ± 3.03	0.25	7.6 ± 4.16	5.4 ± 4.10	0.42
Alnicola	-	-	-	0.8 ± 0.84	2.6 ± 0.89	0.01*
Amanita	0 ± 0	0.2 ± 0.45	0.19	-	-	-
Amphinema	-	-	-	0 ± 0	0.2 ± 0.45	0.19
Boletus	0.6 ± 0.55	0 ± 0	0.04*	-	-	-
Ceratobasidium	1.6 ± 0.55	1.6 ± 0.89	0.50	0 ± 0	0.2 ± 0.45	0.19
Clavicorona	-	-	-	0.4 ± 0.55	0 ± 0	0.09
Clavulina	0.6 ± 1.34	0.4 ± 0.55	0.38	0.6 ± 0.89	0.4 ± 0.55	0.34
Hebeloma	1.6 ± 2.61	0.4 ± 0.89	0.19	2.8 ± 0.84	2.6 ± 1.14	0.38
Hymenogaster	-	-	-	0.6 ± 0.55	0 ± 0	0.04*
Laccaria	-	-	-	1.4 ± 0.89	2.8 ± 0.84	0.02*
Lactarius	0.2 ± 0.44	0.8 ± 0.84	0.10	4.4 ± 3.36	1.8 ± 0.84	0.08
Leccinum	0.6 ± 0.55	0.8 ± 0.45	0.27	2.4 ± 1.34	1.6 ± 1.34	0.19
Membranomyces	0.4 ± 0.55	0 ± 0	0.09	-	-	-
Piloderma	0.2 ± 0.45	0 ± 0	0.19	-	-	-
Pseudotomentella	0.8 ± 1.30	0.4 ± 0.55	0.28	-	-	-
Sebacina	0.8 ± 1.79	0.4 ± 0.55	0.33	2.6 ± 1.34	1.4 ± 0.55	0.06
Sistotrema	1.4 ± 1.34	0.6 ± 1.34	0.19	3 ± 4.47	0.6 ± 0.89	0.15
Tomentellopsis	0.2 ± 0.45	0 ± 0	0.19	0 ± 0	0.4 ± 0.55	0.09
Tulasnella	0.8 ± 0.84	0.2 ± 0.45	0.10	-	-	-
C/ S/ MDS	42.2 ± 19.82	15.6 ± 3.78	0.02*	50 ± 17.13	33.6 ± 15.08	0.07
MDF/ L	11.8 ± 4.82	11 ± 6.63	0.42	22 ± 5	19.4 ± 16.59	0.38
Hi	34.6 ± 20.73	12 ± 4.69	0.04*	37.4 ± 11.68	25.4 ± 13.41	0.09
Ho	16.4 ± 8.02	11.6 ± 7.37	0.18	28.2 ± 8.93	22.6 ± 17.47	0.27
All OTUs	54 ± 23.29	26.6 ± 8.02	0.03*	72.4 ± 22.02	53 ± 31.01	0.15

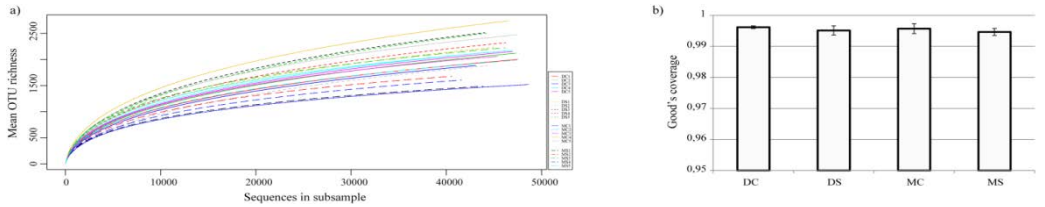

Figure 4.2. a) Observed number of OTUs *per* replicate sample; b) Average Good's coverage per habitat with standard deviation, based on the 5 replicates. Abbreviations are DC: dry tundra ambient snow, DS: dry tundra increased snow depth, MC: moist tundra ambient snow, MS: moist tundra

Overall results

The NMDS analysis of the square-root sequence abundance matrix resulted in a 2-dimensional solution with a final stress and instability of 0.1062 and < 0.00001, respectively. The results of the Monte Carlo test indicated that all two dimensional solutions using the real data were significantly better than occurrences by chance (p < 0.01). The coefficients of determination for the correlations between ordination distances and distances in the original n-dimensional space were axis 1: r^2 = 0.599; axis 2: r^2 = 0.240; total r^2 = 0.839; orthogonality = 88.3%. The NMDS ordination plot was orthogonally rotated by the treatment to visualize correlations between snow depth effect and fungal community composition in general (Fig. 4.3a). The MRPP analysis indicated a clear distinction between dry and moist ECM community composition (p < 0.0000001, A= 0.14601). The NMDS and MRPP analysis with the presence-absence matrix results were similar (apenndix S4.2a). Across the ambient snow plots of both dry and moist tundra, *Tomentella* was the most OTU rich genus with 103 OTU (ca. 29%), followed by *Cortinarius* with 78 OTUs (ca. 22%), *Inocybe* with 66 OTUs (ca. 19%) and *Russula* with 28 OTUs (ca. 8%). All the other genera had less than 5% of the OTUs per taxa and combined solely represented ca. 22% of the OTUs. On the other hand, across the deeper snow plots, *Cortinarius* was the most OTU rich genus with 78 OTUs (ca. 36% of all OTUS), followed by *Tomentella* with 45 OTUs (ca. 20%), *Inocybe* with 28 OTUs (ca. 13%), Russula with 24 OTUs (11%). All the other genera had less than 5% of the OTUs per taxa (Fig. 4.1). Differences between the ambient and deeper snow plots were also evident at the order level. Agaricales and Russulales had an increased OTU richness in deeper snow areas, while Thelephorales and Cantharellales had a decrease. Globally, the majority of the OTUs were only present in the ambient snow plots - ca. 53%, while ca. 24% were solely found in the deeper snow plots and the remainder 23% present in both (data not shown). There was a significant decrease in OTU richness from the ambient to the deeper snow plots (p= 0.0377), with the control plots having on average 66.2 ± 24.5 OTUs, while the deep snow plots had 43.8 ± 28.4 OTUs (Table 4.1). Together the contact, short-distance and medium-distance smooth ET represented the most OTU rich

group in the control plots with an average of 46.1 ± 17.9 OTUs per plot, while the medium-distance fringe and long-distance ETs group had an average of 16.6 ± 7.09 OTUs per plot. Comparing with the OTU richness values of the deep snow plots, the first group had a significant decrease (p = 0.0042, 24.6 ± 14.05 average OTUs per plot); whilst the later did not change significantly (p = 0.36, 15.2 ±12.71 average OTUs per plot) (apenndix S4.3). The overall pattern of changes in functional traits were also depicted when comparing the unique OTUs in the ambient with the deeper snow plots (Fig. 4.1).

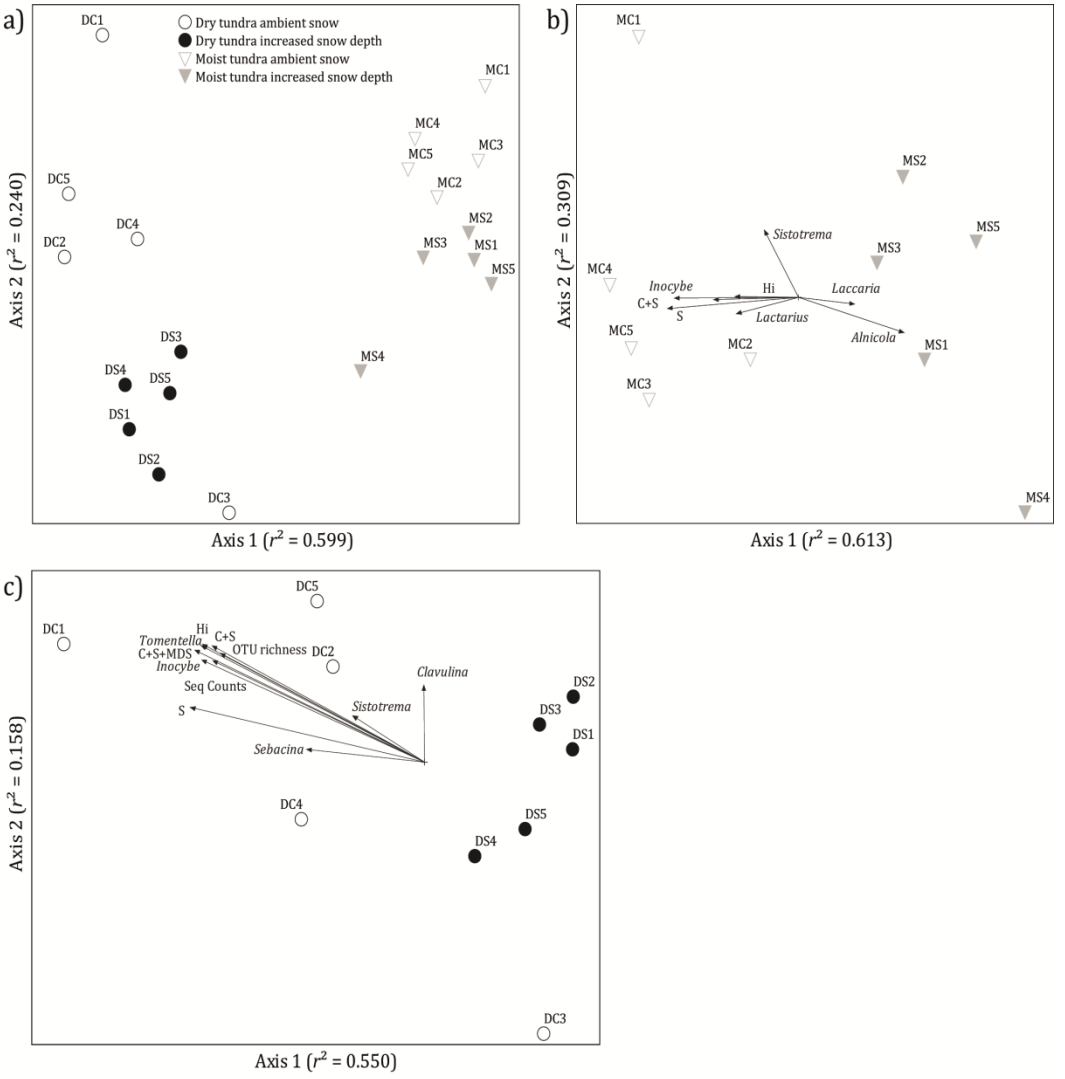

Figure 4.3. Non-metric multidimensional scaling (NMDS) ordination plots of basidiomycete ECM fungal communities from the ambient and increased snow depth plots based on OTU sequence square-root abundance in (a) the whole community (dry and moist tundra), (b) the moist tundra, (c) the dry tundra. Vectors with $|r| \geq 0.5$ are represented on the ordination plot. Abbreviations are C: contact, S: short-distance, MDS: medium-distance smooth, MDF: medium-distance fringe, L: long-distance, Hi: hydrophilic, Seq counts: sequence reads.

Dry heath tundra

The NMDS analysis of the square-root sequence abundance matrix resulted in a 2-dimensional solution with a final stress of 0.0763 and a final instability < 0.00001. The results of the Monte Carlo test indicated that all two dimensional solutions using the real data were significantly better than occurrences by chance ($p < 0.01$). The coefficients of determination for the correlations between ordination distances and distances in the original n-dimensional space were axis 1: $r^2 = 0.667$, axis 2: $r^2 = 0.183$, total $r^2 = 0.849$ and orthogonality = 77.8 %. The NMDS ordination plot was orthogonally rotated by the treatment to visualize correlations between snow depth and fungal community composition in general, and the taxonomic groups and EMM characteristics in particular (Fig. 4.3c). The MRPP analysis indicated a clear distinction between control and deep snow ECM community composition ($p = 0.0039$, $A = 0.04940$). The NMDS and MRPP analysis of the presence-absence matrix results yielded similar conclusions (appendix S4.2c). The groups with the strongest negative correlation (Pearson's correlations) with the increased snow depth were OTUs of the contact, short-distance and medium-distance smooth hyphal ET ($r = -0.950$), *Tomentella* ($r = -0.937$), OTUs with hydrophilic hyphae ($r = -0.935$), *Inocybe* ($r = -0.935$), sequence counts ($r = -0.912$), total OTU richness ($r = -0.896$), *Sebacina* ($r = -0.682$), *Tulasnella* ($r = -0.582$), *Sistotrema* ($r = -0.533$). None of the groups showed a strong positive correlation ($r > 0.5$) with the increased snow depth. The control plots had on average 54 ± 23.29 OTUs per plot, while the treatment plots had 26.6 ± 8.02 OTUs per plot. This difference was statistically significant (p=0.03). *Tomentella* was the most OTU-rich genus in the control plots with 21 ± 13.55 OTUs per plot, followed by *Inocybe* with 10.4 ± 5.3. Interestingly, across the deep snow plots, both genera showed a significant decrease on the average OTUs per plot (p=0.022 and 0.047, respectively). On the other hand, OTU richness in C*ortinarius* was nearly unaffected and this genus had the highest mean richness in the increased snow depth plots (Table 4.1). At the order level, there was an abrupt decrease in the proportion of Thelephorales OTUs, from 36% in the control plots to 17% in the deep snow plots, while most other orders had an increase in proportion between the ambient and increased snow depth plots, with Agaricales having 55% of all OTUs (across the deep snow plots) (Fig. 4.1).

Together the contact, short-distance and medium-distance smooth ETs represent by far the most OTU-rich functional group, with an average of 42.2 ± 19.82 OTUs per plot, while the long-distance and medium-distance fringed ETs solely had on average 11.8 ± 4.82 OTUs per plot, across the ambient snow conditions plots. Interestingly, in the deeper snow plots the first group had a significant decrease ($p = 0.02$) in OTU richness, while the latter group maintained similar OTU richness ($p = 0.42$). The vast majority of OTUs were only present in the control plots (60%), a smaller percentage was present in both the control and the increased snow depth plots (20%), and only a minority (18%) was strictly present in the deeper snow plots (Fig. 4.4). We observed this pattern for the

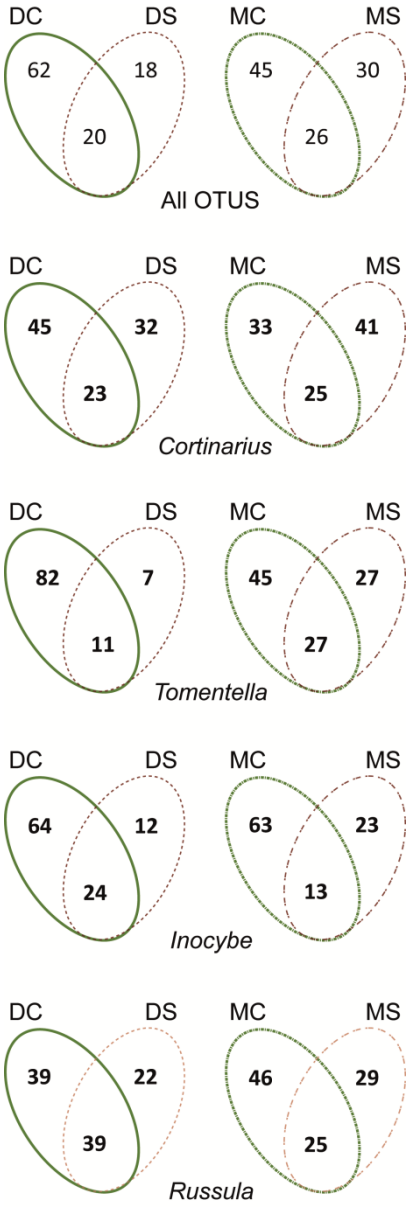

Figure 4.4. Percentage of unique and shared OTUs for all the community and most diverse taxa per treatment (ambient snow and increased snow depth) in the dry and moist tundra types. Abbreviations are DC: dry tundra ambient snow, DS: dry tundra increased snow depth, MC: moist tundra ambient snow, MS: moist tundra increased snow depth.

two most OTU rich genera of the control plots, i.e. *Tomentella* and *Inocybe*. In comparison the percentage of OTUs that are solely present in the control plots in *Cortinarius* and *Russula* is considerably lower (Fig. 4.4). Five (undetermined) *Tomentella* OTUs were indicators of the ambient snow plots, while no OTU was considered indicator of the deeper snow plots (appendix S4.4).

The correlation coefficient between the taxonomic groups and ETs revealed significant positive and negative correlations among specific groups (S7). Across the ambient snow depth plots, *Inocybe* OTU richness showed a significantly negative correlation with *Russula*, and a significantly positive correlation with *Tomentella*. Interestingly, across the increased snow depth plots, the negative correlations of OTU richness between the previously mention genera decreased sharply to non-significant values.

Moist tussock tundra

The NMDS analysis of the square-root sequence abundance matrix resulted in a 2-dimensional solution with a final stress and instability of 0.0759 and < 0.00001, respectively. The results of the Monte Carlo test indicated that all two dimensional solutions using the real data were significantly better than occurrences by chance (p < 0.01). The coefficients of determination for the correlations between ordination distances and distances in the original n-dimensional space were axis 1: r^2 = 0.607; axis 2: r^2 = 0.265; total r^2 = 0.872; orthogonality = 93.1%. The NMDS ordination plot was orthogonally rotated by the treatment to visualize correlations between snow depth effect and fungal community composition in general, and the taxonomic groups in particular (Fig. 4.3b). The MRPP analysis indicated a clear distinction between ambient and deeper snow ECM community

composition (p = 0.0017, A = 0.0943). The NMDS and MRPP analysis of the presence-absence matrix yielded similar results (Appendix S4.2b). *Inocybe* and the group of OTUs with short-distance exploration type had a strong negative correlation with the increased snow depth plots, r = -0.757 and -0.775, respectively, as well as the OTUs with hydrophilic hyphae (r = -0.540) and *Lactarius* (r = -0.536). On the other hand, *Alnicola* and *Laccaria* OTU richness had the strongest positive correlation with the deeper snow plots with r = 0.696 and r = 0.510, respectively. The control plots had on average 72.4 ± 22.02 OTUs per plot, while the deep snow plots had 53 ± 31.01 OTUs per plot. Despite the considerable decrease, the difference was not statistically significant (p = 0.15). The most OTU rich genus in the ambient snow areas was *Tomentella* with 20.6 ± 7.4 OTUs per plot, followed by *Inocybe* with 10.4 ± 5.3. On the deeper snow areas, while *Tomentella* had only a marginally significant (p=0.07) decrease on the average OTUs per plot to 12.6 ± 8.33, *Inocybe* had a significant (p= 0.02) decrease to 3.2 ± 1.92 OTUs per plot. On the other hand, C*ortinarius* was the genus with higher OTU richness in the deeper snow plots with 9.4 ± 6.8, and showed no significant changes (p = 0.47) on OTU richness compared with the ambient snow plots (Table 4.1). Interestingly, in the deeper snow plots, on the order ranking, Thelephorales increased the percentage of OTUs while all the remaining orders decreased (Fig. 4.1), mainly due to the decrease in *Inocybe*, *Russula* and *Lactarius* OTUs richness. Regarding EMM characteristics, in the control plots the contact, short-distance and medium-distance smooth had on average 50 ± 17.13 OTUs per plot, while the long-distance and medium-distance fringed had 22 ± 5 OTUs per plot. In the deeper snow plots the first group had a marginally significant decrease (p = 0.07) to 33.6 ± 15.08 OTUs per plot, while the latter group did not change significantly (p = 0.38 - 19.4 ± 16.59 OTUs per plot). Most OTUs (45%) were only present across the ambient snow areas, 25% were present in both the control and deeper snow plots and 30% were only present in the deeper snow plots (Fig. 4.4). *Tomentella* and *Russula* followed the overall OTU distribution pattern. Conversely, *Cortinarius* had a contrary pattern, with a higher percentage of OTUs solely recovered from the deep snow plots - 41%, 33% solely present on the control plots and 25% present in both the control and deeper snow areas. On the other hand, *Inocybe* had 63% of OTUs only recovered from the ambient snow plots, 23% recovered solely from the deeper snow areas and only 13% were found in both the ambient and deep snow plots (Fig. 4.4). Seven unidentified *Tomentella* OTUs, *Cortinarius huronensis*, *C.* cf. *flos-paludis*, *Inocybe leiocephala*, *I. nitidiuscula*, *Russula renidens*, and *Lactarius torminosus* were indicator OTUs of the ambient snow areas. On the deeper snow plots, *Tomentella lapida*, one unidentified *Russula*, one unidentified *Laccaria*, one unidentified *Inocybe*, one unidentified *Cortinarius*, and tree unidentified *Alnicola* OTUs were indicator OTUs (appendix S4.4).

The correlation coefficient between the taxonomic groups and ETs (appendix S4.5) revealed a significant positive correlation among two groups in the ambient snow plots: *Cortinarius* - *Russula*, and between the group of OTUs with contact, short-distance

and medium-distance smooth ET, and the group of OTUs with medium-distance fringe and long-distance ET. In the increased snow depth plots, the significant positive correlation, among these groups, was also observed, and further extended to the pairs *Tomentella* - *Cortinarius*, and (marginally significantly) for *Russula* - *Tomentella*. Interestingly none of the groups tested had a negative correlation either at the control or the deeper snow plots.

Discussion

The results presented here clearly show that long-term increase in snow depth alters ECM fungal community composition in moist tussock and dry heath tundra, with a considerable portion of OTUs not being resilient to the resulting changes in environmental conditions. These conclusions are based on the significant decrease in average OTU richness in the deeper snow plots and the considerably low percentage of OTUs shared between the two snow depths we studied. Recently it has been shown that ECM fungal community is also sensitive to summer warming with the communities of the moist tundra showing a more evident response altering community composition, trait patterns and OTU richness levels (Geml *et al*., 2015; Morgado *et al*., 2015).

The strongest response to increased snow depth was observed in the dry heath tundra, where besides community composition, there was also an overall significant decrease in richness of the ECM community in response to increased snow depth. The ECM fungal richness of the moist tundra also showed a decreasing trend but not in a significant manner. This was surprising, as we anticipated that ECM fungal community in the moist tussock tundra would have a stronger response to deeper snow, because the plant community and N dynamics had been reported to be more strongly affected by the increased snow depth in the moist tundra (Schimel *et al*., 2004; Wahren *et al*., 2005; Mercado-Díaz, 2011). Together with our previous results (Morgado *et al*., 2015), we argue that the dry tundra ECM fungal community likely is more sensitive to changes in snow depth than to summer warming, and that the ECM fungal community in the dry tundra may be more sensitive to increased winter snow depth than the plant community. Our correlation analysis of OTU richness and sequence abundance with the different snow depths showed different patterns among the tundra types. For example, in the moist tundra OTU richness and sequence abundance between *Cortinarius* and *Tomentella* showed a significant positive correlation in the increased snow depth plots, while no significant correlation was observed in the control plots. On the other hand, in the dry tundra no correlation was observed between these two groups in either snow depth. This suggests that in our study the interaction between OTU richness and potential species abundance likely are habitat-specific, and are in agreement with studies that addressed species-species interactions (Kennedy *et al.*, 2007; Simard *et al.*, 2012; Fransson *et al.*, 2013). Collectively, our findings reflect the complexity of arctic tundra responses to

predicted changes in summer and winter climate and the need to undertake comparative studies that include multiple ecosystem types even at a local spatial scales (Welker *et al.*, 2000; Walker *et al.*, 2008; Sullivan *et al.*, 2008; Sullivan *et al.*, 2010, Rogers *et al.*, 2011; Christensen *et al.*, 2013; Leffler & Welker, 2013; Sharp *et al.*, 2013; Lupascu *et al.*, 2014).

Tomentella, the genus with higher richness and sequence counts in the control plots of both tundra types, showed a sharp negative response to increased snow depth, with a significant six fold decrease in average OTU richness and a majority of the OTUs disappearing in the dry tundra, as well as an overall decrease in proportional sequence counts. In the moist tundra, *Tomentella* richness also showed a decreasing trend, but in a less striking manner than in the dry tundra. The elevated number of indicator OTUs associated with the control plots (5 in the dry and 7 in the moist tundra) also point to the sensitivity of this group to altered conditions. Moreover, two of these OTUs were very closely related with a *Tomentella* OTU (KJ792685) that had a strong negative effect by increased summer temperatures in the dry tundra (Morgado *et al*., 2015), further indicating that besides the general trends for the genus, at least one species of *Tomentella*, which is potentially widespread across the dry tundra, is very sensitive to summer and winter warming. Potential explanations to the observed patterns in our study may be linked with their functional traits and potential ecological roles. *Tomentella* and closely related genera (e.g. *Pseudotomentella* and *Tomentellopsis*) have melanized cell walls (Agerer, 1987-2002; Agerer *et al*., 2006), which is not a common feature in ECM basidiomycetes (Kõljalg *et al*., 2000). Melanins can be produced by fungi, plants and animals, and are dark macromolecules composed of phenolic and indolic monomers, often coupled with protein and carbohydrates (Butler & Day, 1998). They usually constitute a considerable portion of total fungal cell weight and likely require a considerable energetic investment (Rast & Hollenstein, 1977; Butler and Day, 1998). This feature has been extensively argued and was recently shown in physiological experiments (Fernandez & Koide, 2013) to increase tolerance to several environmental stressors, such as freezing (Robinson, 2001) and hydric stress (Singaravelan *et al*., 2008; Fernandez & Koide, 2013). The increased snow depth not only elevates winter soil temperature, but also increases soil moisture content as well (Wipft & Rixen, 2010), the effect of which likely is greater in the dry tundra. These altered conditions might reduce the competitive advantages of melanin-producing *Tomentella* adapted to the above-mentioned stress factors (e.g., drought, and very low temperatures). Additionally, *Tomentella* has either contact, short or medium-distance smooth hyphal ETs, which have been argued to be adapted to labile N soil pools (Hobbie & Agerer, 2010). The plant community responded to increased snow depth with a significant increase in the shrubs and litter layer (Wahren *et al*., 2005; Mercado-Díaz, 2011), indicating a potential change in soil organic matter input and shifts in C:N ratio, that have been argued to be important regulators of arctic N dynamics (DeMarco *et al*., 2011). Therefore, it is possible that in

the altered environmental conditions the combination of traits presented by *Tomentella* might not constitute an advantage in scavenging for soil nutrients, which might lead to a detrimental allocation of photosynthates by the ECM host and/or to competitive exclusion by other ECM fungi better suited to the altered conditions.

The decomposition and turnover of ECM fungal biomass likely has a significant role in C and other nutrient dynamics (Wallander *et al*., 2001; Clemmensen *et al*., 2013; Ekblad *et al*., 2013). Melanized hyphae have been argued to be relatively long lived, slow growing (Robinson, 2001) and to have increased cell wall resistance to decomposition (Coelho *et al*., 1997; Butler & Day, 1998; Butler *et al*., 2005), potentially representing a stable and recalcitrant component in the fungal biomass (Treseder & Lennon, 2015). In two laboratory experiments, Fernandez and Koide (2014) showed that the decomposition rate of ECM fungi was inversely correlated with the concentration of melanin and that the inhibition of melanin biosynthesis in an ECM fungi induced faster rates of decomposition. Moreover, Clemmensen *et al*. (2015), observed a correlation between the abundance of taxa with melanized hyphal content and higher carbon storage in the soil. If future climatic conditions lead to increased snow depth in the arctic tundra, the decreasing richness and relative abundance of *Tomentella* might contribute to soil C loss. Several other groups of root-associated fungi, also have melanized hyphae, such as ericoid mycorrhizal fungi and dark septated endophytes. We therefore highlight the need to address responses of those root-associated fungi to increased snow depth to better understand if the above-mentioned warming-induced trend of decreasing richness is common in melanized fungi or is specific to certain phylogenetic lineages. Despite the uncertainties, our evidence and grounded speculations are in line with the results by Natali *et al.* (2014) that addressed winter warming effects on C cycle dynamics and indicated a net soil C loss due to winter warming.

We observed an abrupt decrease in mean OTU richness of *Inocybe* from the control to the increased snow depth plots in both tundra types. However, a considerable proportion of *Inocybe* OTUs that were found in the increased snow depth plots were also found in the control plots, particularly in the moist tundra. These results suggest that although arctic *Inocybe* spp. seems to be very sensitive to climate changes, a resistant subset of the species seem to be able to withstand changes in the climatic conditions. *Inocybe spp.* were previously argued to be sensitive to altered environmental conditions, such as soil compaction (Hartmann *et al*., 2014) and summer warming (Morgado *et al*., 2015). Additionally, there is evidences that in mature plant stands the rate of root-infection by *Inocybe* might decrease in sites with increased soil moisture (Fleming, 1984). It is possible that in the increased snow depth conditions, the lack of rizhomorphs and hydrophilic ectomycorrhizae of *Inocybe* (Agerer, 2006), a set of characteristics hypothesized to be adapted to labile N uptake (Hobbie and Agerer, 2010), might constitute detrimental traits in relation to other groups of ECM fungi. However, the

increase in shrubs and litter layer might lead to potential patchiness of nutrient soil pools allowing for some species with hydrophilic hyphae to thrive in the increased snow depth.

The lack of significant changes in *Cortinarius* richness and the relative increase in their overall sequence counts (a potential surrogate for relative abundance) between the control and increased snow depth plots indicate that this group might be more resistant and/or resilient to altered conditions, and could become more dominant in the warming Arctic. A similar trend for this group was also observed in our previous work that reported ECM fungal responses to long-term summer warming (Morgado *et al*., 2015). However, in contrast with our previous work, in the present study most OTUs are not shared between the two treatments, potentially indicating that, although average OTU richness does not change, there is a considerable turnover in species composition. This suggests that only a subset of the OTUs present in the control plots are resistant to increased snow depth and that there is substantial functional variation within the genus that allows for the exploitation of new niches created in the altered environment by incoming species. Species of *Cortinarius* form a dense mycelium with medium-distance fringe ET and hydrophobic rhizomorphs (differentiated mycelial cords) (Agerer, 2001). This foraging strategy is adapted for efficient N absorption and nutrient translocation (Hobbie and Agerer, 2010). *Cortinarius* were also reported to have the capability to assimilate organically bounded N (Hobbie & Agerer, 2010), and to transcribe Mn-peroxidase genes (which are involved in the production of exoenzymes) in field conditions and linked through co-localization of DNA abundance with exoenzyme activity that interacts in complex organic matter breakdown (Bödeker *et al*., 2014). Although little is known about ECM fungal physiological responses to extreme cold, Ma *et al*. (2011) compared growth responses to very low temperatures (between -40 and +4 °C) and freeze-thaw events of four ECM species from distinct lineages. Their results indicated that *Cortinarius* had the lowest tolerance to freeze-thaw events and the fastest growth when temperatures reach near 0 °C. Because reduced temperatures, hydric stress and freeze-thaw events inhibit the rate of chemical and microbial activity (Robinson, 2001), and given the characteristics and potential ecological role of *Cortinarius* spp., it seems feasible to argue that long-term increased winter soil temperatures, summer moisture and reduced fluctuations in soil temperatures might convey advantages to this group over other ECM fungal groups.

The increasing dominance of fast growing ECM species with high EMM production and fast N mobilization might lead to increased C storage in this soil pool, however, this will be determined by biomass turnover. In an interesting work relating fungal traits, community structure and nutrient soil pools, Clemmensen *et al.* (2015) found a link between abundance of species with similar ETs similar to that of *Cortinarius* and low accumulation of root-derived soil C. Briefly, they hypothesized that the exploratory hyphae of already explored areas in the soil could be recycled in an autolytic

process, leaving behind just the long-living rhizomorphs connecting the exploratory forefront of the EMM and the ectomycorrhizae. This strategy would enhance their nutrient acquisition and maintain or reduce their biomass, potentially reducing also energetic costs. Due to the potential high turnover of this mycelium biomass this theory also implies a potential reduction in stable soil pools, due to the potential high turnover of this mycelium biomass, and long-term C and N sequestration.

Russula did not show any significant change in richness with increased snow depth, and a considerable portion of the OTUs were found both in the control and the treatment plots, indicating that many OTUs were resistant to altered conditions. While most *Russula* spp. lineages have an hydrophilic ectomycorrhizae and contact or short-distance ETs that lack rhizomorphs, other lineages in *Russula* have a medium-distance smooth ET and hydrophobic hyphae (Agerer, 2006). In our dataset, the *Russula* OTUs with short or contact ET did not show change in richness with increased snow depth. However, one OTU with short or contact ET was indicator of the moist control plots while another was indicator of increased snow depth, suggesting species-specific responses to altered conditions. On the other hand, in the increased snow depth plots in the moist tundra, we observed a significant decrease in richness of OTUs that matched *Russula* species with hydrophobic and medium-distance smooth ET. These results indicate that even within a closely related group of species the functional diversity can differ. While some *Russula* species seem to have a considerable fast growth rate at low temperatures (Ma *et al*., 2011), others are considered to have a slow growth rate (Nygren *et al*., 2008). Moreover, there is evidence of intrageneric variability in N usage as well (Avis, 2012). It is possible that in some species the hydrophilic ectomycorrhizae allows for the rapid intake of labile N forms by the plants, without mineralization, via diffusion through the mantle of the ectomycorrhizae directly to the plant-host root via the apoplast. This process would avoid energetic costs and the necessity of C allocation to the ECM fungi (Nygren *et al*., 2008). This may influence the competitive interactions between species with hydrophilic and hydrophobic mycelia.

In conclusion, our data provide first insights into the taxon-specific effects of increased snow depth on the ECM fungal community of Arctic tundra in Northern Alaska. We discovered major shifts in ECM fungal community composition and its potential functional traits by coupling changes on fine scale taxonomic groups with their extramatrical characteristics. We postulate that ECM fungal community shifts induced by long-term increased snow depth likely stimulate C and N mobilization. However, the final balance induced by arctic ECM basidiomycete community in these nutrient pools will likely depend on the changes in the biomass of specific groups, particularly *Tomentella* and *Cortinarius*. Our results also highlight how the fundamental differences in tundra ecosystem control the nature of the existing fungal communities and their responses to deeper snow.

Landscape at Toolik Lake, Alaska

Chapter 5

Discussion and conclusions

Luis N. Morgado

Discussion and conclusions

Climate changes are driven by temperature increases that affect directly and indirectly ecosystem structure and functions. Even though climate change affects the entire planet, there is considerable spatial variation and high-latitude regions are among the most affected areas. In the last decades, average land surface temperatures in the Arctic have increased at a rate between 0.06 to 0.1 °C per year, while the global average yearly increase was ca. 0.017 °C. At the same time, precipitation in the Arctic also increased, greatly exceeding the global average ratio, especially during the cold season when most precipitation falls as snow. State-of-the-art models predict further increases, possibly by more than 50% of the current precipitation, leading to thicker snow cover. These climatic changes have major consequences in the arctic tundra, including greening of the land surface, tree line advancement, shrub encroachment, altered vegetation composition, plant phenology and mineral nutrition, increased net primary production, warmer winter soil temperatures, increased soil moisture, deeper thaw depth, increased soil microbial respiration and N turnover, and altered C storage potential. However, how arctic soil fungal communities respond to warmer temperatures and increased snow depth is still largely unknown.

Here the long-term effects of summer increased temperatures and winter increased snow depth in arctic soil fungal community composition in dry heath and moist tussock tundra were addressed using a long-term ecological research site at Toolik Lake, Alaska. The long-term warming was achieved through 18 years *in situ* experimental setups with open-top chambers (summer warming) and snow fences (increased snow depth). The control areas were adjacent to the treatments and were maintained at ambient conditions. Soil fungal composition was assessed through soil DNA extraction, massive parallel sequencing of *ITS2* fragment of *ITS* rDNA, the globally accepted barcode of fungi, and 97% sequence similarity cutoff to generate operational taxonomic units (OTUs). OTUs were binned into (1) taxonomic ranks through generally accepted sequence similarity thresholds using a curated taxonomic dataset of publically available fungal sequences, and (2) ecological groups, whenever possible. For community composition comparison a variety of standard statistical analysis tools in community ecology such as multidimensional scaling and multi-response permutation procedure were used. Below the main research questions addressed in this thesis are summarized and discussed, and some of the main findings presented in a schematic form (Fig. 5.1).

How diverse are fungal communities in the dry and moist arctic tundra types found in the Toolik Lake region and what are the most diverse groups?

Using a conservative approach to delimit fungal sequences (i.e., excluding singletons and OTUs with less than 80% ITS2 rDNA sequence similarity or 150 bp pairwise alignment length to a fungal sequence) 5438 fungal OTUs in the Toolik Lake

region were found. In the moist tundra, there were 3534 observed OTUs, with first and second order Jackknife estimates of 4429 and 4725, respectively, while the values were slightly higher in the dry tundra, with 3543 observed OTUs and first and second order Jackknife estimates of 4503 and 4894, respectively. Ascomycota was by far the most OTU-rich phylum accounting for 40.25% of all OTUs, followed by Basidiomycota with 22.18%. Glomeromycota was represented by 0.17%, while basal lineages formerly classified in Zygomycota accounted for 2.66% of all OTUs and Chytridiomycota for 0.09%. In addition, there were 34.65% fungal OTUs that could not be identified due to the incompleteness of the available databases. In Ascomycota, there were several taxonomic orders with a high number of OTUs, such as Helotiales, Chaetothyriales, and Lecanorales, while in Basidiomycota, the order Agaricales was the most diverse, followed by Sebacinales and Thelephorales. Ectomycorrhizal basidiomycetes were the most OTU-rich ecological guild in both tundra types, followed by lichenized ascomycetes in the dry tundra and saprotrophic zygomycetes in the moist tundra.

How did the fungal community responded to long-term summer warming?

The study revealed no significant differences in Shannon's diversity and Simpson's diversity indexes between the control and treatment plots. Beta diversity values were relatively low within the control and treatment plots, although in both the dry and the moist tundra, warmed plots had slightly decreased beta diversity values. Fungal ccommunity composition responded strongly to summer warming in the moist tundra, but not in the dry tundra. Likely, fungi inhabiting the dry tundra are more adapted to the environmental conditions induced by summer warming than the moist tundra community. Perhaps because ambient soils in the moist tundra, being generally cool throughout the summer, tend to experience less fluctuations in temperature than dry tundra soils that are regularly exposed to higher temperatures and pronounced water stress in the upper layer.

Which fungal taxa and ecological groups responded to long-term summer warming?

Although total fungal diversity and richness were not significantly altered by warming and were comparable across moist and dry tundra sites, there were clear patterns of correlations among OTU richness of various ecological and taxonomic groups and long-term warming. In the moist tussock tundra, summer warming induced a decrease in OTU richness of lichens, ericoid mycorrhizal basidiomycetes, endophytes of herbs, dung/litter fungi, and ECM basidiomycetes. In contrast, there was an increase in OTU richness of saprotrophic soil asco- and zygomycetes, dark septate fungi, ericoid mycorrhizal ascomycetes, animal pathogens, and wood-rotting ascomycetes. In the dry heath tundra the patterns were different, only OTU richness of ericoid mycorrhizal ascomycetes decreased significantly with warming, while moss endophytes, saprotrophic soil basidiomycetes, and soil zygomycetes were represented by more OTUs in the warmed plots.

Which ECM fungal genera showed the most pronounced responses to long-term summer warming?

ECM basidiomycetes were the most OTU rich fungal guild in the dry and moist tussock tundra. Although these OTUs were spread across 20 genera, four of these dominated the communities, accounting for approximately 82% of all OTU richness: *Tomentella* (31%), *Cortinarius* (22%), *Inocybe* (18%) and *Russula* (10%). In the moist tundra, warming induced an OTU richness decrease in *Tomentella*, *Inocybe* and *Russula*, while *Cortinarius* OTU richness was not significantly affected. In the dry heath tundra no genera showed a significant change, however, *Tomentella* and *Russula* had a slight warming-induced richness increase.

What are the ecological implications of the responses of the fungal community to long-term summer warming?

The possible ecological implications of the above mentioned observations about fungal community responses to summer warming reflect the complexity of fungal community ecology. In the moist tussock tundra, warming seems to induced an increase in richness of mycorrhizal fungi that have the ability to degrade organic compounds. For example, although there was a richness decrease in many ECM fungal genera, warming appeared to favour taxa with medium-distance fringe mycelial exploration types (such as *Cortinarius*). This exploration type likely is adapted to efficient long-distance transport of nutrients and water, belowground plant-fungal networks, and, perhaps more importantly, to acquire recalcitrant forms of soil N. Additionally, ericoid mycorrhizal ascomycetes, a group of fungi also known for their potential to degrade organic compounds also had a warming-induced richness increase. Collectively, it seems that the ability of mycorrhizal fungi to obtain nutrients from organic compounds may be an important trait in the arctic fungal communities of moist tussock tundra with increased summer temperatures.

The changes in the moist tussock community also have implications for C cycling. Many ericoid mycorrhizal ascomycetes have the ability to synthesize melanin, a long-lived and recalcitrant compound. The increase in OUT richness in this group of fungi may, therefore, increase potential C storage in fungal biomass. However, other melanized fungi, such as *Tomentella* and *Cenococcum*, had a warming-induced richness decrease, suggesting a decreased potential for mycelial C storage. The final C budget of soil fungal biomass will depend on several factors that are beyond the scope of this thesis, such as biomass dynamics of each of these groups of fungi, which likely is linked to their ability to produce extramatrical mycelium and the life span of these fungi. The absence of significant compositional changes in the dry tundra suggests that the resident fungal community is well adapted to the warming-induced conditions. The slight increase in some fungal groups of melanised fungi, such as ericoid ascomycetes and *Tomentella*,

likely is due to their ability to resist water stress and may indicate a certain stability of C storage in fungal biomass in the dry tundra.

Previously reported warming-induced vegetation shifts at the same experimental site were caused by changes in the relative abundance of various plant functional groups rather than changes in richness or species identities. In fungi, most of the differences in community composition among the control and warmed plots were caused by the presence of many OTUs in a particular treatment type and absence in the other. While the currently prevailing view is that altered plant community composition drives fungal community change in the Arctic, it seems that fungal community composition may change more rapidly and independently of plant communities and that fungi may be particularly well-suited to monitor early responses to environmental changes.

How did the ECM fungal community responded to long-term increased snow depth?

The ECM fungal community composition changed significantly in response to long-term increased snow depth in moist tussock and dry heath tundra. Although the most pronounced changes were in the dry tundra with a significant decrease in overall richness, there were similar trends in both moist and dry tundra. Greater snow depth increases winter soil temperature and soil moisture, particularly in early summer, and these effects cannot be decoupled. Therefore, the stronger response by the community of the dry heath tundra may partly be explained by the more pronounced changes in this habitat, because the moist tundra community likely is better adapted to higher winter snow depths and increased soil moisture than the dry tundra community, where soils tend to have little or no snow cover, resulting in very cold temperatures and frequent desiccations.

Which fungal taxa and ecological groups responded to long-term increased snow depth and what are the ecological implications?

Tomentella, *Inocybe*, *Cortinarius* and *Russula* were the ECM basidiomycete genera with highest richness values. While the first two genera had a pronounced decrease in richness due to increased snow depth, the latter pair did not. This may have potential functional implications. The exploration types adapted to labile N uptake showed decreasing richness, while the richness of exploration types adapted to acquire recalcitrant soil N were not affected by increased snow depth. These results indicate that the potential to acquire recalcitrant N may be positively selected for in increased snow depth conditions, leading to an increased potential of the community to utilize different forms of soil N. This shift in the community may implicate a faster N turnover. The decreased richness in *Tomentella* in the dry and moist tundra types may lead to a reduced potential for C storage with increased snow depth. However, the C budget of the ECM fungi will depend on the turnover ratio of the biomass of the group of species that become dominant in the altered conditions, such as *Cortinarius*.

What are the main conclusions from the fungal community responses to long-term summer warming and increased snow depth?

The fungal community composition of the moist tussock tundra was significantly altered due to summer warming and winter snow depth, while the dry tundra community was only significantly altered with increased snow depth conditions. These responses may be driven by direct responses to increased air and soil temperature or may be via the multiple indirect effects that these conditions induce in the ecosystem. As expected, the initial conditions inherent to each habitat are key to understand how the fungal communities respond to climatic disturbances. Maybe not so obvious was that the fungal community, particularly ECM fungi, responded with major shifts in composition, richness and functional trades independently from their plant-hosts, strongly suggesting that the physical and biochemical conditions induced by climatic disturbance are main drivers of community composition and function. In turn the climate-induced changes in the community composition alter the richness of fungal groups that have an important role in C and N cycles.

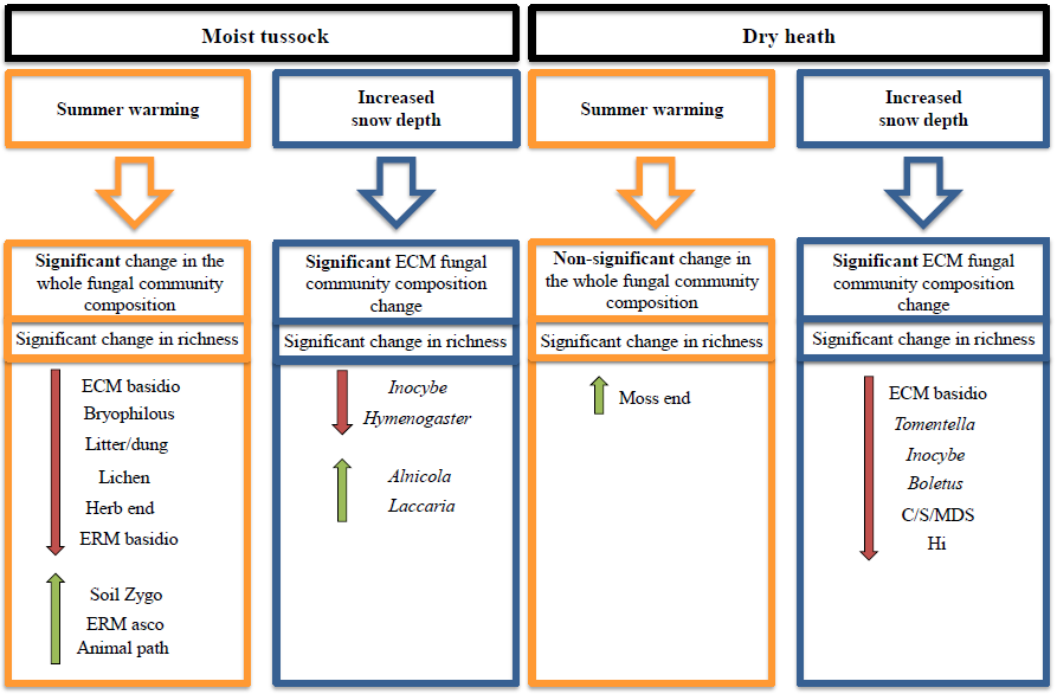

Figure 5.1. Schematic layout of the main findings presented in this thesis per tundra type and climatic disturbance combination. Abbreviations are animal path = animal pathogen, bryophilous = fungi living on mosses, dung / litter = secondary decomposer fungi that live on litter and/or dung, ECM-bas = ectomycorrhizal basidiomycete, ERM asco = ericoid mycorrhizal ascomycete, ERM basidio = ericoid mycorrhizal basidiomycete, Herb end = endophyte of herbs, Lichen = lichenized fungi, moss end = endophyte of mosses, soil Zygo = soil saprotrophic zygomycete, C/S/MDS = ECM fungi with contact, small or medium distance exploration type, Hi = ECM fungi with hydrophilic hyphae.

Methodological considerations

Using next-generation sequencing of soil fungal communities of *in situ* long-term ecological experiments allowed an in-depth insight into the arctic Alaskan fungal taxonomic diversity and responses of community composition to predicted changes in the Arctic. However, the ecological functions of many described fungal species are still unknown. Also, the ecological interpretations are limited by the vast diversity of fungi that are still undiscovered or are described, but do not have publicly available DNA sequence data.

Perhaps the largest critique to the methodology utilized in this work is the absence of replicated habitats. This limitation is beyond feasible methodological choices, because of the existing design of these long-term experiments. None of the existing similar long-term ecological experiments that are part of the circumpolar International Tundra Experiment (and therefore have a similar set up) can be used as a true replicate, because of different environmental conditions as a result of considerable geographic distances. Although individual studies to be done at these other ITEX sites can be used to test the results presented here. In such case interpretations have to be made with caution as fungal communities at distant localities may be driven by different sets of environmental variables.

The methodological approach used allowed to assess fungal community composition, richness and estimate relative taxonomic abundances. However, to gain insight into in-depth ecological implications, total abundance of taxonomic and ecological groups would be an added value. Unfortunately the methodologies available to quantify fungal biomass, such as ergosterol concentration, are mainly informative at the whole biomass community level (i.e., not discriminating among taxa), and not easily correlated with rDNA copies that provide insight into community composition. Also quantitative PCR has been used to estimate fungal abundance through quantification of rDNA copies and them correlated with relative abundance among samples, but quantitative PCR analyses are subject to biases due to differences in DNA extraction efficiencies and varying DNA amplification efficiencies across samples, hampering the comparison among DNA extracts. Hopefully, technical advances combined with elegant solutions will soon increase the ability to estimate and correlate rDNA abundance among DNA extracts enabling a better insight into the microbial community functions (Smets *et al*., 2015).

Future research

The work developed and presented in this thesis provides a crucial baseline for future studies. The thesis is largely focused on a subset of the whole fungal community, namely the ECM fungi. However, I am co-authoring a manuscript (under preparation) on

how the total fungal community responds to increased snow depth. The main goal of that study is to unravel the various trends of the different taxonomic and ecological groups to long-term increased snow depth, with potential implications for future arctic tundra ecology. It will be particularly interesting to see how fungi possessing certain key functional traits, such as melanin production, because it can be related to potential C storage in the soil fungal biomass, respond to snow depth increase.

The core of this thesis is built upon 2 independent experiments, the open top chamber and the snow fence that increase summer temperatures and winter snow depth, respectively. It would be useful to study how the fungal community responds to the combined effects of summer temperature increase and increased winter snow depth, because that is one of the possible future scenarios. Although most groups did not show contradictory effects among experiments, *Tomentella* showed slightly opposite response trends between summer warming and increased snow depth in the dry tundra, indicating that the combinatorial outcome of the experiments should be taken as an independent experiment in order to provide accurate estimates in future conditions. Nevertheless, both general and specific hypotheses can be drawn from the results present in this thesis. The more pronounced responses to summer warming were in the moist tundra community, while snow depth increase induced more profound changes in the dry heath tundra fungal community, potentially indicating a complementary effect that will likely induce profound changes in the communities of both tundra types. Perhaps the more important inferences will be related with the extent of these alterations, and how particular groups of fungi, for which potential functional traits are known, will respond to the combined effects of summer warming and increased snow depth.

The experiments where the sampling was performed are part of an international consortium of researchers that aims to investigate climate changes effects on tundra ecosystems (ITEX). Because fungi are a vital part and play a crucial role in the functioning of the tundra ecosystem and are particularly suited to assess changes in ecosystem functioning, it would be of added value to society in general and to the scientific community in particular to understand to what extent it is possible to generalize the results obtained in these studies. Therefore, studying fungal communities at other arctic long-term experimental sites would reveal the spatial variation of arctic fungal communities and their responses to climate change on a circumpolar scale. Because the sampling plots are part of the ITEX network that constitutes the scientific basis for the vast majority of scientific studies on terrestrial arctic ecology and climate change, this network should constitute a primary option for further investigations on arctic fungal ecology, preferably using similar methodology for full compatibility. Such a comprehensive study could be used to integrate the fungal responses to climate change in various geographic regions of the Arctic with implications for climate change models and for more realistic predictions.

The studies presented here represent two different disturbance treatments in two different habitats. Each habitat showed specific responses to a specific disturbance, indicating that in order to fully understand community responses to climate changes, it is necessary to include landscape variations in the equation. Although the studies focused on two main tundra types that occupy a large area in the studied region (northern Alaska) and represent contrasting sets of environmental conditions present in arctic tundra habitats, there are many other vegetation types with different underlying environmental conditions. Assessing how fungal communities vary along topographic and edaphic gradients will allow us to better understand the full diversity of low arctic fungi and their spatial complexity and habitat partitioning. There have been vegetation studies linking tundra plant communities and abiotic environmental factors, however, fungal communities are absent from such studies. It would be interesting to understand how arctic fungal communities vary along these habitats and how they correlate with the various biotic and abiotic factors. In this way, one could potentially predict the pan-arctic distribution of key groups of fungi and provide insight into potential areas that are more or less threatened by climate changes and other disturbance, such as increasing land use pressure.

The studies presented in this thesis showed that the ECM fungal community composition is extremely rich and that ECM fungi likely represent the most taxonomically diverse fungal functional guild in the arctic tundra. Although ECM fungi are species rich, this richness is not evenly distributed among the taxonomic groups. Indeed, most ECM taxa belong to four genera: *Tomentella*, *Cortinarius*, *Inocybe* and *Russula*. These groups have different hyphal exploration types that have implications for nutrient acquisition. Tissue of fruit-bodies belonging to species with different exploration types have different isotopic signatures (either more or less ^{15}N enriched), leading to the hypothesis that correlates hyphal exploration types with distinct strategies of nutrients uptake. However, these patterns may have intrageneric and even intraspecific variations. Moreover, these patterns are known to differ among geographical areas. It would probably be useful to perform an explorative study targeting the groups of ECM fungi that showed the strongest responses to summer warming and increased snow depth, such as *Tomentella* and *Inocybe* and compare it with the groups that did not show change in richness, such as *Cortinarius* and *Russula.* Additionally, future studies should assess variation in isotopic signatures at intrageneric and intraspecific levels, as well as within given experimental plots and control areas. This experimental delineation would address the main issues regarding the variability of isotopic patterns, and would provide insights into the hyphal exploration theory in the context of climate change. Although the ideal situation described above may not be feasible in practical terms due to the scarcity of fruit-bodies produced in any given small area, such as that of the experimental plots, the species identified in this study as indicators of certain treatment types might provide a good starting point.

The community structure was assessed through ITS2 rDNA sequencing of the soil fungal community. Although this methodology allowed the determination of community composition, community functioning was inferred through taxonomic identity. However, the presence of a species does not necessarily informs us about its function in the community. Additionally, rDNA sequencing assesses both active and inactive members of the community. Recent advances in sequencing techniques are enriching the public databases and enabling the investigation of below-ground patterns to move towards gene expression and potential functions. Although constrains still exist regarding the sequencing of below-ground communities that target selected genes in order to obtain community functions, future studies of microbial communities responses to climate changes may target genes that have crucial roles in specific pathways (such as, exoenzyme production and melanin biosynthesis), allowing direct quantification and comparison of functions between different experimental treatments and/or habitats.

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Appendices

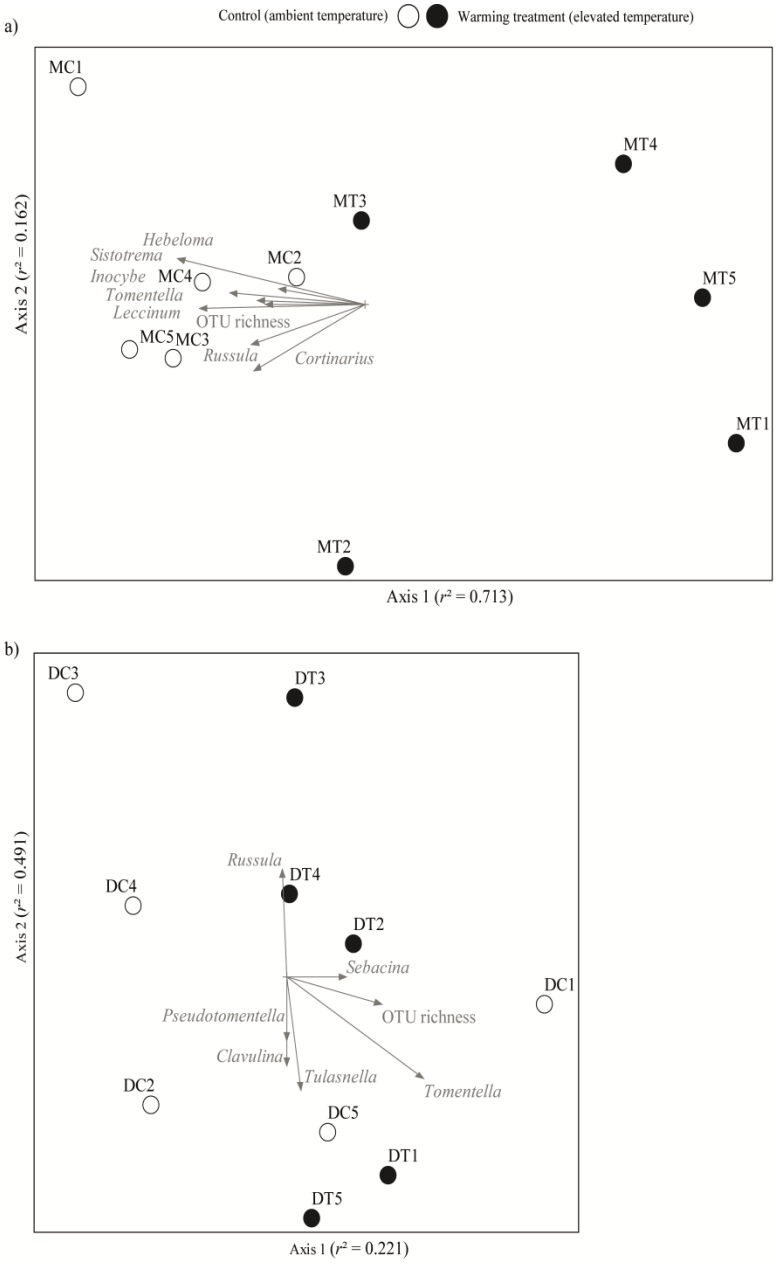


Figure S2.1. a) NMDS analysis with the square-root sequence abundance dataset of the ECM fungal communities of the moist tussock tundra plots with final stress 0.0523, final instability < 0.00001, total r^2 = 0.876 and orthogonality = 96.5%. MRPP *A* = 0.0927 and *p* = 0.0051. b) NMDS analysis of the ECM fungal communities of the dry tundra replicates with final stress 0.1266 and final instability < 0.00001. MRPP *A* = 0.0163 and *p* = 0.1750. Legend: DC – dry control, DT – dry warming treatment, MC – moist tussock control, MT – moist tussock warming treatment.

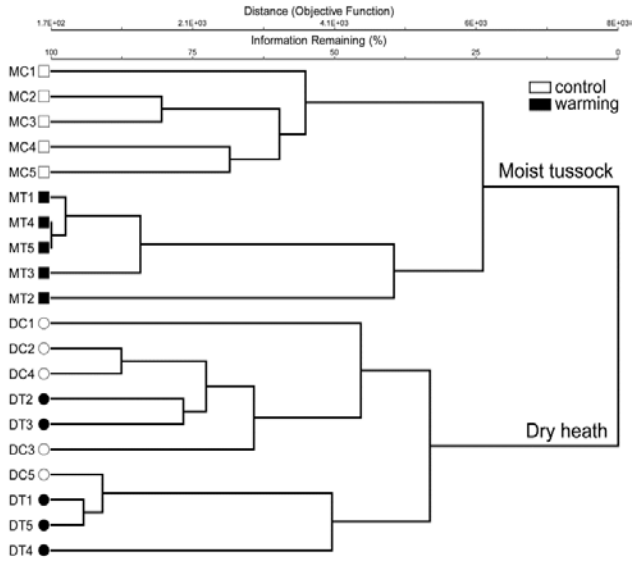


Figure S3.1. Cluster diagram for fungal communities from the warmed and control plots in the dry and moist tundra types.

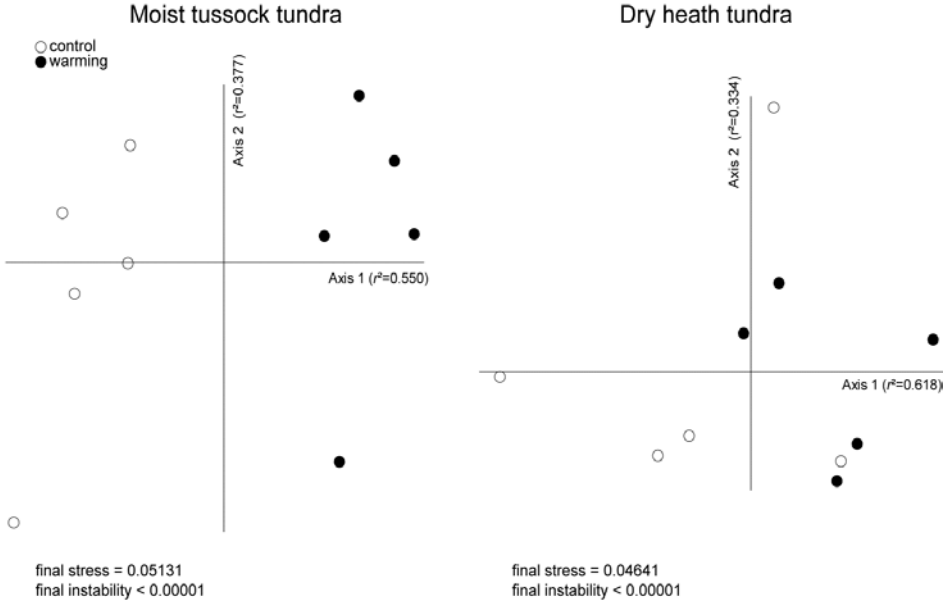


Figure S3.3. Non-metric multidimensional scaling (NMDS) ordination plots for fungal communities from the warmed and control plots in the dry and moist tundra types based on presence-absence.

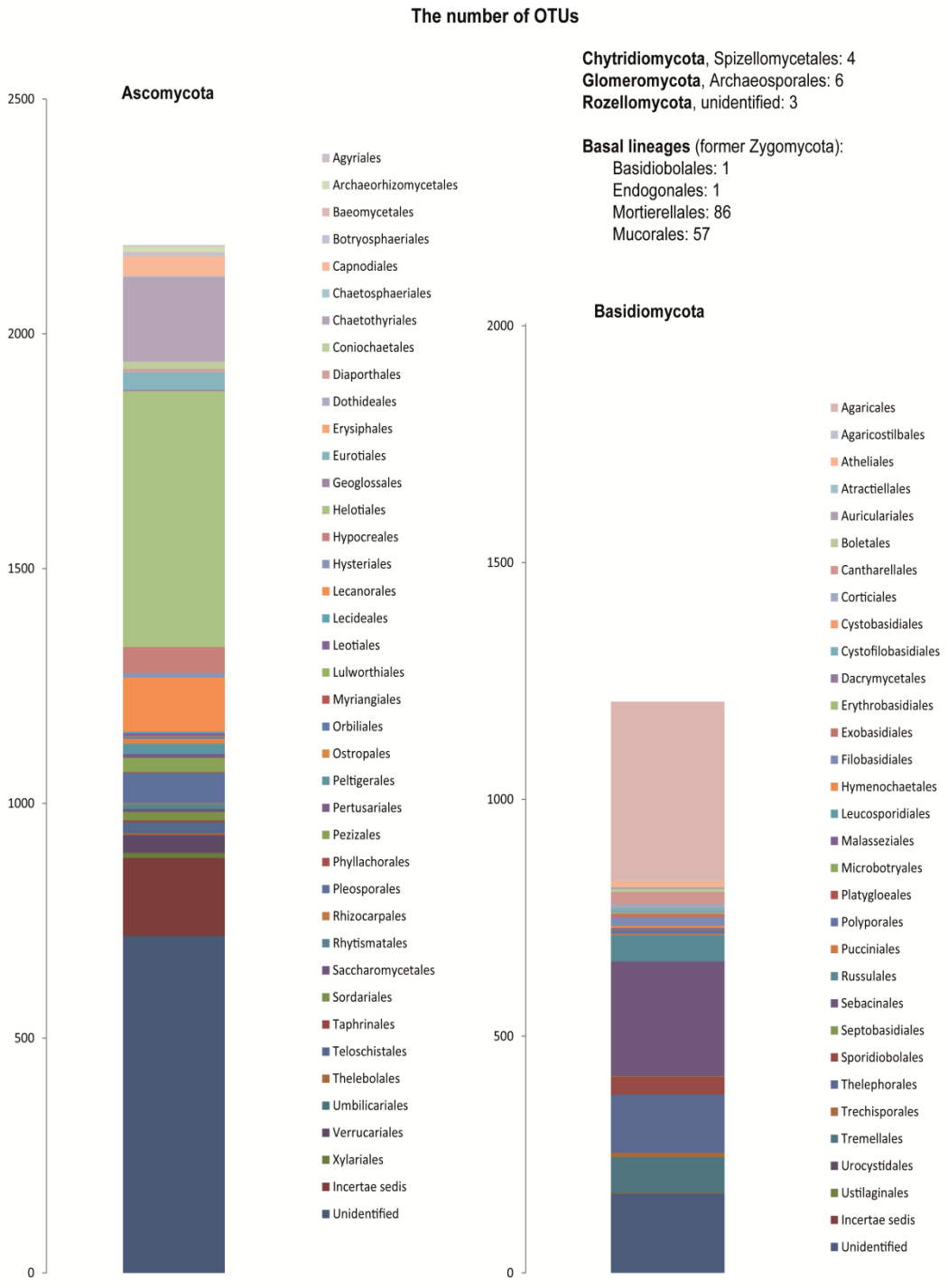


Figure S3.2. The proportional distribution of the 3554 fungal 97% ITS sequence similarity OTUs identified to taxonomic phyla (of 5438 fungal OTUs). Assignment to phyla and taxonomic orders was based on closely related sequences found in the UNITE database.

Table S3.1. OTUs considered as significant indicators of the treatment types with corresponding p-values, and with accession numbers, sequence similarity, pairwise alignment length, name, and taxonomic classification of the most similar sequence in the UNITE+INSD database. Only OTUs with at least 97% ITS2 sequence similarity to a closely related sequence are shown. When multiple OTUs matched the same sequence and were indicators of the same treatment type, only the OTU with the highest sequence similarity to the reference sequence is shown. Where available, the Species Hypothesis (SH) numbers are given for the corresponding sequence as published by Köljalg et al. (2013). Abbreviations for treatments are M=moist tundra, D=dry tundra, C=control, T=warming.

OTU	Treatment	*p*	Accession	%	bp	Name	SH	Order	Phylum
5606	MC	0.0454	HQ446078	98.2	276	*Acephala* sp.	SH234174.06FU	Helotiales	Ascomycota
3355	MC	0.0314	AY204589	98.5	273	*Alatospora acuminata*	SH207334.06FU	Leotiales	Ascomycota
846	MC	0.008	JX984742	99.7	290	*Aureobasidium pullulans*	SH053566.06FU	Dothideales	Ascomycota
1089	MC	0.0204	DQ001277	97.7	299	*Cladonia macroceras*	SH215969.06FU	Lecanorales	Ascomycota
3493	MC	0.025	GU082964	98.5	262	*Cladophialophora* sp.	SH228315.06FU	Chaetothyriales	Ascomycota
201	MC	0.008	EF433981	98.3	350	*Clavaria acuta*	SH262546.06FU	Agaricales	Basidiomycota
281	MC	0.008	EF434000	97.7	258	*Clavaria* sp.	SH201752.06FU	Agaricales	Basidiomycota
43	MC	0.008	EF434027	100	315	Clavariaceae sp.	SH218632.06FU	Agaricales	Basidiomycota
122	MC	0.0456	EF434098	97.7	259	Clavariaceae sp.	SH201751.06FU	Agaricales	Basidiomycota
853	MC	0.008	HQ211947	99.4	178	Clavariaceae sp.	SH203501.06FU	Agaricales	Basidiomycota
3000	MC	0.0456	GU234153	99.6	274	*Coprinus* sp.	SH229041.06FU	Agaricales	Basidiomycota
1229	MC	0.048	GU174325	98.9	280	Dermataceae sp.	SH286768.06FU	Helotiales	Ascomycota
798	MC	0.0456	HQ211766	99.3	285	Dothideomycetes sp.	SH239019.06FU	Capnodiales	Ascomycota
2837	MC	0.008	EF434102	97.3	264	Dothideomycetes sp.	SH227703.06FU		Ascomycota
1676	MC	0.0456	AM901816	99.2	245	*Dothiorella* sp.	SH195688.06FU	Botryosphaeriales	Ascomycota
602	MC	0.0456	GU083075	97.8	277	ectomycorrhizal fungus	SH209187.06FU	Helotiales	Ascomycota
3427	MC	0.035	GU998263	98.6	280	ectomycorrhizal fungus	SH208255.06FU	Helotiales	Ascomycota
634	MC	0.0456	AY112924	99.4	330	ericoid mycorrhizal fungus	SH113701.05FU	Sebacinales	Basidiomycota

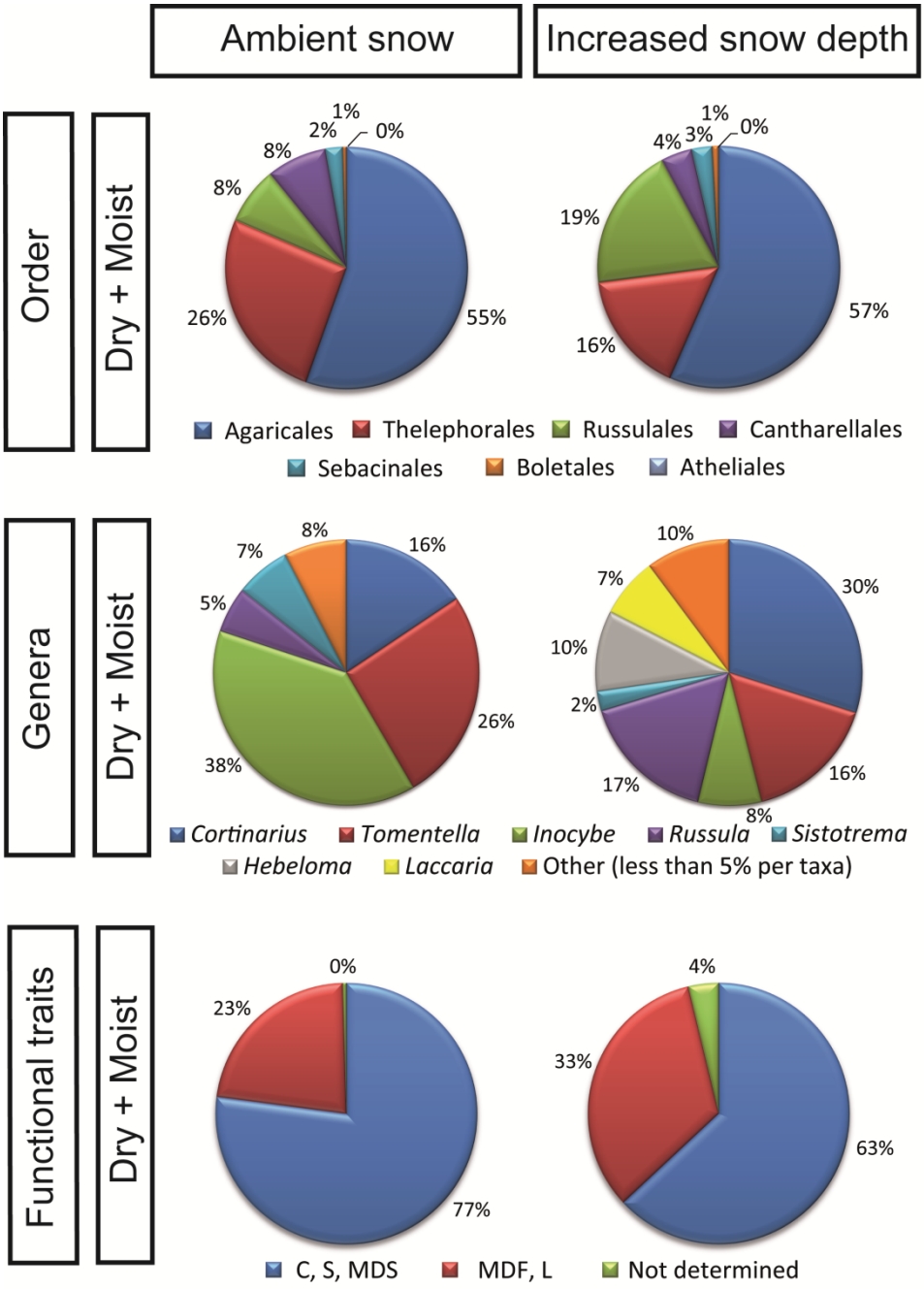
3453	MC	0.0456	EF030927	97.7	216	ericoid mycorrhizal fungus	SH101765.05FU	Sebacinales	Basidiomycota
1058	MC	0.008	JQ420951	99.1	340	ericoid mycorrhizal fungus	SH265789.06FU	Sebacinales	Basidiomycota
500	MC	0.008	UDB017969	98.2	221	*Hebeloma radicosum*	SH039561.06FU	Agaricales	Basidiomycota
1088	MC	0.008	HQ211525	97.8	277	Helotiaceae	SH209578.06FU	Helotiales	Ascomycota
1257	MC	0.0456	EU292248	98.9	279	Helotiales sp.	SH275085.06FU	Helotiales	Ascomycota
357	MC	0.008	FJ475785	99.3	280	Helotiales sp.	SH195751.06FU	Helotiales	Ascomycota
934	MC	0.0238	HQ212298	97.8	276	Helotiales sp.	SH241585.06FU	Helotiales	Ascomycota
64	MC	0.0166	HQ211772	98.8	162	Hyaloscyphaceae sp.	SH195749.06FU	Helotiales	Ascomycota
125	MC	0.025	HQ212020	99.3	279	Lecanoromycetes sp.	SH198710.06FU		Ascomycota
30	MC	0.008	JN889819	98.5	201	Lecanoromycetes sp.	SH197703.06FU		Ascomycota
375	MC	0.008	EF433993	98.1	257	Leotiomycetes sp.	SH217860.06FU		Ascomycota
2446	MC	0.0466	FJ552958	97.8	268	*Leptosphaeria doliolum*	SH228249.06FU	Pleosporales	Ascomycota
1260	MC	0.0152	DQ112559	99.4	325	*Lycoperdon utriforme*	SH244710.06FU	Agaricales	Basidiomycota
3396	MC	0.0372	KC152885	100	256	*Malassezia restricta*	SH206219.06FU	Malasseziales	Basidiomycota
4316	MC	0.0304	EF434060	97.1	272	*Meliniomyces bicolor*	SH161266.06FU	Helotiales	Ascomycota
1259	MC	0.0456	JN655597	97.4	191	*Meliniomyces sp.*	SH207177.06FU	Helotiales	Ascomycota
2702	MC	0.008	GQ219843	97.6	255	*Mortierella antarctica*	SH211068.06FU	Mortierellales	Zygomycota
374	MC	0.0248	HQ211914	99.6	281	Pezizomycotina sp.	SH213006.06FU		Ascomycota
2690	MC	0.008	GU909828	100	211	*Preussia* sp.	SH190471.06FU	Pleosporales	Ascomycota
865	MC	0.0164	JQ420956	100	327	root endophyte	SH265824.06FU	Sebacinales	Basidiomycota
1232	MC	0.0456	JQ420999	99.1	339	root endophyte	SH167198.06FU	Sebacinales	Basidiomycota
1105	MC	0.008	JQ421000	97.4	342	root endophyte	SH285151.06FU	Sebacinales	Basidiomycota
1120	MC	0.008	UDB015975	100	333	*Russula renidens*	SH025103.06FU	Russulales	Basidiomycota
3392	MC	0.0456	FJ827241	97.8	321	*Sebacina* sp.	SH231852.06FU	Sebacinales	Basidiomycota
126	MC	0.0486	HQ211970	99.7	309	Sebacinaceae sp.	SH214727.06FU	Sebacinales	Basidiomycota

3483	MC	0.0452	EF433989	99.7	310	Sebacinales Group B	SH265810.06FU	Sebacinales	Basidiomycota
1231	MC	0.0142	FJ475668	99.6	282	*Sporormiella* sp.	SH190484.06FU	Pleosporales	Ascomycota
2036	MC	0.0162	GU817126	99.6	279	*Tetracladium furcatum*	SH216427.06FU	Helotiales	Ascomycota
3158	MC	0.0456	AJ893339	100	219	Thelephoraceae sp.	-	Thelephorales	Basidiomycota
4645	MC	0.0456	EU645643	98.9	180	Thelephoraceae sp.	SH108158.05FU	Thelephorales	Basidiomycota
3351	MC	0.0456	JN198080	97.8	324	Thelephoraceae sp.	SH202475.06FU	Thelephorales	Basidiomycota
1124	MC	0.0352	HQ433170	99.6	265	*Tomentella* sp.	-	Thelephorales	Basidiomycota
181	MC	0.008	DQ974780	98.1	268	*Tomentella* sp.	SH220174.06FU	Thelephorales	Basidiomycota
188	MC	0.041	JF304353	100	300	*Tomentella* sp.	SH202711.06FU	Thelephorales	Basidiomycota
81	MC	0.0456	HQ215815	100	300	*Tomentella* sp.	SH202533.06FU	Thelephorales	Basidiomycota
484	MC	0.0456	JX630588	99.6	284	*Tomentella* sp.	SH220001.06FU	Thelephorales	Basidiomycota
1009	MT	0.043	AY249072	98.9	275	*Cadophora melinii*	SH267533.06FU	Helotiales	Ascomycota
2178	MT	0.043	JQ312919	100	197	*Calcarisporium arbuscula*	SH205605.06FU	Hypocreales	Ascomycota
270	MT	0.008	FR773398	99.5	219	Capnodiales	SH235804.06FU	Capnodiales	Ascomycota
3674	MT	0.008	EU035413	99.6	262	*Cladophialophora* sp.	SH228303.06FU	Chaetothyriales	Ascomycota
3749	MT	0.0142	HQ211817	98.9	264	*Cryptosporiopsis brunnea*	SH108598.06FU	Helotiales	Ascomycota
2925	MT	0.008	JN032540	98.8	259	Cystofilobasidiales sp.	SH198310.06FU	Cystofilobasidiales	Basidiomycota
1323	MT	0.0212	FJ213516	100	281	*Davidiella tassiana*	SH093668.06FU	Capnodiales	Ascomycota
3375	MT	0.0166	AM260897	99.2	266	Dothideomycetes sp.	SH196053.06FU		Ascomycota
3458	MT	0.0228	FN298754	97.3	258	ectomycorrhizal fungus	SH236313.06FU	Chaetothyriales	Ascomycota
2099	MT	0.043	HQ211764	97.8	185	ectomycorrhizal fungus	SH207207.06FU	Helotiales	Ascomycota
2415	MT	0.016	FJ896135	98.5	333	*Exobasidium arescens*	SH236085.06FU	Exobasidiales	Basidiomycota
211	MT	0.036	HQ211632	99.4	329	*Exobasidium woronichinii*	SH204830.06FU	Exobasidiales	Basidiomycota
337	MT	0.0396	HQ211516	98.6	277	Helotiales sp.	SH215692.06FU	Helotiales	Ascomycota

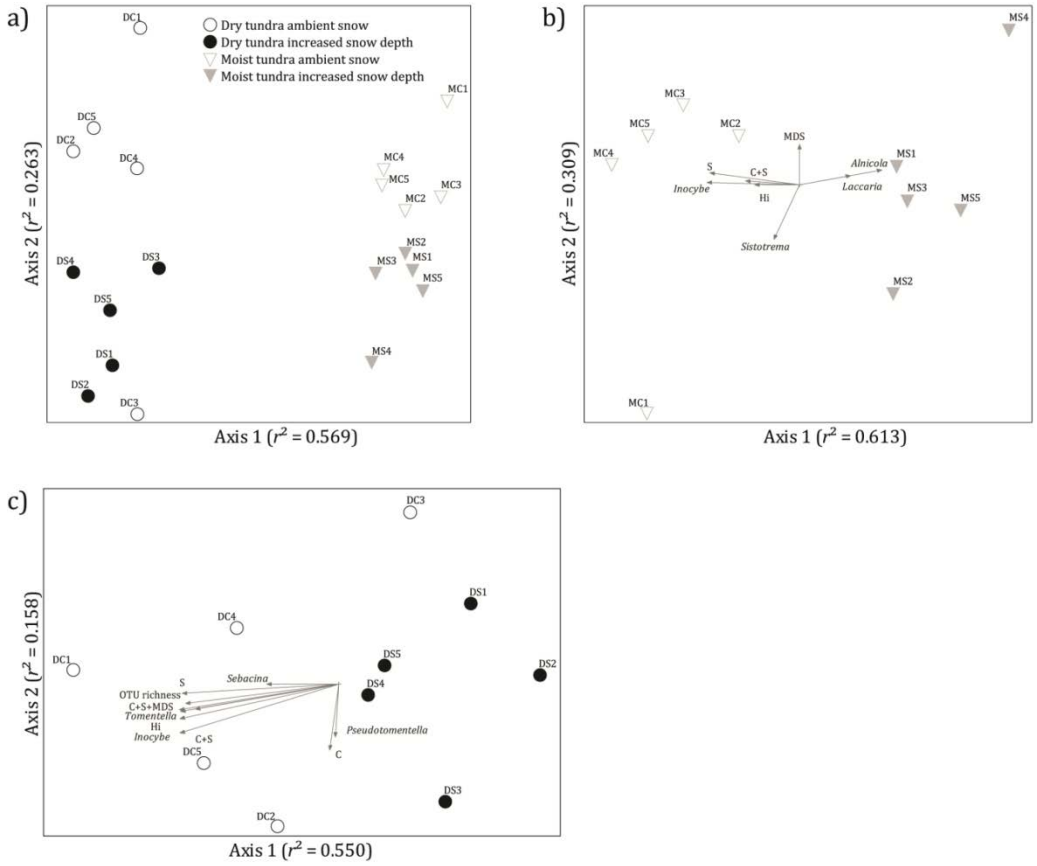
5447	MT	0.018	HQ211644	97.8	277	Hyaloscyphaceae sp.	SH189791.06FU	Helotiales	Ascomycota
1060	MT	0.043	HE605234	99.7	293	*Isaria* sp.	SH196291.06FU	Hypocreales	Ascomycota
1174	MT	0.008	HQ211903	99.6	256	*Lachnum* sp.	SH189825.06FU	Helotiales	Ascomycota
2592	MT	0.018	HQ212064	98.7	229	*Lachnum* sp.	SH189775.06FU	Helotiales	Ascomycota
5657	MT	0.0426	JQ347180	97.1	210	*Lachnum* sp.	SH189775.06FU	Helotiales	Ascomycota
1073	MT	0.008	FJ490755	99.3	293	*Lecanicillium* sp.	SH196376.06FU	Hypocreales	Ascomycota
88	MT	0.0258	JQ346845	99.3	281	*Leptodontidium* sp.	SH236035.06FU		Ascomycota
2082	MT	0.0444	HQ211729	97.4	191	*Meliniomyces bicolor*	SH207165.06FU	Helotiales	Ascomycota
2438	MT	0.043	AJ608979	98.2	283	*Mortierella hyalina*	SH214833.06FU	Mortierellales	Zygomycota
3537	MT	0.008	AB521986	99.1	234	*Mortierella* sp.	SH233133.06FU	Mortierellales	Zygomycota
1191	MT	0.0148	HM136654	100	240	*Mortierella* sp.	SH218432.06FU	Mortierellales	Zygomycota
378	MT	0.008	JQ272348	99.2	257	*Mortierella* sp.	SH233132.06FU	Mortierellales	Zygomycota
785	MT	0.043	KC773828	99.3	297	*Penicillium angulare*	SH213212.06FU	Eurotiales	Ascomycota
768	MT	0.008	HQ211540	99.6	282	Pezizomycotina sp.	-		Ascomycota
890	MT	0.0432	HM044625	98.9	278	*Phacidium lacerum*	SH108595.06FU	Helotiales	Ascomycota
247	MT	0.008	HF947841	99.1	211	*Phialocephala fortinii*	SH113844.06FU	Helotiales	Ascomycota
3435	MT	0.0386	AB378555	99.5	222	*Pochonia bulbillosa*	SH293054.06FU	Hypocreales	Ascomycota
1778	MT	0.0456	FJ948141	99.6	277	*Pseudocercosporella* sp.	SH195092.06FU	Capnodiales	Ascomycota
2153	MT	0.0212	JQ666376	97.1	174	*Pseudogymnoascus roseus*	SH236509.06FU	*Incertae sedis*	Ascomycota
1275	MT	0.015	HQ115661	97.1	279	*Pseudogymnoascus roseus*	SH236509.06FU	*Incertae sedis*	Ascomycota
46	MT	0.0222	JX131373	98.2	169	*Pseudogymnoascus roseus*	SH236509.06FU	*Incertae sedis*	Ascomycota
161	MT	0.0236	HQ211674	97.1	174	*Pseudogymnoascus roseus*	SH236509.06FU	*Incertae sedis*	Ascomycota
6044	MT	0.008	EU019299	97.1	276	*Trimmatostroma betulinum*	SH193248.06FU	Helotiales	Ascomycota
1322	MT	0.0284	JF440625	99.7	329	*Umbelopsis isabellina*	SH056344.06FU	Mucorales	Zygomycota
6121	MT	0.0364	HQ211801	98.8	347	*Umbelopsis* sp.	SH205590.06FU	Mucorales	Zygomycota

328	MT	0.0212	EF434088	98.8	327	*Umbelopsis* sp.	SH205589.06FU	Mucorales	Zygomycota
1219	MT	0.043	FJ386891	99.3	293	unidentified fungus	-		
3683	MT	0.0168	JQ666464	98.3	292	unidentified fungus	SH195737.06FU		
1227	DC	0.0214	HQ212085	99.2	258	*Archaeorhizomyces* sp.	SH198360.06FU	Archaeorhizomycetales	Ascomycota
3372	DC	0.035	FJ475667	99.6	262	Dothideomycetes sp.	SH207413.06FU		Ascomycota
2436	DC	0.0422	HQ211576	99.3	293	Dothideomycetes sp.	SH231472.06FU		Ascomycota
1294	DC	0.0442	HQ625478	99.1	319	ectomycorrhizal fungus	SH220993.06FU	Agaricales	Basidiomycota
572	DC	0.0342	DQ497936	99.3	296	ectomycorrhizal fungus	SH305874.06FU	Chaetothyriales	Ascomycota
1253	DC	0.0422	UDB014235	99.7	326	*Entoloma cetratum*	SH203333.06FU	Agaricales	Basidiomycota
1281	DC	0.0136	FM202814	97.9	242	*Tomentella* sp.	SH112690.05FU	Thelephorales	Basidiomycota
4022	DC	0.0442	JF519380	96.8	277	*Trechispora* sp.	SH203290.06FU	Trechisporales	Basidiomycota
1301	DT	0.0068	JQ759462	99.6	285	Amphisphaeriaceae sp.	SH210430.06FU	Xylariales	Ascomycota
846	DT	0.021	JX984742	99.7	290	*Aureobasidium pullulans*	SH053566.06FU	Dothideales	Ascomycota
3497	DT	0.0068	FJ554074	98.1	267	*Bullera* sp.	SH237040.06FU	Tremellales	Basidiomycota
1093	DT	0.026	HQ260104	97.3	291	*Capronia* sp.	SH227237.06FU	Chaetothyriales	Ascomycota
1323	DT	0.0068	FJ213516	100	281	*Davidiella tassiana*	SH093668.06FU	Capnodiales	Ascomycota
3194	DT	0.0068	EU725679	97.3	257	*Davidiella tassiana*	SH253764.06FU	Capnodiales	Ascomycota
602	DT	0.0068	GU083075	97.8	277	ectomycorrhizal fungus	SH209187.06FU	Helotiales	Ascomycota
1335	DT	0.041	GU083029	98.3	179	ectomycorrhizal fungus	SH108616.06FU	Helotiales	Ascomycota
4782	DT	0.0436	GU083075	97.1	170	ectomycorrhizal fungus	SH209187.06FU	Helotiales	Ascomycota
211	DT	0.0412	HQ211632	99.4	329	*Exobasidium woronichinii*	SH204830.06FU	Exobasidiales	Basidiomycota
3586	DT	0.0476	FN392312	100	199	fungal endophyte	SH232493.06FU	Helotiales	Ascomycota
248	DT	0.0154	HQ211781	99.6	263	fungal endophyte	SH207406.06FU	Venturiales	Ascomycota
1073	DT	0.0264	FJ490755	99.3	293	*Lecanicillium* sp.	SH196376.06FU	Hypocreales	Ascomycota

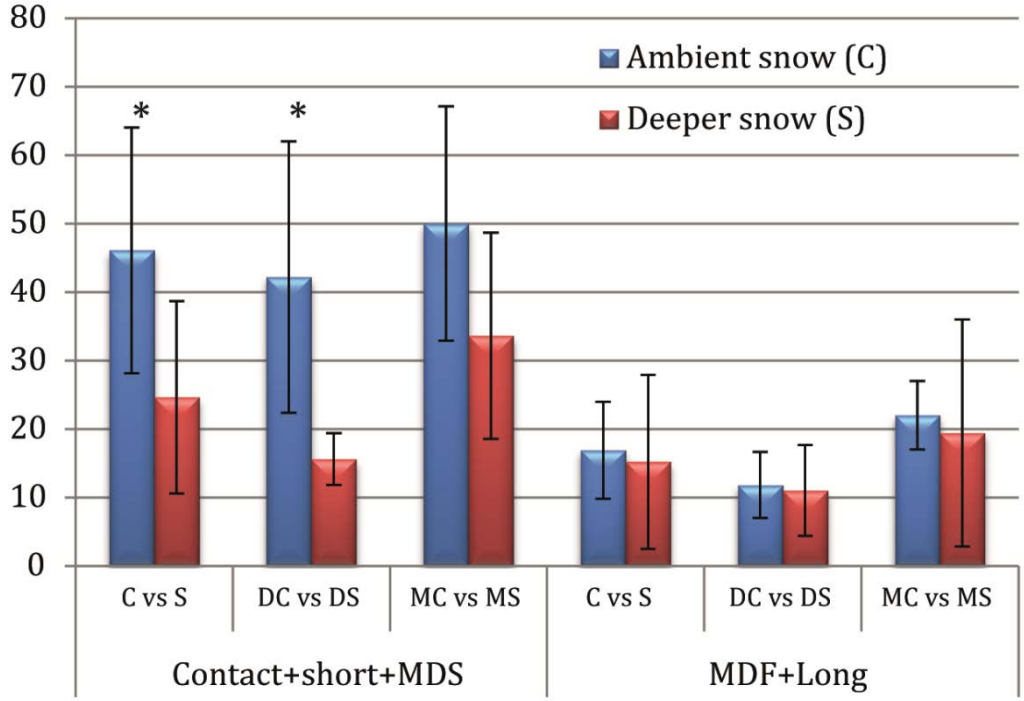
5392	DT	0.027	HQ446010	97.5	279	Lecanoromycetes sp.	SH198715.06FU		Ascomycota
3702	DT	0.027	HQ021932	98.5	267	*Meliniomyces bicolor*	SH207295.06FU	Helotiales	Ascomycota
6621	DT	0.0412	JQ346979	97.8	185	*Meliniomyces* sp.	SH207172.06FU	Helotiales	Ascomycota
2702	DT	0.0068	GQ219843	97.6	255	*Mortierella antarctica*	SH211068.06FU	Mortierellales	Zygomycota
3460	DT	0.0476	HM044656	97.7	353	*Mortierella* sp.	SH217238.06FU	Mortierellales	Zygomycota
1239	DT	0.0068	JX984721	97.5	201	*Pseudogymnoascus roseus*	SH321318.06FU	*Incertae sedis*	Ascomycota
2153	DT	0.0462	JQ666376	97.1	174	*Pseudogymnoascus roseus*	SH236509.06FU	*Incertae sedis*	Ascomycota
1868	DT	0.02	FJ235993	99.6	244	*Rhodotorula* sp.	SH227569.06FU	Sporidiobolales	Basidiomycota
132	DT	0.0352	GQ924001	98.5	274	root endophyte	SH194401.06FU	Helotiales	Ascomycota
1212	DT	0.0372	HQ446017	99.3	285	*Trapeliopsis* sp.	SH223082.06FU	Agyriales	Ascomycota
1911	DT	0.0458	JX984720	100	234	Tremellomycetes sp.	SH230598.06FU		Basidiomycota
4883	DT	0.037	GU307354	98.2	170	unidentified fungus	SH106459.05FU		
2409	DT	0.0154	KC588625	99.4	176	unidentified fungus	-		
5197	DT	0.02	EU035446	97.8	275	*Venturia alpina*	SH238439.06FU	Pleosporales	Ascomycota



S4.1. Total ECM fungal sequence counts, classified by taxonomic and functional traits, comparing ambient snow with increased snow depth plots. The legend for each pair of graphics is organized by colors disposed in clockwise. Abbreviations are C: contact, S: short-distance, MDS: medium-distance smooth, MDF: medium-distance fringe, L: long-distance



S4.2. Non-metric multidimensional scaling (NMDS) ordination plots of basidiomycete ECM fungal communities from the ambient and increased snow depth plots based on OTU presence-absence primary matrix, and taxonomic and extramatrical mycelium groups in: a) the whole community (dry and moist tundra) with final stress and instability of 0.1207 < 0.00001, respectively, axis 1: r2 = 0.569, axis 2: r2 = 0.263, total r2 = 0.832, orthogonality = 98.2%. MRPP analysis: p = 0.0000027, A= 0.1202. b) The moist tundra with a final stress and instability of 0.0621 and < 0.00001, respectively, axis 1: r2 = 0.613, axis 2: r2 = 0.309, total r2 = 0.922, orthogonality = 95.4%. MRPP analysis: p = 0.0017, A = 0.1254. c) The dry tundra, final stress and instability of 0.1239 and < 0.00001, respectively, axis 1: r2 = 0.550, axis 2: r2 = 0.158, total r2 = 0.708, orthogonality = 96.3%. MRPP analysis: p = 0.0191, A= 0.04425. Vectors with |r|≥ 0.5 are represented on the ordination plot. Abbreviations are C= contact hyphal exploration type (ET), S = short-distance ET, MDS = medium-distance ET, Hi: hydrophilic hyphae.



S4.3. Average OTU richness per ECM fungi hyphal exploration groups in dry and moist tundra types of the communities with ambient (left) and increased snow depth (right). Abbreviations are C: ambient snow, S: increased snow depth, DC: dry tundra ambient snow, DS: dry tundra increased snow depth, MC: moist tundra ambient snow, MS: moist tundra increased snow depth, MDS: medium-distance smooth, MDF: medium-distance fringe. *Significant treatment effect (α = 0.05).

S4.4. Indicator OTUs (resultant from indicator species analysis, $\alpha = 0.05$) per treatment (correlated site), with classification, similarity and origin of the reference sequence. Legend – Dry heath tundra with ambient snow, MC: Moist acidic tussock tundra with ambient snow, MS: Moist acidic tundra with increased snow depth.

OTU	Correlated site	Kõljalg *et al.* (2013) and UNITE classification	Similarity (%)	Best match sequence origin
473	DC	*Tomentella* sp. (JX630707)	97.4	Happy Valley, AK
6290	DC	*Tomentella* sp. (UDB018363)	92.4	North India
6579	DC	*Tomentella* sp. (JX630707)	95.3	Happy Valley, AK
6686	DC	*Tomentella* sp. (FJ581421)	95.6	China: southwestern alpine meadow
8011	DC	SH108139.05FU *Tomentella* sp. (HQ211689)	93.8	Toolik Lake, AK
6073	MC	SH106684.05FU *Tomentella* sp. (EF218830)	96.5	British Columbia Interior Cedar Hemlock Forest, Canada
1991	MC	*Tomentella* sp. (JQ347212)	97.8	Subalpine medow, China
55	MC	*Tomentella* sp. (JX630431)	97.5	Thule, Greenland
12782	MC	*Tomentella* sp. 33E (FN687652)	95.2	Mid alpine environment, Sweden
1354	MC	*Tomentella* sp. (JX630589)	100	Prince Patrick Island, Canada
12656	MC	SH103086.05FU *Tomentella badia* (JQ711987)	93.6	BC, Canada
251	MC	SH108158.05FU *Tomentella* sp. (JF304372)	97.6	North America Arctic Transect
390	MC	SH166458.05FU *Cortinarius huronensis* (UDB015917)	100	Kilingi-Nõmme, Estonia
80	MC	SH105172.05FU *Cortinarius* cf. *flos-paludis* (FJ039560)	98.1	Canada
1030	MC	SH099601.05FU *Inocybe leiocephala* (AM882793)	99.2	Sweden
872	MC	SH111588.05FU *Inocybe nitidiuscula* (HQ604382)	95.4	BC, Canada?
301	MC	SH102330.05FU *Russula renidens* (UDB011117)	99.1	Kilpisjärvi, Finland
828	MC	SH164699.05FU *Lactarius torminosus* (UDB011509)	99.4	Estonia
10373	MS	*Laccaria* sp. (JX630414)	97.9	Ellef Ringnes Island, Canada
328	MS	*Inocybe* sp. (JX630878)	96.8	Baffin Island, Canada

3465	MS	SH105206.05FU *Cortinarius* sp. 17C (FN687635)	94.3	Sweden
11539	MS	SH101144.05FU *Russula* sp. (HQ212276)	96.9	Toolik Lake, AK
1165	MS	SH101328.05FU *Alnicola* sp. (FJ197860)	98.4	Primary successional glacier foreland soil, Austria
7259	MS	SH101328.05FU *Alnicola* sp. (FJ197860)	96	Primary successional glacier foreland soil, Austria
7802	MS	SH101328.05FU *Alnicola* sp. (FJ197860)	95.9	Primary successional glacier foreland soil, Austria
1058	MS	SH112490.05FU *Tomentella lapida* (JQ724049)	99.6	Natural/naturalized willow site, Sweden

S4.5. Paired correlation of OTU richness and sequence abundance (for 5 replicates) between the genera with higher richness, and between grouped hyphal exploration types (ET). Color code: white: ambient snow plots; grey: increased snow depth plots. Legend – C: contact, S: short-distance ET, MDS: medium-distance smooth ET, MDF: medium-distance fringe ET, L: long-distance ET. In bold lettering significant effect (α = 0.05).

OTU Richness (presence-absence)

Dry heath tundra

	Cortinarius	*Tomentella*	*Inocybe*	*Russula*	C/ S/ MDS	MDF/ L	
Cortinarius	-	0.62	0.63	-0.51	-	-	Ambient snow
Tomentella	0.20	-	**0.91**	-0.74	-	-	
Inocybe	0.62	0.41	-	**-0.94**	-	-	
Russula	-0.24	0.10	-0.46	-	-	-	
C/ S/ MDS	-	-	-	-	-	0.66	
MDF/ L	-	-	-	-	0.12	-	
	Increased snow depth						

Moist acidic tussock tundra

	Cortinarius	*Tomentella*	*Inocybe*	*Russula*	C/ S/ MDS	MDF/ L	
Cortinarius	-	0.69	0.65	**0.89**	-	-	Ambient snow
Tomentella	**0.92**	-	0.40	0.50	-	-	
Inocybe	0.21	0.21	-	0.44	-	-	
Russula	0.80	0.81	0.37	-	-	-	
C/ S/ MDS	-	-	-	-	-	**0.88**	
MDF/ L	-	-	-	-	**0.92**	-	
	Increased snow depth						

Sequence abundance

Dry heath tundra

	Cortinarius	*Tomentella*	*Inocybe*	*Russula*	C/ S/ MDS	MDF/ L	
Cortinarius	-	0.27	0.58	-0.07	-	-	Ambient snow
Tomentella	-0.26	-	0.41	-0.32	-	-	
Inocybe	0.60	-0.04	-	-0.84	-	-	
Russula	-0.22	-0.17	0.43	-	-	-	
C/ S/ MDS	-	-	-	-	-	0.75	
MDF/ L	-	-	-	-	-0.41	-	
	Increased snow depth						

Moist acidic tussock tundra

	Cortinarius	*Tomentella*	*Inocybe*	*Russula*	C/ S/ MDS	MDF/ L	
Cortinarius	-	0.08	0.85	0.61	-	-	Ambient snow
Tomentella	**0.98**	-	0.29	0.61	-	-	
Inocybe	0.17	0.12	-	0.65	-	-	
Russula	**0.89**	**0.90**	0.38	-	-	-	
C/ S/ MDS	-	-	-	-	-	0.01	
MDF/ L	-	-	-	-	0.85	-	
	Increased snow depth						

Arctic Alaska ectomycorrhizal fungal diversity. From top left: *Inocybe flavella, Cortinarius delibutus, Leccinum variicolor, Boletus edulis.* From bottom left: *Russula* aff. *bicolor, Cortinarius* aff. *aurantiobasis.*

English summary

Climate change is expected to be among the major threats to biodiversity influencing ecosystems structure and functioning over the coming decades. Although it is a global phenomenon, there is considerable spatial variation and high-latitude regions are among the most affected areas. In the last decades, average land surface temperatures in the Arctic have increased at rates up to six times higher than the global average increase of 0.017 °C per year. Similarly, precipitation in the Arctic also increased, especially during the cold season when most precipitation falls as snow. State-of-the-art models predict further increases in the 21st century, possibly by more than 50% of the current precipitation, leading to thicker snow cover. In this thesis, the long-term effects of summer increased temperatures and increased winter snow depth in arctic soil fungal community composition in dry heath and moist tussock tundra were addressed using long-term ecological experiments at Toolik Lake, Alaska, with special attention to ectomycorrhizal fungi. Experimental manipulations mimicking predicted changes in temperature and precipitation were achieved through 18 years of *in situ* treatments using open-top chambers (summer warming) and snow fences (increased snow depth). Soil fungal composition was assessed through soil DNA extraction and massive parallel sequencing of rDNA ITS2. The fungal community composition responded strongly to summer warming in the moist tundra, but not in the dry tundra. Although total fungal richness was not significantly affected by warming, there were clear correlations among richness of various ecological and taxonomic groups and long-term warming. Richness of ectomycorrhizal, ericoid mycorrhizal and lichenized fungi generally decreased with warming, while richness of saprotrophic, plant and animal pathogenic, and root endophytic fungi tended to increase in the warmed plots. Regarding the effects of increased winter snow depth, the ectomycorrhizal fungal community composition of the dry tundra had a stronger response than the moist tundra community. In the dry tundra both community composition and richness were significantly altered while in the moist tundra, only community composition was significantly altered while richness was not. Nevertheless, in both tundra types we observed a decrease in richness of taxa adapted to scavenge the soil for labile N forms. On the other hand, richness of species with the ability to scavenge the soil for recalcitrant N forms did not change. Richness of mycorrhizal taxa containing melanin, a long-lived recalcitrant compound, responded with opposite trends to the summer warming and decreased with increased snow depth. Changes in these taxa will likely affect soil C storage and have the potential to feedback to climate changes. Future research should focus on the fungal community composition response to combined effects of long-term summer warming and increased winter snow depth in the different tundra types.

Dutch summary

Klimaatverandering is een van de belangrijkste bedreigingen voor de biodiversiteit vanwege het effect op de structuur van ecosystemen en het functioneren ervan in de komende decennia. Hoewel klimaatwijziging een wereldwijd fenomeen is, is er een grote ruimtelijke variatie in de effecten ervan, waarbij de regio's gelegen op hogere breedtegraden het meest aangetast zijn en ook het meest bedreigd worden. In de laatste tientallen jaren is de gemiddelde oppervlaktetemperatuur op het land in de Arctische regio tot zesmaal hoger dan de wereldwijde gemiddelde jaartoename van 0,017 °C. Terzelfder tijd nam ook de neerslaghoeveelheid toe, meer bepaald in de winter wanneer de neerslag vooral als sneeuw valt. Actuele modellen voorspellen een verdere toename van de neerslag in de 21ste eeuw, mogelijk zelfs met meer dan 50% van de huidige neerslaghoeveelheid, wat bijgevolg zal leiden tot een dikkere sneeuwlaag. In dit proefschrift werden de lange-termijn effecten onderzocht van toenemende temperaturen in de zomer en van dikkere sneeuwtapijten in de winter op de samenstelling van de schimmelgemeenschappen in de bodems van droge heide en natte toendra, gebruikmakend van een langlopend ecologisch experiment in Toolik Lake, Alaska. De aandacht ging hierbij vooral uit naar ectomycorrhiza schimmels. Het onderzoek is gebaseerd op experimenten waarin de voorspelde veranderingen in temperatuur en neerslag in situ werden nagebootst gedurende 18 jaar, gebruikmakend van open-top kamers (voor het meten van de effecten van stijgende temperatuur) en sneeuwhekken (voor het testen van toenemende sneeuwlaagdiepte). De samenstelling van de bodemschimmels werd bepaald via de extractie van bodem DNA en het massale sequensen van rDNA ITS2. De bodemschimmelgemeenschappen in de natte toendra reageerden sterk op de verhoogde temperaturen, maar deze in de droge toendra bleven ongewijzigd. Hoewel de totale rijkdom aan schimmels niet significant gewijzigd werd door de opwarming, werd er een duidelijke correlatie vastgesteld tussen de rijkdom van specifieke ecologische en taxonomische groepen en de opwarming gedurende een langere periode. De rijkdom van ectomycorrhiza, ericoïde mycorrhiza en lichen-schimmels daalde in het algemeen door de toenemende temperatuur, terwijl de rijkdom van saprofytische, plantpathogene en zoöpathogene schimmels en endofytische wortelschimmels steeg in de warmere experimentele plots. Wat betreft de effecten van toenemende sneeuwdiepte in de winter, was er dan weer een sterker effect op de ectomycorrhiza schimmelgemeenschappen van de droge toendra in vergelijking tot de natte toendra. In de droge toendra waren zowel de samenstelling als de rijkdom van de gemeenschappen significant gewijzigd terwijl in de natte toendra enkel de samenstelling was gewijzigd en er geen wijziging was in de soortenrijkdom. Desalniettemin werd in beide toendratypes een afname waargenomen van de diversiteit van taxa die aangepast zijn aan de opname van labiele stikstofverbindingen uit de bodem. De rijkdom aan

soorten die de mogelijkheid hebben om recalcitrante stikstof uit de bodem op te nemen wijzigde echter niet. Bij mycorrhiza taxa die melanine bevatten, een langlevende recalcitrante component, werden tegengestelde trends waargenomen met betrekking tot temperatuurstijging in de zomer, en ze namen af bij toenemende diepte van de sneeuwlaag. Veranderingen in deze taxa zullen wellicht de C-opslagcapaciteit van de bodem beïnvloeden met mogelijke terugkoppelingseffecten op de verandering van het klimaat. Toekomstig onderzoek zou dan ook meer moeten gericht worden op onderzoek naar het gecombineerde effect van stijgende temperatuur in de zomer en toenemende sneeuwdiepte in de winter op de samenstelling van schimmelgemeenschappen, met name in verschillende toendratypes.

Curriculum Vitae

Luis Neves Morgado was born in Lisbon, Portugal, on March 27, 1982. He carried out his licence degree (4.5 years) in biology at Évora University (Portugal). His final project to complete the degree focused on the macrofungal community associated with *Quercus coccifera* and the mushroom poisoning cases in the Alto Alentejo region in collaboration with Évora Hospital. He then obtained a Leonardo da Vinci scholarship to train and study the molecular phylogeny and biogeography of *Entoloma* (Agaricales, Fungi) in the National Herbarium of the Netherlands at Leiden University. Next, he pursued a MSc. in management and conservation of natural resources at Évora University and Lisbon Technical University (Portugal). His final project focused on the traditional use of mushroom species by locals in the Alto Alentejo region. During his MSc. he co-founded a mycological group in an NGO and organized several workshops about mushroom identification and management of mycological resources. After obtaining his MSc. in 2011, he started his PhD. study under the supervision of Dr. József Geml and Prof. Dr. Erik Smets at Leiden University and Naturalis Biodiversity Center. The PhD. project was focused on effects of climate changes on arctic fungal communities. Besides his main research project, he engaged in other research projects related with tropical fungal ecology, fungal molecular phylogeny, evolution and taxonomy. During his PhD. study he did field work in the Netherlands, Alaska (USA), and Sabah (Malaysia). He attended and presented his work in national and international conferences, such as the Mycological Society of America Annual Meeting, the International Mycological Congress, BioSyst.EU, the Netherlands Annual Ecological Meeting, among others. After completing his PhD., he will be appointed as a postdoctoral researcher at the University of Oslo, focusing on the ecology of boreal fungi.

Publications in peer reviewed journals

Published

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Semenova TA, **Morgado LN**, Welker JM, Walker MD, Smets E, Geml J. Compositional and functional shifts in arctic fungal communities in response to long-term experimental snow depth increase. (in preparation).

Oliveira P, Arraino-Castilho R, Vila-Viçosa C, Castro, MR, **Morgado LN**. Discovery of a new cryptic taxon among sporocarp collections of the edible *Amanita ponderosa* (Basidiomycota, Agaricales). *Cryptogamie* (submitted).

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Noordeloos ME & **Morgado LN** (2015). New insights into *Entoloma bloxamii*. *Coolia* **58**: 41-47.

Morgado LN, Martins L, Gonçalves H, Oliveira P (2006). Estudo de intoxicações causadas por ingestão de macrofungos na região do Alto Alentejo. *Anais da Associação Micológica A Pantorra* **6**: 65–74.

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Morgado LN (2015). Phylogenetic overview of the Entolomataceae with insights into biogeographical patterns and character evolution. *XXIII meeting of the European Confederation of Mediterranean Mycology – CEMM*. Fornos de Algodres, Portugal. (Invited speaker).

Geml J, Pastor N, **Morgado LN**, Semenova T, Nouhra ER (2015). Mycota of understudied biodiversity hotspots –DNA metabarcoding reveals hyperdiverse communities and strong habitat partitioning along altitudinal gradients in Borneo and in the Andes. *DNA Barcoding – The gold standard for species recognition.* Utrecht, the Netherlands. (Oral presentation)

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Semenova TA, **Morgado LN**, Welker JM, Walker MD, Smets E, Geml J (2014). Ascomycetous fungal communities respond to experimental warming in the mesic and dry arctic tundra. *The 10th International Mycological Congress*. Bangkok, Thailand. (Oral presentation)

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