Fungi OF THE GREENING ARCTIC

COMPOSITIONAL AND FUNCTIONAL SHIFTS IN RESPONSE TO CLIMATIC CHANGES

TATIANA SEMENOVA-NELSEN

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Fungi of the greening Arctic: compositional and functional shifts in response to climatic changes

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FUNGI OF THE GREENING ARCTIC:
Compositional and Functional Shifts in Response to Climatic Changes

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Chapter 1

GENERAL INTRODUCTION AND THESIS OUTLINE

Arctic tundra is the northernmost terrestrial biome formed ca. 2-3 million years ago. The area is surrounded by the Boreal zone to the South and by the Arctic Ocean to the North. Short summers of ca. 50 days and long cold winters formed this unique biome characterized by low diversity of vascular plants and mostly migrant animals. Low species diversity, specific adaptations of tundra inhabitants to harsh environmental conditions, and the long time required for the biome to return to a steady state following perturbation, contribute to the biome's fragility (Banfield, 1972; Bliss et al, 1973). For example, construction activities related to oil and gas production have had immediate negative effect on polar bear and caribou populations due to disruption of migratory and feeding paths, and disturbance of denning areas (Kaplan, 1996). Nowadays, the fragility of arctic tundra is of serious concern due to on-going climatic changes. Although Arctic biota has been subjected to dramatic environmental changes over the last 2.5 million years, the ongoing warming of the climate is very rapid and the tundra is being increasingly "compressed" between the boreal forest zone and Arctic Ocean (Wookey, 2007). The effect of climatic changes on vulnerable arctic species and habitats implies changes in lower latitude ecosystems as well, through feedback loops and strong climatic connections between meteorological and environmental phenomena that occur a long distance apart (Wookey, 2007; Kug et al, 2015). Arctic tundra is important for the global energy budget: primarily due to its impact on the Earth's albedo (reflective capacity). Secondly, Arctic stores large amounts of the planet's Carbon (C) in the form of frozen organic matter (Tarnocai et al, 2009), which relates to the concentrations of green-house gases in the atmosphere. The importance of arctic climate research also relates to the fact that the Arctic is experiencing higher warming rates compared to temperate regions and, therefore, local changes in Arctic organisms and communities are considered "an early detection system" to predict the impacts of environmental change on the planet Earth (Wookey, 2007).

Fungi play important roles in nutrient-poor terrestrial arctic ecosystems as decomposers and symbionts (Gardes and Dahlberg, 1996; Hobbie et al, 2009;

Newsham et al, 2009). Given that as much as 60-80% of nitrogen (N) is obtained by arctic plants through association with their symbiotic fungi (Hobbie and Hobbie, 2006), and that fungi contribute 10 times more to Arctic soil microbial biomass than cohabitating bacteria (Dahlberg and Bultmann, 2013), the importance of the fungal role in the functioning of the tundra ecosystem is difficult to overestimate. However, our knowledge of arctic fungal diversity remains largely incomplete due to the cryptic nature of the majority of fungal species and the fact that today's knowledge of Arctic fungi relies on a very small number of experienced and skilled mycologists (Dahlberg and Bultmann, 2013). Rapid assessments of fungal community compositions became possible with recent development of next-generation sequencing (NGS) techniques that are based on screening fungal DNA in various environmental samples, including soils. In this thesis, DNA metabarcoding of soil samples was utilized to study the long-term effects of experimental climate manipulations on fungal community compositions in low arctic tundra of Northern Alaska. This introduction provides the general background of the study and briefly describes the major focus of the following Chapters.

Natural and anthropogenic climate warming

Climate on Earth is determined by the balance between energy uptake through sunlight absorption and losses of energy through emission to space. The Earth is absorbing ca. 70% of the sunlight and reflects ca 30%, although in recent years the Earth's albedo is decreasing with subsequent warming of the climate. Climatic changes became recognized internationally in 1988, when the Intergovernmental Panel on Climate Change (IPCC) provided its first report (available in 1990) suggesting strong scientific evidence for human-induced warming of the climate (IPCC, 1990). This warming relates to increases in concentrations of greenhouse gases (GHG, i.e., CO_2, CH_4, N_2O, fluorinated gases and aerosols) in the atmosphere. Because GHG have the potential to absorb infrared radiation of the Earth, they prevent energy emission to space, while short-wave solar radiation passes through the Earth's atmosphere. Warming of the climate, therefore, is due to reduced emissions in the infra-red spectrum coupled with stable energy uptake (through absorption of sunlight). GHG concentrations in the atmosphere have been increasing rapidly since the mid-18th century, likely due to the Industrial Revolution, when humankind started burning fossil fuels (coal, oil and natural gas) to power manufacturing. Even though chemical composition of the atmosphere may vary substantially due to natural factors, most of the warming over the last 50 years has been attributable to human activities (IPCC, 2007; Fig 1.1). Climate

models that take into account natural factors and anthropogenic forces that influence climatic changes, suggest that recent warming very likely results from human-induced increases in atmospheric GHG, rather than being attributed to natural fluctuations (IPCC, 2007; Fig 1.2). For instance, the atmospheric CO_2 concentration reached its maximum over the last 800 000 years in 2015 (Lüthy al, 2008).

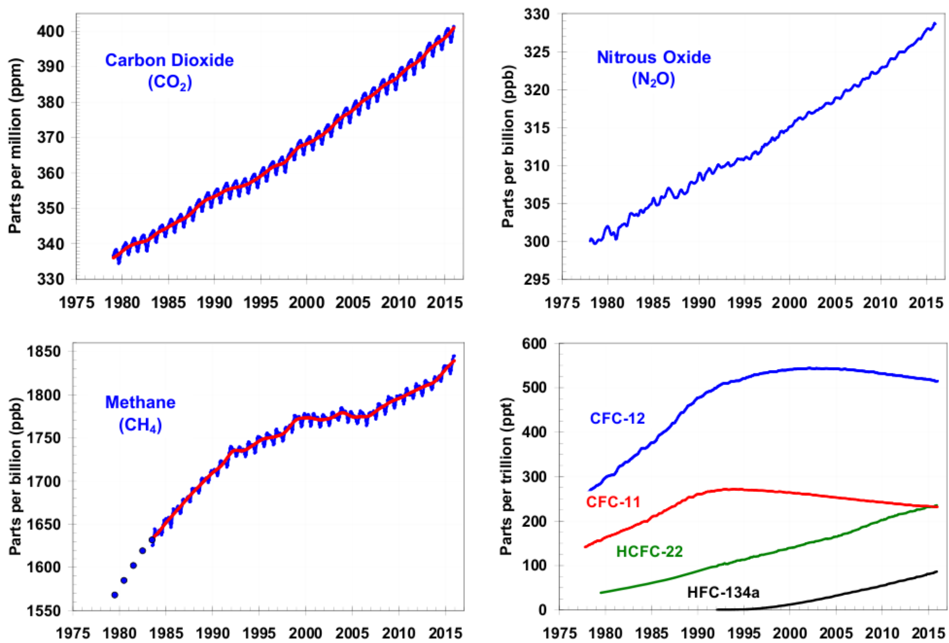

Figure 1.1. *Global average abundances of the major greenhouse gases - carbon dioxide, methane, nitrous oxide, CFC-12 and CFC-11 - from the National Oceanic and Atmospheric Administration (NOAA) global air sampling network are plotted since the beginning of 1979. These five gases account for about 96% of the direct radiative forcing by long-lived greenhouse gases since 1750. The remaining 4% is contributed by an assortment of 15 minor halogenated gases including HCFC-22 and HFC-134a, for which NOAA observations are also shown in the figure. Methane data before 1983 are annual averages from D. Etheridge (Etheridge et al, 1998), adjusted to the NOAA calibration scale (Dlugokencky et al, 2005). Adapted from Butler and Montzka (2016); http://esrl.noaa.gov/.*

The Earth's rising temperature and its consequences

Over the last 150 years, the Earth's surface temperature increased by 0.68°C (http://climate.nasa.gov), and an even higher rate (0.74±0.18 °C) has been reported for the last 100 years due to more pronounced warming in recent decades (Fig 1.3; Peterson et al, 2009; Allison et al, 2009). Measurements derived from sediment,

tree rings, and ice cores all suggest that recent temperatures are largely exceeding those of the past four millennia (Mann and Jones, 2003; Salzer et al, 2014), even though there is a large spatial variation in temperature change across the continents (Fig 1.4). Important consequences of these rising temperatures and increased GHG emissions are the reduction of the extent of Arctic sea ice (Fig 1.5-1.6) and acidification of the oceans (Feely et al, 2004). Subsequently, there is evidence for altered global precipitation patterns (Wilby and Wigley, 2002; Kharin and Zwiers, 2005; Meehl et al, 2005; Barnett et al, 2006).

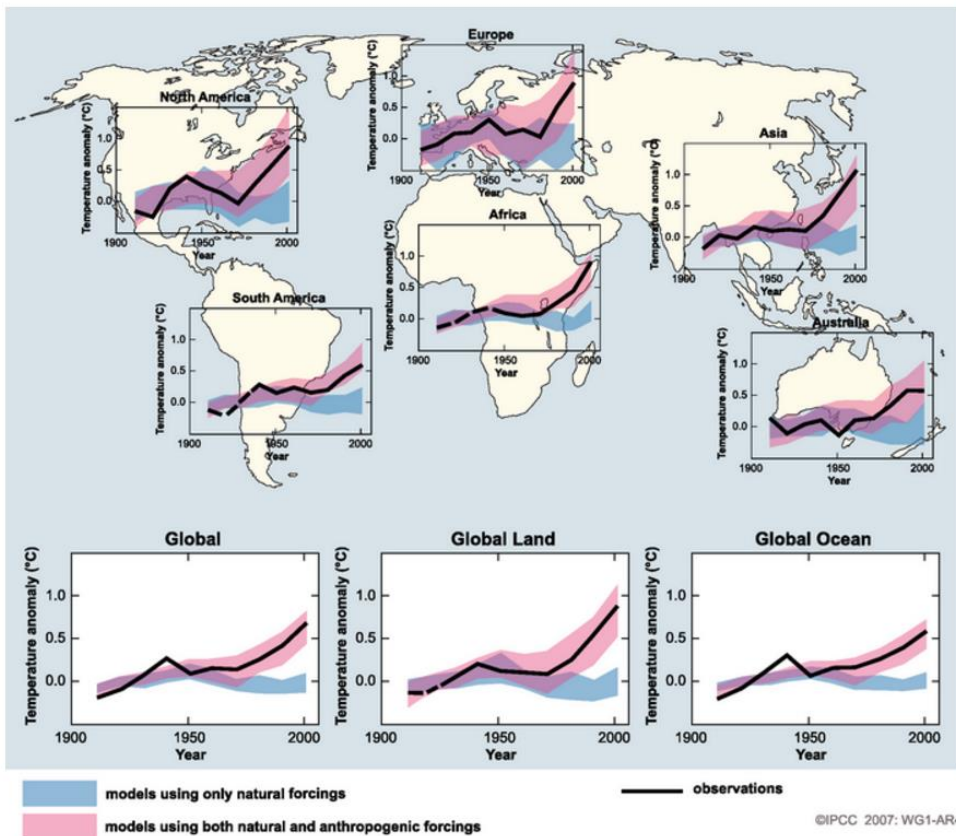

Figure 1.2 *Comparison of observed continental- and global-scale changes in surface temperature with results simulated by climate models using natural and anthropogenic forcings. Decadal averages of observations are shown for the period 1906 to 2005 (black line) plotted against the center of the decade and relative to the corresponding average for 1901–1950. Lines are dashed where spatial coverage is less than 50%. Blue shaded bands show the 5–95% range for 19 simulations from five climate models using only the natural forcings due to solar activity and volcanoes. Red shaded bands show the 5–95% range for 58 simulations from 14 climate models using both natural and anthropogenic forcings. (Adapted from IPCC, 2007).*

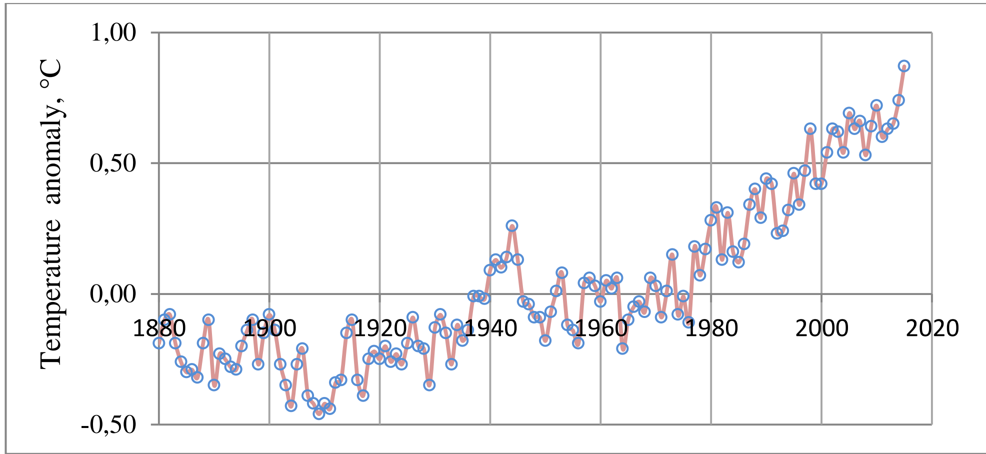

Figure 1.3 *Change in global surface temperature relative to 1951-1980 average temperatures, °C. The 10 warmest years in the 134-year record all have occurred since 2000, with the exception of 1998. The year 2015 ranks as the warmest on record. (Source: NASA's Goddard Institute for Space Studies (GISS).*

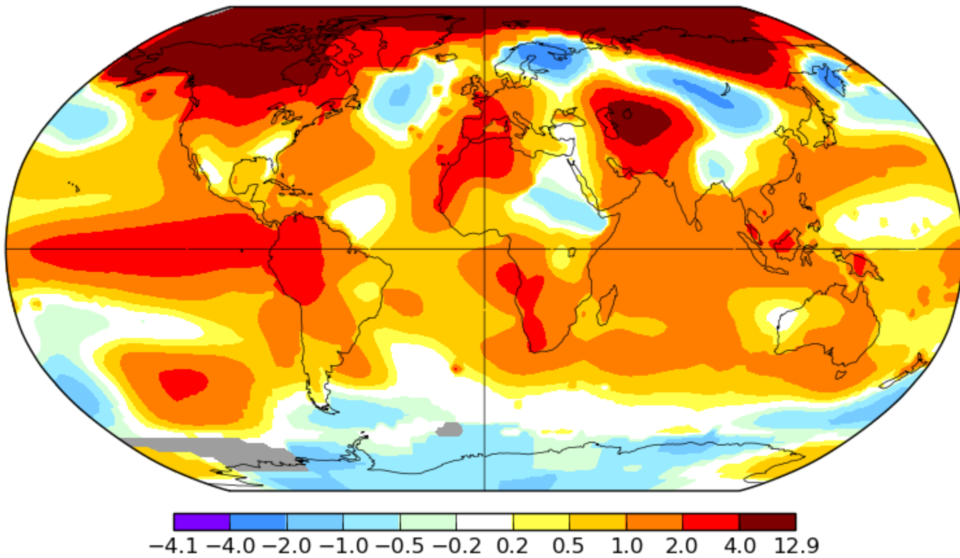

Figure 1.4 *Global temperature anomaly in January 2016 in relation to the period between 1950 and 1980. Overall, the global average temperature was 1.13 °C warmer than the long-term average. Blank areas indicate the regions for which the data was not collected or incomplete (Source: NASA Goddard Institute for Space Studies).*

The warming climate has already altered the structure and dynamics of a broad range of ecosystems, with responses observed in phenology and physiology of organisms, ranges and distributions of species, and compositional and interaction shifts within communities (Walther et al, 2002). For example, these changes involve treeline advancement towards higher altitudes (Kullman, 2001; Meshinev et al, 2000; Wardle et al, 1992; Grace et al, 2002), expansion of shrubs into shrub-free tundra (Sturm et al, 2001), elevational shifts in alpine plants (Grabherr et al, 1994) and changes in the distribution of both Antarctic plants and invertebrates (Kennedy, 1995). In marine ecosystems, increased abundance of warm-water species has been reported (Holbrook et al, 1997; Southward et al, 1995; Alheit and Hagen, 1997). On the level of populations, range shifting, northward and upward, has been reported for butterfly species (Parmesan et al, 1999), bird species (Pounds et al, 1999) and foxes (Hersteinsson and MacDonald, 1992). Documented phenological responses involve earlier flowering and leaf-unfolding in plants (Menzel and Estrella, 2001), earlier appearance of butterflies (Roy and Sparks, 2000) and earlier breeding and spring migration in a variety of animal species (Beebee, 1995; Crick et al, 1997; Brown et al, 1999; Dunn et al, 1999; Inouye et al, 2000). These numerous examples, however, are difficult to summarize into a large-scale projection due to complex interactions between species and populations. Due to heterogeneity in ecological dynamics, future communities will likely reorganize and function differently than those of today (Montoya and Raffaelli, 2010), suggesting a high importance of predictive ecological studies and computer models (Bellard et al, 2012).

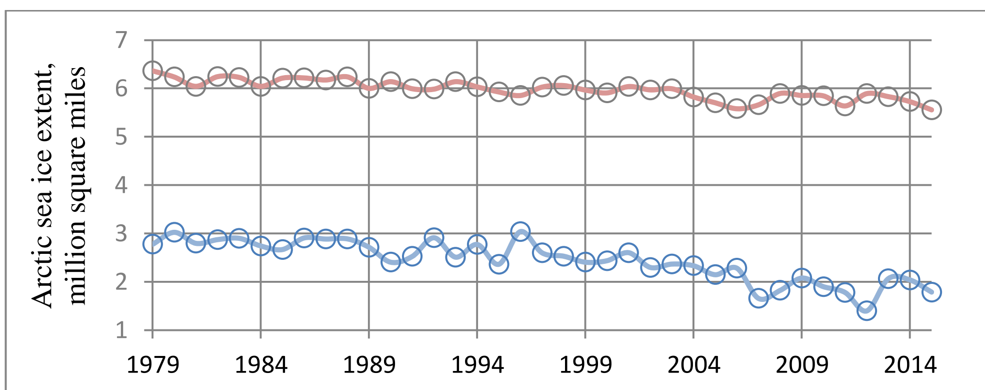

Figure 1.5. *Arctic sea ice extents for the months of March (maximum extent, shown in red) and September (minimum extent, shown in blue) from 1979 to 2015. (Adapted from https://www3.epa.gov)*

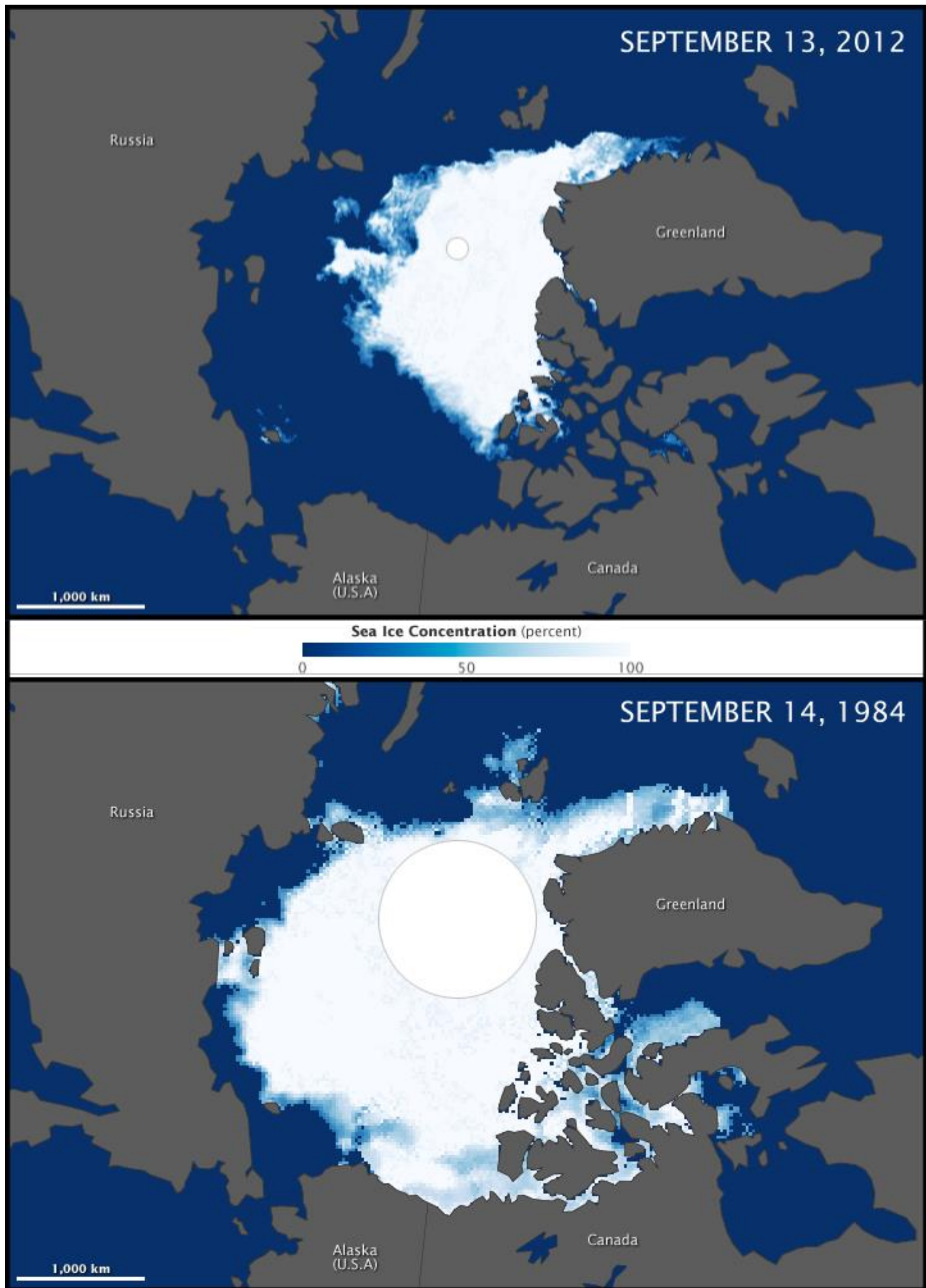

Figure 1.6. *Arctic sea ice extent of September 2012 versus 1984. (Images: NASA)*

Climate change in the Arctic

The rate of climate warming in the Arctic is nearly double the global mean warming rate in recent decades (IPCC, 2007; Richter-Menge and Jeffries, 2011). This phenomenon has been referred as “Arctic amplification” (Sukyoung, 2014) and relates to retreating in sea ice and snow cover, especially during the spring and summer months. Ice and snow retreat, in turn, have subsequent albedo feedbacks (Serreze et al, 2009; Screen and Simmonds, 2010) and alter cloud- and water vapor patterns (Graversen and Wang, 2009). Another local factor that intensifies Arctic warming is the so-called thermal surface inversion, i.e., abnormal increase of air temperatures with altitude. Thermal surface inversion decreases cooling of arctic clear-sky atmosphere in winter and thus contributes to Arctic amplification (Bintaja et al, 2011). Besides the local effects, changes in atmospheric mid-latitude circulation, i.e. enhanced meridional energy transport, contributes to warming of the Arctic (Graversen, 2006; Koenigk et al, 2013). Briefly, over the last 30 years, arctic temperatures have been increasing by ca. 0.6°C-1.0°C per decade (Jeffries et al, 2012), while sea ice extent has decreased in all months and virtually all regions (Ford et al, 2014), overall ice thickness is reduced, and earlier spring ice break-up with corresponding later autumn freeze-up was reported (Comiso et al, 2014).

Rapid warming of the Arctic is expected to strongly impact the global climate. According to estimations, the amount of the Earth’s reactive carbon stored in the form of frozen organic matter in arctic permafrost may approach as much as 50% (Tarnocai et al, 2009). Thawing of the permafrost will, therefore, lead to massive releases of stored carbon, as organic matter will become progressively available for microbial degradation (Anisimov et al, 2007; Comiso and Hall, 2014) and release of CO_2 to the atmosphere. Higher temperatures have already altered nutrient cycling in low Arctic (Schiemel et al, 2004; Pattison and Welker, 2014) and have had profound effect on tundra vegetation, observed as advancement of the treeline into tundra (Kharuk et al, 2013; Zhang et al, 2013) and increases in shrub cover and biomass (Sturm et al, 2005; Tape et al, 2012).

Another important consequence of climate warming is an increase in arctic precipitation (Kattsov and Walsh, 2000; Stocker et al, 2013; Bintaja and Selten, 2014). The models suggest that precipitation increases due to greater moisture inflow from lower latitudes and more intensive surface evaporation of the Arctic ocean (Bintaja and Selten, 2014). With the current levels of global GHG emission, precipitation in the Arctic is projected to increase by 30-60% by the end of the 21st

century (Fig. 1.7; IPCC, 2013). Most of the precipitation will likely fall as snow, because arctic winters may last up to 9 months, which would result in a largely increased snow depth (Kattsov and Walsh, 2000). Deeper snow cover is expected to have multiple consequences for tundra ecosystems, including soil insulation, delayed snowmelt and increased soil moisture in spring (Jones et al, 1998). In addition to higher water content and soil temperatures, deeper snow protects arctic vegetation from frost and wind disturbances, favoring the growth of taller plants over the shade-intolerant lichens and bryophytes (Schimel et al, 2004; Sturm et al, 2005; Welker et al, 2005; Pattison and Welker, 2014).

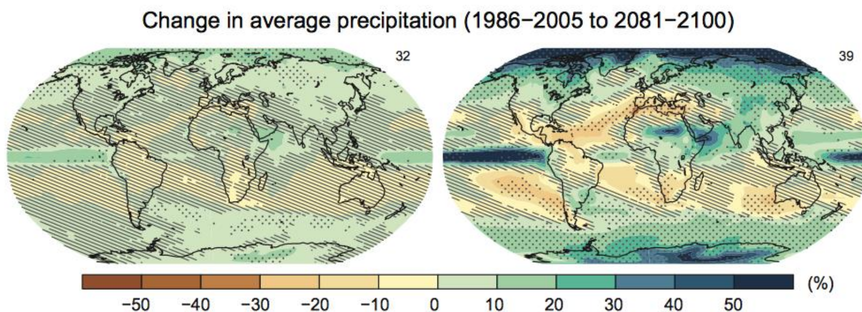

Figure 1.7. *Projected changes in global mean precipitation for the low emission (RCP 2.6, on the left) and high emission (RCP 8.5, on the right) scenarios for the years 2081-2100 in relation to 1986-2005, as revealed by CMIP5 models. The number of models used is indicated in the upper right corner (Adapted from IPCC, 2013).*

To quantify the effects of climate warming on arctic ecosystems, an International Tundra Experiment (ITEX) was established in the 1990s (Henry and Molau, 1997). Initially, the main focus of ITEX was to measure phenological and growth responses of arctic plants to moderate (+1-3°C) near-surface warming by open-top chambers (OTCs) (Wookey, 2007). These experiments revealed strong warming-induced turnover in arctic plant communities, with increase in the biomass of graminoids and deciduous shrubs, higher plant canopy, accumulation of leaf litter (Arft et al, 1999; Hollister et al, 2005; Wahren et al, 2005) and strong declines in lichens and bryophytes (Walker et al, 2006; Cornelissen et al, 2001; Jägerbrand et al, 2009). Another ITEX experiment addressed the effect of increased winter precipitation (ca 0.5 – 2.5 m snow addition) on arctic vegetation using wooden snow fences (Henry and Molau, 1997; Welker et al, 1997, 2000; Jones et al, 1998). Increased snow pack also strongly altered arctic vegetation; plant growth rates and canopy height increased, especially in two deciduous shrubs

– *Betula nana* and *Salix pulchra* and a graminoid *Eriophorum vaginatum* (Mercado-Diaz, 2011). With more intensive growth of leafy biomass, the thickness of leaf litter in autumn increased accordingly (Mercado-Diaz, 2011). Later, ITEX research addressed some of the below-ground processes associated with climatic warming, such as nitrogen (N) cycling (Schimel et al, 2004; Borner et al, 2008; Natali et al, 2012; Schaeffer et al, 2013; Pattison and Welker, 2014), soil water content (Natali et al, 2012), respiration (Chapin et al, 1995; Shaver et al, 1998), and microbial activity (Clemmensen et al, 2006; Campbell et al, 2010; Deslippe et al, 2011, 2012). However, almost no research was carried out on soil fungal communities, despite their well-known role in nutrient acquisition in cold, harsh and nutrient-poor arctic soils (Hobbie and Hobbie, 2006; Buckeridge and Grogan, 2008; Hobbie et al, 2009).

Arctic fungal research

The total richness of arctic fungi is estimated to be as high as 13 000 species, based on the suggested ratio 1:5 – 1:7 between vascular plants and fungi, and known arctic vascular plant diversity of 2,218 species (Hawksworth, 2001; Schmit and Mueller, 2007; Dahlberg and Bültmann, 2013). The number of described fungal species in Arctic approaches only ca. 4500 (Dahlberg and Bültmann, 2013), suggesting that more than 10,000 species may still remain unknown. Lichens account for the large portion of the known diversity (ca. 1750 species). Among the non-lichenized species, fungi of the phylum Ascomycota dominate arctic soils (Geml et al, 2012; Timling et al, 2014). The importance of arctic mycological research involves matters of both scientific and practical value, including numerous adaptations arctic fungi have evolved for growth in cold and dry conditions. For instance, such practical applications involve spoilage of refrigerated foods or production of cold-active enzymes (Margesin and Schinner, 1994), as fungal growth and C uptake continues in arctic soils even when temperatures decrease to -2°C (McMahon et al, 2009).

Specific adaptations of arctic fungi to growing in low temperatures and water potential include increases in concentrations of intracellular trehalose and polyol, and unsaturated lipids in cell membrane, as well as the secretion of antifreeze proteins and production of cold- active metabolites (Robinson, 2001). Another mechanism of stress-tolerance relates to melanin production (Robinson et al, 2001; Fernandez and Koide, 2013). Melanins are the components of fungal cell walls or extracellular matrix, composed of indolic and phenolic monomers complexed with

proteins and carbohydrates (Butler and Day, 1998). Supposedly, melanization contributes to resistance of fungal mycelium to low temperatures, as melanized fungal hyphae are known to predominate in polar soils (Robinson et al, 2001). In addition to altered cell chemistry, many arctic fungi evolved ecological adaptations to cold and dry environment. For example, arctic macrofungi form smaller sporocarps with reduced number of gills as compared to mushrooms of temperate regions; in ascomycetes, the role of sexual reproduction for dispersal is limited and spreading largely relies on asexual spores and mycelial growth (Knudsen, 2006). Due to the shorter growing season, plant pathogenic species are characterized by simplified lifecycles (Savile, 1982; Dahlberg and Bültmann, 2013). However, as climate becomes warmer in the Arctic, fungi with these specific adaptations may be outcompeted by other species better adapted to growth in the altered conditions. Supposedly, this will have consequences for the functioning of arctic ecosystems, due to key roles of fungi as plant symbionts (e.g., mycorrhzae, endophytes, lichens) and decomposers of organic matter (Geml et al, 2015).

The majority of arctic plants depend on their mutualistic fungi for accessing nutrients (Hobbie et al, 2009; Gardes and Dahlberg, 1996; Bjorbækmo et al, 2010). Degradation of dead organic (mostly plant) material predominantly relies on fungi as activity of bacteria is limited in cold arctic soils (Dahlberg and Bültmann, 2013). Mycorrhizal types include ectomycorrhizae (ECM), ericoid-, arbutoid- and arbuscular mycorrhizae (Väre et al, 1992; Michaelson et al, 2008; Newsham et al, 2009) that play key roles in plant nutrient acquisition and water uptake. For example, the ECM fungus *Cortinarius favrei* contributes up to 90% of N to its host plant, while using only 8-16% of the plants net photosynthesis products (Hobbie, 2008). Among ascomycetes, dark-septate endophytes (DSE) are ubiquitous in the roots of arctic plants (Jumpponen and Trappe, 2008; Newsham et al, 2009), although functional relations between the DSEs and their hosts remain largely unknown. The research on arctic root-associated fungi (Väre et al, 1992; Olsson et al, 2004; Kohn and Stasovski, 1990) suggest that DSE may be more frequent in the roots of arctic plants than any form of mycorrhiza (Newsham et al, 2009). High diversity of endophytic fungi has also been observed in above-ground plant parts (Arnold et al, 2009; Higgins et al, 2007), and similar to root endophytes, this community is expected to increase plant stress-tolerance to low moisture and temperatures (Botnen et al, 2014; Rodriguez et al, 2008). Even though current knowledge of arctic endophytic diversity relies on a limited number of publications (e.g., Zhang and Yao, 2015; Blaalid et al, 2014; Bjorbækmo et al, 2010; Botnen et al, 2014; Higgins et al, 2007), it is expected to expand with further development of

NGS techniques. DNA metabarcoding techniques have facilitated arctic fungal research with respect to diversity and biogeography (Geml et al, 2008; Bjorbækmo et al, 2010; Blaalid et al, 2012; Geml et al, 2012; Timling et al, 2012) and complemented traditional sporocarp-based studies. Research focused on fungal ecology and responses to climate change have so far received less attention, except for a few studies on microbial responses to experimental climatic warming on ECM fungi (Clemmensen et al, 2006; Deslippe et al, 2011; Morgado et al, 2015; Morgado et al, 2016). These studies showed strong warming-induced changes in ECM communities (Morgado et al, 2015, 2016) and fungi associated with roots of *Betula nana* (Deslippe et al, 2011). Aside from these studies, our knowledge of compositional and functional shifts in fungal communities in response to climate warming in the Arctic remains rudimentary.

Research aims and outline of this thesis

This thesis is a contribution to a large-scale project aiming to understand how arctic communities respond to long-term experimental changes in climate in dry heath and moist tussock tundra types of Northern Alaska. The field site was at Toolik Lake area, which is a part of a Long-Term Ecological Research (LTER) experiment included in larger International Tundra Experiment (Henry and Molau, 1997; Welker et al, 1997). Toolik Lake is located on the northern foothills of the Brooks Range (68°38´N, 149°36´W, 670 m asl), in low tundra of Arctic Alaska. The area belongs to the warmest zone of arctic tundra - bioclimatic subzone E. This subzone is characterized by mean July temperatures of 9-12°C (Walker et al, 2005), and an annual mean temperature of -7°C. The annual precipitation in the region is ca 200-400 mm, and the majority of it falls as snow. The average snow depth approaches 0.5 m (De Marco et al, 2011).

This work focused on changes in soil fungal community compositions and relative abundance of fungal functional groups under summer warming and increased winter snow depth. Summer temperatures were passively increased by OTCs (Fig. 1.8) that were placed on the same experimental plots for 18 summers, and removed during the winters. OTCs have been widely used for climatic studies in the Arctic (Marion et al, 1997; Sharkhuu et al, 2013; Morgado et al, 2015; Geml et al, 2015) to predict the consequences of warming over the next decades, when temperatures are expected to exceed the current levels by ca. 2-3°C. Increase in snow depth was achieved by the implementation of snow fences (Fig. 1.9), wooden fences ca. 60 m long and 2.8 m tall (Henry and Molau, 1997; Welker et al, 1999; Pattison and

Welker, 2014). Snow fences create a leeward drift of ca 60 m that keeps the soils warmer and protects vegetation from frost disturbances, as well as altering soil nutrient cycling and microbial activity. In all experiments presented in this thesis, soil samples were collected from the control and the treatment plots with a soil corer (20 cm long and 2 cm in diameter). Soils were frozen until lyophilization, and thoroughly mixed prior to DNA extraction. One gram of freeze-dried soil was used for two independent DNA extractions. Fungal community compositions were assessed by deep DNA sequencing (Ion Torrent) of the ITS2 rDNA region. Sequencing data were subjected to thorough quality control. Only high-quality data were used for the subsequent analyses that involved a variety of statistical methods to compare fungal community compositions across the control and treatment communities. In addition to the present work, part of these data provided the basis for the doctoral thesis of L. Morgado (2016) who focused on ectomycorrhizal fungal communities of the arctic tundra and their responses to simulated climatic changes.

Figure 1.8. *Open top chambers (OTCs) – experimental devices used for simulating climate change that passively warm air and soil inside for ca 2°C.*

Figure 1.9. *Snow fence - wooden fence ca 60 m long and ca. 2.8 m tall to accumulate snow brought by the predominant wind from Brooks range. Snow fences increase the snow depth from ca 0.5 m (ambient depth) to ca. 1 - 3 m depending on the distance from the fence.*

This thesis contains of five chapters. First Chapter provides the general outline for the thesis. Chapter two describes changes in the most diverse phylum of arctic fungi - Ascomycota – in response to 18-year experimental increase of summer air and near-surface soil temperatures. Changes in community composition and abundance of various functional groups of Fungi induced by the OTC treatment are presented in Chapter 3. Chapter 4 describes changes in the overall fungal community composition caused by increased precipitation, i.e. 18-year increased snow depth. Chapter 5 contains general conclusions and discussion of the results presented in the thesis.

The data obtained suggest strong shifts in fungal community compositions, fungal species richness, and relative abundance of functional guilds, induced by the climate manipulations. The results are discussed in relation to shifts in arctic vegetation, and changes in edaphic factors formerly reported for the same experimental plots as used in this study.

On the way to Toolik Lake field station

Chapter 2

LONG-TERM EXPERIMENTAL WARMING ALTERS COMMUNITY COMPOSITION OF ASCOMYCETES IN ALASKAN MOIST AND DRY ARCTIC TUNRA

Tatiana A Semenova, Luis N Morgado, Jeffrey M Welker, Marilyn D Walker, Erik Smets, József Geml

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Abstract

Arctic tundra regions have been responding to global warming with visible changes in plant community composition, including expansion of shrubs and declines in lichens and bryophytes. Even though it is well-known that the majority of arctic plants are associated with their symbiotic fungi, how fungal community composition will be different with climate warming remains largely unknown. In this study, we addressed the effects of long-term (18 years) experimental warming on the community composition and taxonomic richness of soil ascomycetes in dry and moist tundra types. Using deep Ion Torrent sequencing we quantified how OTU assemblage and richness of different orders of Ascomycota changed in response to summer warming. Experimental warming significantly altered ascomycete communities with stronger responses observed in the moist tundra compared to dry tundra. The proportion of several lichenized and moss-associated fungi decreased with warming, while the proportion of several plant and insect pathogens and saprotrophic species was higher in the warming treatment. The observed alterations in both taxonomic and ecological groups of ascomycetes are discussed in relation to previously reported warming-induced shifts in arctic plant communities, including decline in lichens and bryophytes and increase in coverage and biomass of shrubs.

Introduction

The greatest rates of climate warming have been observed in the Arctic, where the mean rate of temperature increase is nearly double of that in lower latitudes, and approaches 0.1°C per year over the last three decades (Anisimov et al, 2007; IPCC, 2007; Kaufman et al, 2009). This warming is leading to a suite of changes including: a) altering the extent and thickness of the arctic sea ice, b) shifts in plant community composition visible as tree line advancement into tundra and increase in shrub density, and c) permafrost thawing and alterations in the net exchange of CO_2 and CH_4 with the atmosphere (Welker et al, 2000, 2004; IPCC, 2013; Oechel et al, 2014). Warming-induced changes in the arctic ecosystems are of serious concern because most of them are leading to large positive feedback effects, promoting even greater warming of the climate. For example, rising concentrations of the greenhouse gases are followed by temperature increase and thawing of the permafrost - process that in turn, leads to increased efflux of CO_2 and CH_4 due to enhanced rates of decomposition in the warmer soils (Zona et al, 2009). Because northern circumpolar regions store approximately 50% of the Earth's soil carbon in seasonally or permanently frozen organic matter (Tarnocai et al, 2009), warming in the Arctic likely has tremendous consequences for atmospheric greenhouse gas concentrations that continue to rise (now at 400 ppm) (Cox et al, 2000). Another important feedback loop that is developing on tundra landscapes is the warming-induced expansion of shrubs (Hollister et al, 2005; Wahren et al, 2005), that trap snow during the winter and significantly increase winter soil temperature and, thus, soil microbial activity, promoting further the expansion of shrubs (Sturm et al, 2001).

Climate-induced changes in the arctic plant communities are among the most evident ones on our planet, and have been the focus of intensive research (e.g., Welker et al, 1997; Arft et al, 1999; Wahren et al, 2005; Walker et al, 2006). In addition to long-term field monitoring, the responses of arctic vegetation to elevated temperatures have been estimated in experimental manipulations that revealed rapid shifts in arctic plant communities, including an increase in the biomass of graminoids and deciduous shrubs, higher plant canopy, accumulation of leaf litter and decrease in relative cover of shade-intolerant lichens and bryophytes (Hollister et al, 2005; Walker et al, 2006). The extent of these changes has been dependent on the duration of experiments and initial composition of the plant community (Hollister et al, 2005), with greatest responses exhibited by low arctic ecosystems, compared to high arctic and alpine areas (Arft et al, 1999). Within the

low arctic, in both natural and experimental conditions, stronger responses to warming were exhibited by plant communities of the so-called moist tussock tundra. This vegetation type is clearly distinguished from another tundra types, e.g., dry heath tundra characterized by *Dryas octopetala*, by visible differences in plant community composition that is dominated by the tussock-forming sedge *Eriophorum vaginatum*, deciduous and evergreen shrubs (Grime, 2001; Walker et al, 2006; Mercado-Diaz, 2011). In addition, there is ca. 8-fold difference in the aboveground vascular plant biomass in the two tundra types (ca. 800 g/m^2 in the moist tundra and ca. 100 g/m^2 in the dry tundra) (Shaver and Jonasson, 1999). Dry and moist tundra types are described in detail in Walker and Maier (2008; http://www.arcticatlas.org) including their surficial and glacial geology, soil carbon contents, elevations, relative percentage of the area, pH levels and plant community compositions.
Although the responses of arctic plant communities to warming have been well studied, our understanding of how warming in the Arctic will affect soil fungi remains rudimentary (Schaeffer et al, 2013). Fungi regulate nutrient-cycling processes and influence the plant fitness by forming various types of plant-fungus associations (Ludley and Robinson, 2008; Hobbie et al, 2009; Newsham et al, 2009; Dahlberg and Bültmann, 2013). These fungal associations can be especially important as they enhance plant acquisition of scarce nutrients, especially N and P, that generally limit plant growth in cold-dominated ecosystems (Nadelhoffer et al, 1996; Kytöviita and Ruotsalainen, 2007; Hobbie et al, 2009). Previous research carried out on the responses of arctic fungi to rising temperatures focused on ectomycorrhizal basidiomycetes associated with the arctic shrub *Betula nana*, and showed warming-induced increase in fungal diversity and biomass (Clemmensen et al, 2006; Deslippe et al, 2011). A more recent study by Morgado et al. (2014) reported a sharp decrease in richness of ectomycorrhizal basidiomycetes due to warming in the moist tundra of arctic Alaska, with the shifts in the hyphal exploration types that likely indicate increased potential for mineralization of recalcitrant nutrient pools in the soil. Neither basidiomycete richness nor community composition was altered by warming in the dry tundra type (Morgado et al, 2014). On the other hand, phylum Ascomycota is the most diverse group of fungi in the Arctic, (Geml et al, 2012; Timling et al, 2014) and their community composition and possible responses to increased temperatures are almost entirely unknown. Along with the other groups of fungi and bacteria, ascomycetes contribute to the functioning of tundra ecosystems through a broad spectrum of their ecological roles, such as: a) decomposers of organic substances as

saprotrophs, b) symbionts of photosynthetic algae and N-fixing cyanobacteria in the forms of lichens, c) ericoid, arbutoid and ectomycorrhizal partners of shrubs, d) mutualist or commensalist endophytes, and e) pathogens of plants, fungi and animals. Therefore, changes in the community compositions of ascomycetes due to warming are likely linked to changes in key ecological processes in tundra ecosystems. We investigated the responses of ascomycetes to experimentally increased summer air and soil temperatures in dry heath and moist tussock tundra in Northern Alaska. We hypothesized that: a) community composition and richness of taxonomic groups of ascomycetes will change under warmed conditions, b) the responses of ascomycete communities will be stronger in the moist tussock tundra type compared to dry heath tundra in agreement with trends observed for plant communities, and c) warming will favour the growth of shrub-associated and saprotrophic fungi but will suppress lichenized ascomycetes. To test these hypotheses, we used deep Ion Torrent DNA sequencing of the ITS2 rDNA region, to compare ascomycete community compositions in ambient and elevated temperatures in the two tundra types that have been exposed to ca. 18 years of experimental warming as part of the International Tundra Experiment (Welker et al, 1997, 2005; Pattison and Welker, 2014).

Materials and Methods

Study site and sampling

Our research area was near the Toolik Lake Research Station, situated on the northern foothills of the Brooks Range, Alaska (68°38´N, 149°34´W). Two main vegetation types, dry heath tundra and moist tussock tundra are found throughout the region; dry heath tundra is dominated by *Dryas octopetala*, *Salix polaris, Vaccinium* species and fruticose lichens, while the moist tussock tundra is dominated by *Betula nana*, *Salix pulchra* and the sedge *Eriophorum vaginatum* (Walker et al, 1999). Experimental plots of warming treatments were established in 1994 as part of the International Tundra Experiment. Warming was accomplished using open-top chambers (OTCs) (Jones et al, 1998; Walker et al, 1999; Welker et al, 2000). An OTC is a hexagonal device of ca. 1 m^2 constructed of translucent fiberglass that passively increases daytime air temperature by 1-5°C during the snow-free period (Marion et al, 1997; Welker et al, 1999). OTCs are placed over experimental plots in every spring as soon as 50% of the plot becomes snow-free, and are removed at the end of the growing season in late August or early September (Walker et al, 1999).

The sampled plots had previously been used for vegetation studies that reported: significant shifts in the plant community composition inside the OTCs, especially in the moist tundra type (Welker et al, 1999). In each tundra type, we sampled soil at five OTCs (warming treatment) and five control plots, resulting in twenty plots used for the whole analysis. Five soil cores, 2 cm in diameter and ca. 20 cm deep, were taken randomly to provide a composite sample for each plot that included all the organic and parts of the mineral soil horizon. Coarse litter and aboveground vegetation parts were removed from the sample, although some fine roots were present in the samples. Composite samples were kept frozen until lyophilization. Although we sampled soils in two different tundra types, our methodological approach was not intended to compare the ascomycete communities in the dry *versus* the moist tundra. Instead we aimed to investigate the effect of warming on ascomycete communities in both tundra types separately to eliminate all potentially contributing factors other than the warming treatment itself.

DNA isolation, PCR and sequencing

Genomic DNA was extracted from ca. 0.4-1 g of dry soil (that corresponded to the maximal amount of soil that could be loaded to the tube) using NucleoSpin® Soil kit (Macherey-Nagel Gmbh & Co., Düren, Germany), according to the manufacturer's protocol. Because of the relatively small amount of the soil that could be processed in one tube (ca. 0.2-0.5 g), for each sample DNA extraction was carried out twice and replicates were combined. The DNA concentration of the samples were normalized. PCR amplification and Ion Torrent sequencing of the ITS2 region (ca. 250 bp) of the nuclear ribosomal rDNA repeat were carried out with primers fITS7 (Ihrmark et al, 2012) and ITS4 (White et al, 1990) (see Table 2.S1 for the primer sequence information), and as described in detail in both Geml et al. (2014) and Morgado et al. (2014). ITS is the universal DNA barcode marker for fungi and has been used in a wide variety of taxonomic and ecological studies (e.g., Bruns et al, 1991; O'Brien et al, 2005; Geml et al, 2014 and references therein). The ITS4 primer was labeled with sample-specific Multiplex Identification DNA-tags (MIDs, see Table 2.S1 for the complete MID list). The amplicon library was sequenced using an Ion 318™ Chip by an Ion Torrent Personal Genome Machine (PGM; Life Technologies, Guilford, CT, USA) at the Naturalis Biodiversity Center. The raw sequence data (FASTQ files) are available at Dryad (doi:10.5061/dryad.2fc32).

Ion Torrent adaptor			
Sequencing adaptor P1	5' - CCTCTCTATGGGCATCGGTGAT - 3'		
Sequencing adaptor A	5' - GACTCAGCCTCTGTGCGTCCCTACTCTACC - 3'		
Gene Primer			
fITS7-trP1 (forward)	5'-GTGARTCATCGAATCTTTG-3'		
ITS4 (rewerse):	5'-TCCTCCGCTTATTGATATGC-3'		
Molecular Identifiers (MID) tags			
MID sequence	***Tundra type***	***Temperature treatment***	***MID, No***
IonXpress_010 CTGACCGAAC	MESIC	Control	MID 10
IonXpress_033 TTCTCATTGAAC	MESIC	Control	MID 33
IonXpress_009 TGAGCGGAAC	MESIC	Control	MID 9
IonXpress_031 TCCAAGCTGC	MESIC	Control	MID 31
IonXpress_011 TCCTCGAATC	MESIC	Control	MID 11
IonXpress_013 TCTAACGGAC	MESIC	OTC	MID 13
IonXpress_024 AACCTCATTC	MESIC	OTC	MID 24
IonXpress_023 TGCCACGAAC	MESIC	OTC	MID 23
IonXpress_036 AAGGAATCGTC	MESIC	OTC	MID 36
IonXpress_040 CTGACATAATC	MESIC	OTC	MID 40
IonXpress_018 AGGCAATTGC	DRY	Control	MID 18
IonXpress_039 TAACAATCGGC	DRY	Control	MID 39
IonXpress_029 TCGACCACTC	DRY	Control	MID 29
IonXpress_038 TGGAGGACGGAC	DRY	Control	MID 38
IonXpress_037 CTTGAGAATGTC	DRY	Control	MID 37
IonXpress_003 AAGAGGATTC	DRY	OTC	MID 3
IonXpress_034 TCGCATCGTTC	DRY	OTC	MID 34
IonXpress_007 TTCGTGATTC	DRY	OTC	MID 7
IonXpress_035 TAAGCCATTGTC	DRY	OTC	MID 35
IonXpress_028 ATCCGGAATC	DRY	OTC	MID 28

Table 2.S1. *Ion Torrent adaptor, primer, and multiplex tag (MIDs) sequences.*

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. (2014). The FASTQ files were converted to FASTA and QUAL files, and the sequences were subjected to quality filtering, whereby each sequence was screened for thresholds for the average Phred score of Q≥25 in a sliding window of 50 bp (qwindowaverage=25; qwindowsize=50), no ambiguous bases (maxambig=0) and homopolymers no longer than 8 bp (maxhomop=8). Sequences shorter than 150 bp or longer than 400 bp were omitted from further analysis (minlength=150, maxlength=400). Because next-generation sequencing libraries generally vary in size, we normalized the number of sequences for all samples, as recommended by Gihring et al. (2012), to ensure that estimators across all samples were comparable. For this purpose, we randomly subsampled the number of trimmed and quality-filtered reads to the size of the smallest library (of 56 483 sequences). The resulting sequences were clustered into operational taxonomic units (OTUs) with 97% ITS sequence similarity using OTUPIPE 1.1.9 (Edgar et al, 2011). Simultaneously, the putatively chimeric sequences were removed by *de novo* and reference-based filtering using the curated dataset for fungal ITS sequences (http://www.emerencia.org/ chimerachecker.html) of Nilsson et al. (2011). We assigned sequences to genera based on pairwise similarity searches using USEARCH (Edgar, 2010) against the quality-checked UNITE fungal ITS sequence database containing identified fungal sequences. Of the total fungal OTUs, the ones assigned to the phylum Ascomycota were selected for further analysis. Representative sequences of these ascomycete OTUs have been submitted to GenBank with the following accession number KJ826608-KJ828710. The depth of the OTU coverage across the treatments was examined by Good's coverage (as in Brown et al, 2013) and by rarefaction analysis using the Vegan package (Oksanen et al, 2012) in R software for statistical computing (R core Team, 2013).

Comparing ascomycete fungal communities across the sampling sites

We first compared the communities among all sites by performing one-way cluster analysis, using Euclidean distances and Ward's group linkage method in PC-Ord v.5.32 (McCune and Grace, 2002). The effect of warming on community compositions was estimated using non-metric multidimensional scaling (NMDS)

on a primary presence/absence matrix of plots by OTUs, also, in PC-Ord, following the protocol described in detail in Geml et al. (2014). Because of uncertainties regarding the reliability of read count as an estimator of species abundance (Amend et al, 2010), we carried out two sets of ordination analyses: 1) based on presence/absence and 2) taking into account OTU abundance values. 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 the others). The secondary matrix consisted of treatment (control or warming) and number of ascomycete OTUs per taxonomic order (see Table 2.S4 for ordination matrices). We also tested whether the warmed and control ascomycete communities were statistically different across the sites using a multi-response permutation procedure (MRPP) and permutation-based nonparametric MANOVA (Anderson, 2001), and determined any preferences of individual OTUs for specific experimental treatment using Indicator Species Analyses (Dufrêne and Legendre, 1997), also in PC-Ord. Additionally, for the most diverse orders of Ascomycota, Venn diagrams were generated with the BioVenn web tool (Hulsen et al, 2008) to visualize the distribution of OTU composition across the experimental treatments. In addition, the significance of the observed differences in the OTU richness across the different experimental treatments was tested by Student t-test.

Analysis of ecological functions

We estimated the proportions of different ecological groups among the OTUs that showed strong ($|R| \geq 0.5$) positive or negative correlation with warming in the NMDS analyses. Ecological functions for these OTUs were selected based on the information for the isolation source for the reference sequences (with at least 97% similarity) presented in GenBank. For the OTUs with similarity levels of 95-96% to the reference sequence, the ecological function was set as "putative", and in case there were no sequences with at least 95% similarity in the database, the ecological function for the OTU was set as "unknown". Fungi isolated from non-living materials (i.e. litter, rocks, marble, feathers, decaying wood, rotten fruits and mushrooms) were defined as "saprotrophic", and different types of mycorrhizal and root endophytic fungi were considered "root-associated". The group of "endophytes" involved the fungi isolated from asymptomatic photosynthetic tissues of plants, lichens and bryophytes. Mycobionts of lichens were grouped as "lichenized" fungi. For the OTUs that matched only to the sequences obtained

from the arctic soils, the putative ecological function was defined as "soil". We calculated the proportion (%) of OTUs representing various ecological groups across the control and warmed plots. The percentages were *arcsin*-transformed and differences between the treatments were tested by Student's *t*-test.

Results

Sequence data analysis

The Ion Torrent dataset contained 4 046 811 sequences with the median length of 303 bp. After the initial filtering step we obtained 2 068 216 sequences characterized by the sufficient length (150-400 bp), reasonable quality scores (Q>25) and homopolymers with no more than eight nucleotides.

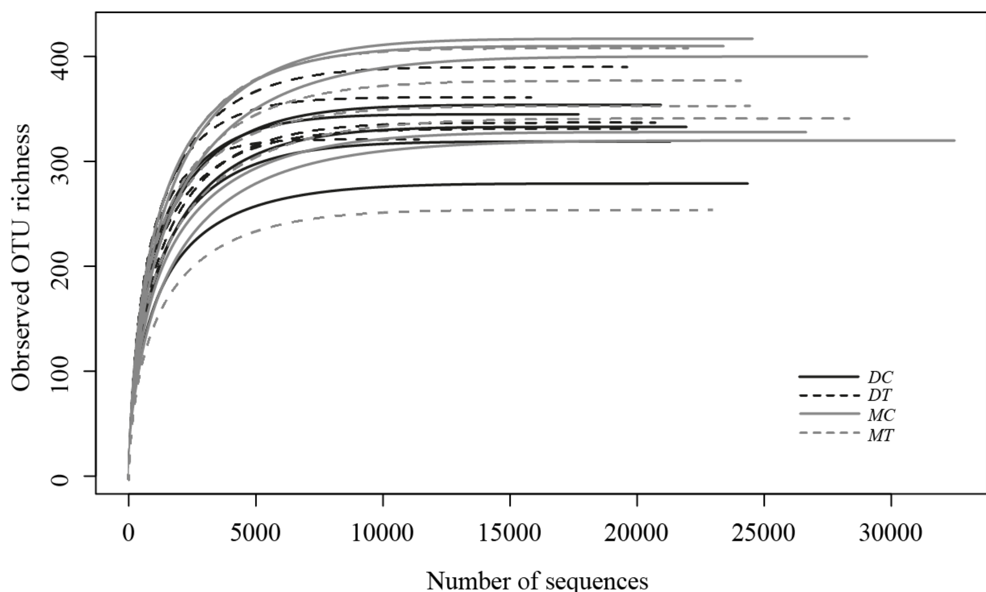
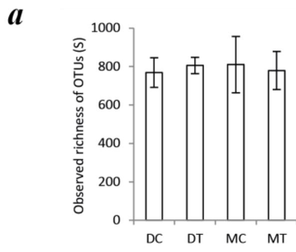


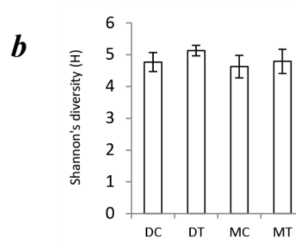
Figure 2.1. *Rarefaction curves obtained for the fungal OTUs for the two tundra types (D-dry, M-moist) at two temperature treatments (C-control plots, T-experimentally warmed plots).*

Figure 2.2. (next page) *Community compositions, richness and coverage estimators across the sampled sites, i.e. two tundra types (D-dry, M-moist) at two temperature treatments (C-control plots, T -experimentally warmed plots): (a) observed number of OTUs (S), (b) Shannon's diversity index (H), (c) Simpson's diversity index, (d) Good's coverage, (e) evenness (H/lnS). All estimators are shown ±SD. (f) cluster diagram for ascomycete community compositions, (g,h) non-metric multidimensional scaling (NMDS) ordination plot for ascomycete communities of the moist (g) and dry (h) tundra type. Vectors are shown for variables correlated with ordination axes at |R| > 0.5.*

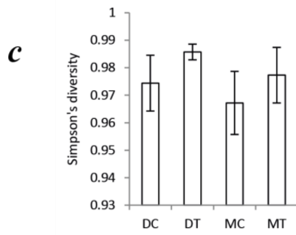
a



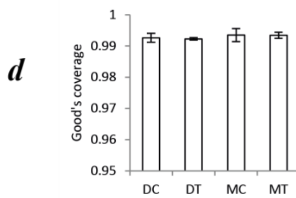
b



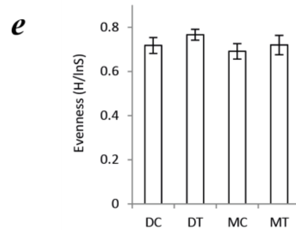
c



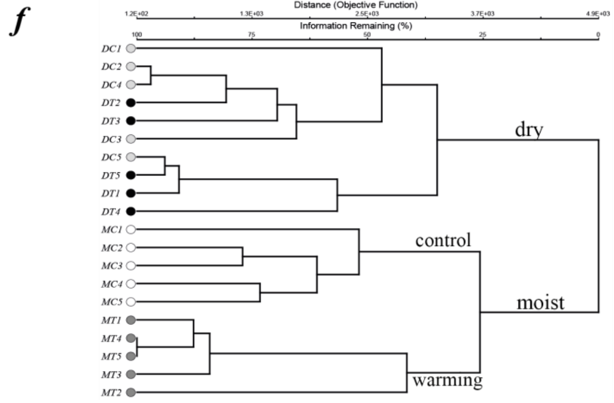
d



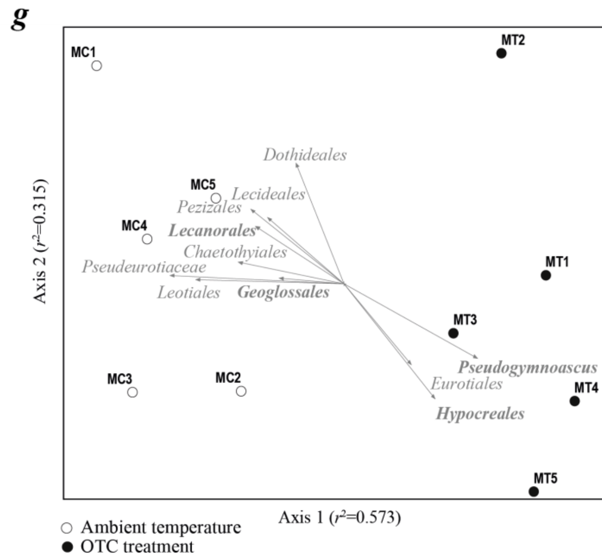
e



f

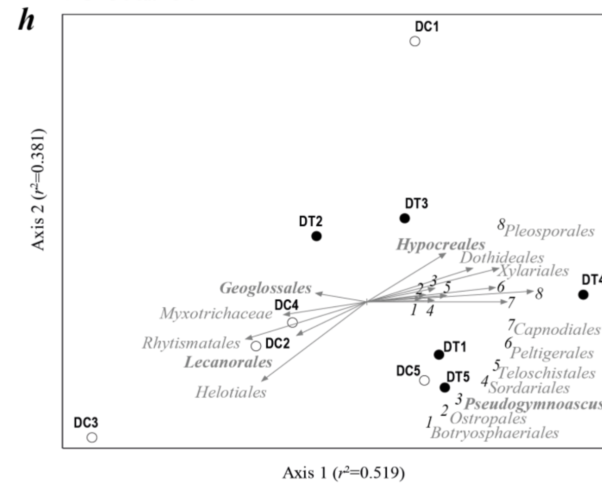


g



○ Ambient temperature
● OTC treatment

h



Sequence data were subsampled according to the size of the smallest sequence library of 56483 sequences. Total number of 1 129 660 sequences obtained for twenty samples had the mean read length of 255 ± 56 bp (± SD); OTU clustering of these sequences resulted in 5638 non-singleton OTUs. The rarefaction curves approached the saturation plateau for all of the samples, suggesting an equally deep OTU recovery across the treatments (Fig. 2.1). We observed no correlation between warming treatment and OTU richness (Fig. 2.2a). Similarly, there was no difference between Shannon's and Simpson's diversity indexes (Fig. 2.2b,c) and evenness values (Fig. 2.2e) among the control and warmed sites. Good's coverage estimators (99.3 ± 0.14 across all the treatments) indicated that the deep sequencing allowed for a very high OTU coverage (Fig. 2.2d). We identified 2103 OTUs belonging to the phylum Ascomycota. Among them, only about 20% showed ≥97% similarity with their closest relatives in reference databases, and therefore, could putatively be regarded as conspecific with the reference sequences. We detected 35 taxonomic orders, with most of the OTUs belonging to Helotiales (480 OTUs), Chaetothyriales (243), Lecanorales (91), Pleosporales (73), Verrucariales (56), Hypocreales (51), Capnodiales (48), Leotiales (45), Coniochaetales (43), Eurotiales (25) and Pezizales (25), followed by numerous orders with less than 20 OTUs. We detected representatives of 311 genera. For example, observed genera of putative root endophytes included *Cladophialophora* (114 OTUs) – the genus with the highest obtained OTU richness in the dataset, *Meliniomyces* (66), *Phialocephala* (39), *Cadophora* (19), *Phialophora* (14) and *Leptodontidium* (12). The genera of putative lichenized fungi with the high OTU richness included *Lecidea* (98 OTUs), *Cladonia* (40), *Peltigera* (13) and *Allocetraria* (10). Relatively high OTU richness was observed for the saprotrophic genus *Capronia* (94 OTUs) and ericoid mycorrhizal fungi of the genera *Rhizoscyphus* (89) and *Pseudogymnoascus* (83). Additionally, the group of aquatic hyphomycetous fungi was represented by a high OTU number, with the affinities to the following genera: *Alatospora* (51 OTUs), *Articulospora* (35), *Helicodendron* (25) and *Spirosphaera* (20).

Moist tundra communities

For the moist tundra site, we obtained a dataset of 1253 ascomycete OTUs. OTU richness did not vary significantly with the treatment (t_8=0.85; P=0.42), although the total number of OTUs in the control plots, 901 (mean value 378±47 OTUs per plot), was slightly higher than the richness observed for the warming treatment (802 OTUs, mean value 350±58 OTUs per plot).

The orders with the highest observed OTU richness – Helotiales and Chaetothyriales – comprised ca. 38% and 15% of the OTUs in control communities, and respectively ca. 38% and 10% of the OTUs in warmed communities. The rest of the OTUs belonged to numerous taxonomic orders with lower numbers of OTU hits: for the control communities Leotiales (5%) and Pleosporales (4%) followed the OTU richness ranking, and for the warmed plots Hypocreales (5%), Pleosporales (4%), Eurotiales (3.4%) and Leotiales (3.2%) orders followed the richness ranking of the ascomycete communities.

Because the results of the presence/absence and abundance-based NMDS analyses were very similar, we present the former below and the latter in the Supplementary Information (Fig. 2.S3, Table 2.S4). We found a strong effect of warming on the community composition of ascomycetes, as shown by one-way clustering (Fig 2.2f), MRPP (P = 0.0025; size effect A = 0.134) and MANOVA (F=3.99; P=0.01) analyses, and depicted by the NMDS (final stress = 7.10) plot (Fig 2.2g). Temperature treatment accounted for 37.4% of the variation in ascomycete community compositions (MANOVA). Richness in seven orders and one family of uncertain taxonomic placement negatively correlated ($|R| > 0.5$) with warming: Leotiales (R = -0.874), Chaetothyriales (R = -0.767), Pezizales (R = -0.752), Lecanorales (R = -0.718), Lecideales (R = -0.659), Dothideales (R = -0.571), Geoglossales (R = -0.569) and Pseudeurotiaceae (R = -0.952). On the other hand, two orders and one genus with unknown systematic position (*Incertae sedis*) showed positive correlation with increased temperatures: Eurotiales (R = 0.632), Hypocreales (R = 0.761) and *Pseudogymnoascus* (R = 0.876).

The effect of experimental warming on OTU composition of the two orders with highest OTU richness – Helotiales and Chaetothyriales - was also displayed in the Venn diagram (Fig. 2.3a). In both orders, we observed a relatively high proportion of OTUs unique to a temperature treatment. Although OTU richness in the order Helotiales was not affected by the treatment, and thus, not depicted on NMDS plot, Venn analysis suggested a visible effect of the warming treatment on OTU composition in this order. Pearson's correlation analysis for the individual OTUs resulted in 153 OTUs associated with the control conditions and 143 OTUs correlated with the warming treatment. Identification of these OTUs to the species level, however, was hampered by the scarcity of publicly available identified sequences of closely related taxa. From the 153 OTUs negatively correlated with warming, only 16 were identified to the species level.

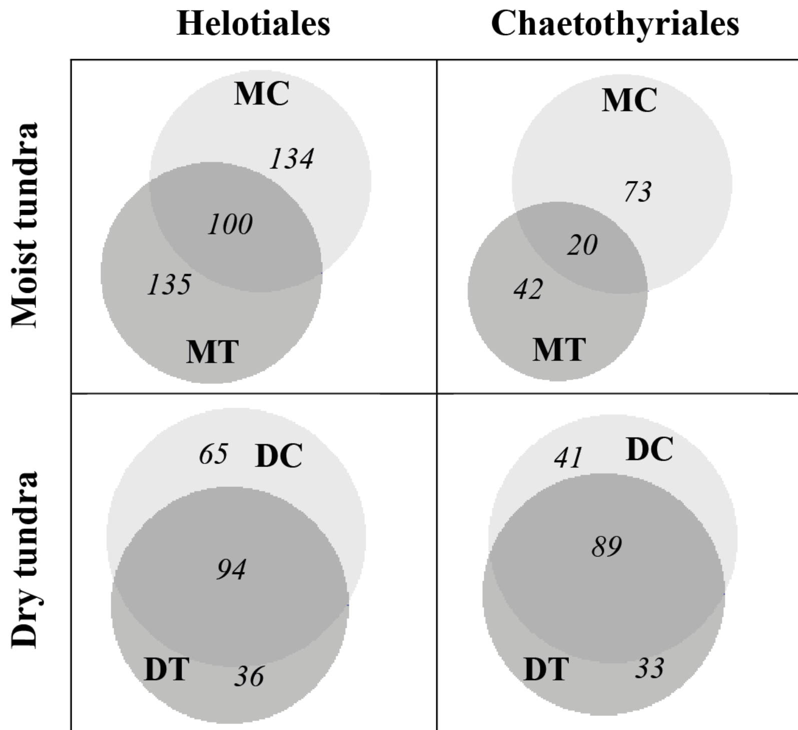


Figure 2.3a. *Warming-induced compositional and ecological shifts in ascomycetes. D-dry tundra, M-moist tundra, C-control plots, T-experimentally warmed plots. (**a**) Venn diagram displaying the proportion of shared and unique OTU counts for two dominant ascomycete orders.*

Of the 143 OTUs positively correlating with the OTC treatment, 59 OTUs were identified to species, and 27 of them related to a single genus – *Pseudogymnoascus.* 296 OTUs that correlated with one of the treatment were used for the analysis of ecological functions (Fig. 2.3b). Student *t*-test analysis revealed a significant increase in the richness of saprotrophs, endophytes and putative pathogens of plants and insects with warming. At the same time, the richness of lichenized ascomycetes declined significantly. Indicator Species analysis unraveled 33 OTUs characteristic to control plots and 42 OTUs indicator for the warmed plots across the moist tundra type (Table 2.S2). With the obtained similarity levels,

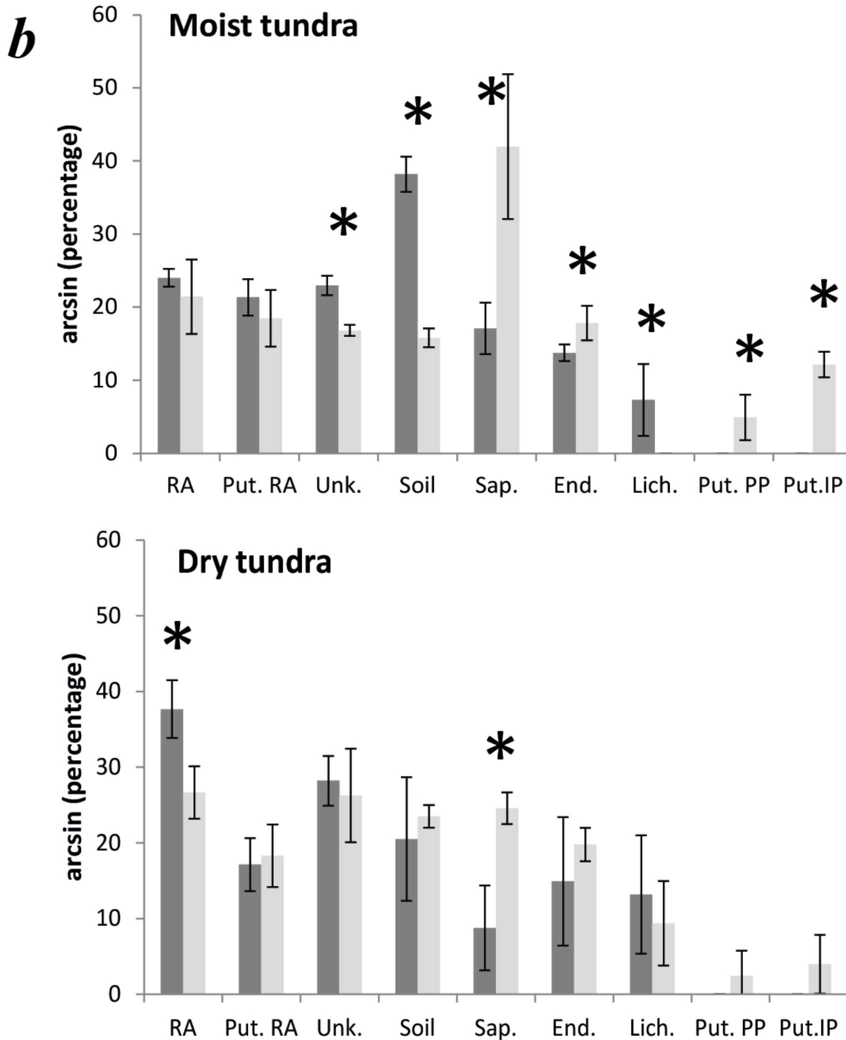


Figure 2.3b. ***b*** *Proportions (±SD) of the OTUs belonging to different ecological groups of fungi among the OTUs correlated with control or warming treatment: RA-root-associated, Put.RA-putative root-associated, Unk-unknown, Soil-known from soil sequencing, Sap-saprotrophic, End-endophytic, Lich – lichenized, Put PP – putative plant pathogen, Put IP-putative insect pathogen.*

we identified to species two OTUs as indicators for the control plots - *Alatospora acuminata* (AY204589) and *Aureobasidium pullulans* (EU272483). 15 OTUs were associated with the warming treatment, among them, ten showed high sequence

similarity and affined to species of *Pseudogymnoascus* (AJ608972; EF540755) (Table 2.S2).

Dry tundra communities

Data analysis indicated a highly diverse community of 1159 ascomycete OTUs in the dry tundra. We did not observe significant change in OTU richness under the experimentally elevated temperatures (t_8=1.22; *P*=0.26). In total, 849 OTUs were found across the ambient temperature plots (mean value 329±29 OTUs per plot) and 831 OTUs were obtained for the OTC communities (mean value 351±28 OTUs per plot). Most of the OTUs (ca. 80%) were identified to taxonomic order, while the percentage of the OTUs identified to the level of the species was much lower (17%). The orders Helotiales and Chaetothyriales were characterized by the highest OTU richness across the control and warmed sites in the dry tundra, representing 24% and 19%, and 20% and 18.8% of the OTUs, respectively.

Cluster analysis did not reveal a clear distinction between the overall compositions of ascomycete communities in the control and warmed sites (Fig 2.2f). Similarly non-significant results were obtained by MRPP analysis (*P* = 0.10; *A* = 0.24) and by NMDS (final stress = 0.19) (Fig. 2.2h). Treatment explained only 8% of the variation, as revealed by MANOVA statistics (*P* = 0.087; *F* = 1.46). Similarly, the proportion of shared OTUs of Helotiales and Chaetothyriales among the ambient temperature and warmed plots was greater than in the moist sites (Fig. 2.3a). However, we were able to observe changes in the OTU richness of several taxonomic orders in correlation ($|R| > 0.5$) with the temperature treatment (Fig. 2.2h). Similar results were obtained in the analysis of relative abundance of ascomycete taxa (see Fig. 2.S3 for the ordination plot and correlation values). OTU richness in the following taxonomic groups negatively correlated with warming: Rhytismatales (*R* = -0.786), Helotiales (*R* = -0.732), Lecanorales (*R* = -0.6) and Geoglossales (*R* = -0.51) and the family Myxotrichaceae (*Incertae sedis*) (*R* = -0.651).

Ten taxonomic orders exhibited higher OTU richness under the simulated warming. These orders included Botryosphaeriales (*R* = 0.509), Ostropales (*R* = 0.546), Sordariales (*R* = 0.588), Teloschistales (*R* = 0.632), Hypocreales (*R* = 0.639), Dothideales (*R* = 0.735), Peltigerales (*R* = 0.807), Xylariales (*R* = 0.821), Capnodiales (*R* = 0.847) and Pleosporales (*R* = 0.92). Correlation between OTU richness and warming was also shown for the genus *Pseudogymnoascus* (*R* = 0.584), (Fig. 2.2h). On the level of individual OTUs, Pearson's correlation analysis

resulted in 179 OTUs that negatively correlated with warming. Of these, only 25 OTUs could be identified to species and 14 OTUs related to a single species, *Rhizoscyphus ericae*. On the other hand, 235 OTUs correlated positively with the experimentally warmed sites. We identified 45 OTUs to the species level and obtained a diverse group of ascomycete species, with one dominant genus, *Pseudogymnoascus*, represented by 11 OTUs. 414 OTUs with high Pearson's correlation values were checked for possible ecological functions (Fig 2.3b). Student's *t*-test analysis showed a significant decrease in the richness of root associated fungi and increase in saprotrophic species with warming. Few OTUs were characteristic for either control or warming treatment, as revealed by the Indicator Species analysis. One OTU that matched with the reference sequence for *Pertusaria excludens* (SH117228.05FU), was indicator for the control plots (Table 2.S2); nine OTUs were characteristic for the warmed sites, and among the nine, five related to the following species: *Cladosporium cladosporoides* (AJ300335), *Fusciсladium catenosporum* (EU035427), *Pseudogymnoascus vinaceus* (AJ608972), *Venturia alpina* (EU035446) and *Venturia polygoni-vivipari* (EU035466) (Table 2.S2).

Discussion

Warming alters ascomycete communities

We found significant shifts in ascomycete community composition induced by eighteen years of summer warming. According to our research hypothesis and in agreement with the results of the previous vegetation studies, we observed greater changes in the ascomycete community compositions in the moist tundra compared to the dry tundra. Stronger responses of the plant communities in the moist tundra may correlate with greater responses in soil fungal communities due to tight associations that arctic fungi form with living and/or dead parts of specific vascular plants (Hobbie et al, 2009; Bjorbækmo et al, 2010; Dahlberg and Bültmann, 2013). It is difficult to compare the findings of our study with previous works addressing the effects of experimental warming on soil fungal communities (Allison and Treseder, 2008; Papanikolaou et al, 2010; Gutknecht et al, 2012; Hayden et al, 2012; Anderson et al, 2013; Jumpponen and Jones, 2013), because most of these projects studied different vegetation types, used different warming methods (e.g., greenhouse, infrared lamps) for much shorter periods (up to 6 years) and used different molecular techniques best suited for biomass and abundance estimations. For example, Jumpponen and Jones (2013) and Papanikolaou et al. (2010) found

no effect of warming on community composition in temperate grasslands and agricultural soils, respectively, similarly to what we observed in the dry tundra. On the other hand, Allison and Treseder (2008) found that 3-year warming had significant effects on the composition of the active fungal community (ca. 90% basidiomycetes, 5% ascomycetes) at an Alaskan boreal forest site, which is consistent with our results from the moist tundra. It is interesting to note that the samples analyzed by Allison and Treseder (2008) originate from the acidic black spruce (*Picea mariana*) vegetation type that generally shows substantial floristic resemblance in the understory to the low arctic moist tussock tundra (e.g., Hollingsworth et al, 2006).

With respect to the observed richness of different taxonomic groups, Jumpponen and Jones (2013) found that warming did not affect the richness in most ascomycete classes, except for the Lecanoromycetes that declined with warming. In our study, the order Lecanorales (involving lichenized ascomycetes) was one of the two orders that showed significant decrease in OTU richness in the warmed plots of both tundra types. Non-significant or inconsistent effect of OTC and infrared warming on total fungal abundance and biomass were also reported by Hayden et al. (2012), Papanikolaou et al. (2010) and Gutknecht et al. (2012). On the other hand, Allison and Treseder (2008) reported a significant positive effect of warming on the taxonomic richness of active fungi, particularly in ascomycetes that showed an increase in relative abundance from 5 to 14.4%. Neither total ascomycete richness nor total abundance across the treatments (Table 2.S4) changed in our study after 18 years of warming, although there were several groups with higher number of OTUs in the warming treatment plots (e.g., Eurotiales, Hypocreales and *Pseudogymnoascus*).

Responses in shrub-associated ascomycetes

The formerly observed, warming-induced expansion of shrubs was expected to favor plant pathogenic, mycorrhizal and root-endophytic fungi. Reported larger shrub density and biomass (Mercado-Diaz, 2011) was assumed to reflect increased root biomass (Sullivan et al, 2007), which in turn, may broaden niches for the root-associated ascomycetes. Analysis of ecological functions revealed a significant increase in the proportion of endophytic ascomycetes and plant pathogens among the OTUs correlated with warming treatment in the moist tundra, suggesting that warming-induced increase in plant growth may provide more suitable microhabitats for shrub-associated ascomycetes. Possibly, increase in OTU richness and abundance in the genus *Pseudogymnoascus* is also related to its ability

to form ericoid mycorrhiza (Vohnik et al, 2007) with arctic shrubs. In our dataset, several *Pseudogymnoascus* OTUs were at least 98% similar to sequences derived from plant roots or photosynthetic tissue (AJ608972). Unexpectedly, among the OTUs that correlated with the warming treatment in the moist tundra dataset, we observed no significant change in the proportions of root-associated ascomycetes (Fig. 2.3b). Moreover, in the dry tundra the proportion of root-associated ascomycetes decreased with warming. Sharp warming-induced decrease in the richness has been also shown for ectomycorrhizal basidiomycetes (Morgado et al, 2014). It is possible, therefore, that either warming provides unfavorable conditions for root-associated fungi in general or, more likely, warming favors few root-associated taxa that may competitively exclude other groups, leading to the overall decrease in richness, despite the reported trend of warming-induced increase in total fungal biomass (Clemmensen et al, 2006).

Responses in lichenized and moss-associated ascomycetes

We expected the decline in lichenized ascomycetes and species associated with bryophytes in response to warming due to reported previously overall decrease in bryophyte and lichen coverage (Hollister et al, 2005; Walker et al, 2006). In agreement with the former observations, our analysis revealed a reduction in OTU richness in the order Geoglossales, many of which are moss-associated (Hustad et al, 2013). In both ecological and taxonomic analyses and for both tundra types, we observed the reduction in lichenized fungi (order Lecanorales). In addition, in the moist tundra, we observed the warming-induced decline in richness for at least one more order that includes lichenized fungi (Lecideales). Due to the key-role of lichens in caribou food-diet, the warming-induced decline in lichen coverage is of high concern, as it could affect the populations of caribou and related chains of tundra food-webs (Dahlberg and Bültmann, 2013).

Responses in saprotrophic and insect pathogenic ascomycetes

Leaf litter accumulation reported for the OTC plots (Hollister et al, 2005; Wahren et al, 2005; Walker et al, 2006) was expected to favor the growth of litter-inhabiting saprotrophic ascomycetes. Indeed, in the both tundra types the proportion of saprotrophic ascomycetes increased under the warming treatment (Fig. 2.3b). Possibly, increased litter accumulation in the warmed plots may contribute to higher richness and abundance of several saprotrophic and psychrophilic *Pseudogymnoascus* species (see Table 2.S2 for the list of OTUs that were at least 97% similar to sequences derived from non-living materials).

Interestingly, irrespective of the initial vegetation type, OTU richness in the order Hypocreales correlated positively with the warming treatment. Analysis of the OTU affinities in this order revealed high sequence similarities to various species in the family *Cordicipitaceae* that comprises many insect pathogenic fungi. It is possible that the increased abundance of certain arctic insect groups due to warming (Dollery et al, 2006; Adler et al, 2007) contributes to the higher OTU richness of insect pathogenic ascomycetes. Also, previous studies in temperate and subtropical ecosystems have consistently found that OTU richness of hypocrealean fungi correlates positively with temperature, as shown in various altitudinal gradient studies (Devi et al, 2012; Geml et al, 2014). Therefore, the underlying mechanisms for the increase in richness in Hypocreales are uncertain, but it is safe to speculate that the increased temperature likely favors the decomposer capabilities of hypocrealean fungi and that other warming-induced changes (e.g., increase in the abundance of insects) may contribute to the higher OTU richness in this group as well. On the other hand, some of the OTUs that were highly similar to the sequences of well-known insect pathogenic fungi (e.g., *Beauveria bassiana*) had also high similarity to the sequences amplified from roots (e.g., EF093153, KC243962). Therefore, defining the ecological roles for these ascomycetes is complicated, and some of the putative insect pathogens may have a root-associated or endophytic lifestyle (White et al, 2003).

Arctic ascomycete diversity

Although the main focus of our study was to assess responses of soil ascomycete communities in dry and moist arctic tundra to long-term experimental warming, our data offer unprecedented insights in the richness of arctic fungi. It is well-known that functioning of the arctic ecosystems is reliant on fungi, nonetheless, fungal communities in the arctic tundra, with the exception of ectomycorrhizal basidiomycetes, remain largely unstudied. This is particularly true for ascomycetes that comprise more than 60% of all known fungi and are often difficult to study because the vast majority of them are inconspicuous “microfungi”, including most of soil fungi, leaf and root endophytes, plant and animal pathogens that do not form macroscopic fruiting structures (with few exceptions, e.g., lichens). The existing regional checklists mention 1245 non-lichenized ascomycete species for the whole Arctic region (Dahlberg and Bültmann, 2013) that is less than the number of non-lichenized OTUs (1967) found in our study for one location only. Although the 97% ITS sequence similarity OTUs routinely used in molecular studies, including this one, likely do not correspond one-to-one to species, they nevertheless represent

the best approximation in rapid richness assessments with wide taxonomic focus and, when applied with rigorous sequence quality checks, they can provide reasonably accurate estimates of the number of species. Our analysis of OTU richness supported suggested previously high diversity within several fungal taxa, for example, *Rhizoscyphus ericae*, that is considered a “species aggregate” (Hambleton and Sigler, 2005; Grelet et al, 2010) and for which 19 species hypotheses have been proposed by UNITE experts, or *Pseudogymnoascus* sp. that is known to be far exceeding the number of described species (Minnis and Lindner, 2013).

The total fungal richness for the Arctic was estimated by Schmit and Mueller (2007) as 11,000 species, based on plant-fungal diversity ratio of Hawksworth (2001). However, the diversity estimates provided in this paper suggest that the total fungal richness in the Arctic may be even greater, particularly because fungal-per-plant species richness was shown to increase towards the poles (Tedersoo et al, 2014). In our data analysis, a relatively low percentage (<20%) of OTUs were identified to species (with >97% sequence similarity) due to the scarcity of reference sequence data. Approximately 40% of the OTUs that correlated with the treatment had highly similar, but unidentified sequences known only from other soil sequencing studies, while the proportion of OTUs that had no close reference sequences with >95% similarity was ca. 20%. It is well known that the sequence data currently available in public databases only represent a fraction of total fungal diversity: it has been estimated that less than 5% of all fungi are known (Blackwell, 2011) and only 20% of the described species have been sequenced and, thus, can be identified using DNA (Kõljalg et al, 2013). In our dataset, there was a vast inequality in the average sequence similarity values obtained for economically “important” vs. “non-important” ascomycete genera. For example, sequence similarity values to the most similar publicly available sequences for OTUs in relatively well-studied genera, i.e. those with economic importance in food production, medicine or biotechnology, were generally high: *Penicillium* (98.5±1.21%), *Torula* (97.3±2.63%), *Botrytis* (99.1±0.31%), *Hypocrea* (98.4±0.9%). On the other hand, sequence similarity values were much lower for numerous species lineages that do not have any industrial application, e.g., *Lecidea* (86.5±3.4%), *Capronia* (85.5±2.16%), and, therefore, likely are underrepresented in public databases. Consequently, it is very difficult to speculate on the ecological roles of most of the ascomycete OTUs and to link the observed changes in ascomycete community composition to changes in ecological functions. For example, many aquatic hyphomycetous species (*Alatospora, Articulospora,*

Spirosphaera etc), that have been traditionally considered saprotrophic, have been isolated from surface-sterilized roots of the arctic shrubs *Dryas octopetala*, *Betula nana* and *Salix polaris* (Semenova, unpubl. data), suggesting that these fungi may be root-associated. Clearly, more research on the taxonomy, phylogenetic diversity and ecological functions of arctic fungi is needed.

Our data suggest that arctic ascomycete communities are extremely diverse and vary in composition depending on the tundra type. Their community composition is altered by warming, with a much stronger response exhibited in the moist tussock tundra compared to dry heath tundra. Yet, the lack of adequate taxonomic and ecological knowledge of fungi severely compromises our ability to disentangle causal relationships, to infer likely changes in ecological functions, and to provide predictions about the Arctic. Numerous fungi in our samples likely are still undescribed, while others may remain unidentified because of the lack of reference sequences from known species. In addition, even for known species, more research is required to obtain information on their ecological functions. Addressing these questions will be helpful in predicting how arctic ecosystems respond to warming, from nutrient cycling to trophic relationships.

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Table 2.S2. (next page) *OTUs considered a significant (all P<0.05) indicators of the warming treatment or control. The best match is provided for the OTUs according to Bayesian classifier (http://rdp.cme.msu.edu/), UNITE database for the identified fungal ITS (A) (Abarenkov et al., 2010) and UNITE+INSD database (B) (Koljalg et al., 2013). Putative ecological function was determined based on isolation source for the closest reference sequences in GenBank database: ecm - ectomycorrhizal, end - endophytic, in path - insect pathogenic, lich - lichenized, p path - plant pathogenic, put root-as - putative root-associated, sapr - saprotrophic, soil - known from soil sequencing only, unk - unknown, i.e. no close reference sequence available.*

Table S2.3 *is too large to include in this document. A digital version is available at http://onlinelibrary.wiley.com/doi/10.1111/mec. 13045/ abstract*

Dry tundra type									
OTU	Treatment	Ecology	Bayesian classifier	BC %	Best match	Taxonomic affinity	Taxonomic order	% similarity	Coverage length
1458	Control	unk	*Pertusaria pertusa* SH231089.06FU	25	A:DQ534475 B:DQ534475	*Pertusaria excludens* *Pertusaria excludens*	Pertusariales Pertusariales	83,47 81,2	357 351
3194	Warming	end	*Davidiella tassiana* SH196750.06FU	100	A:AJ300335 B:EU725679	*Cladosporium cladosporioides* Fungi sp.	Capnodiales	99,22 97,3	258 257
3294	Warming	unk	*Pochonia* sp. SH228971.06FU	21	A:U57667 B:AB294424	*Dussiella tuberiformis* *Clavicipitaceae* sp.	Hypocreales Hypocreales	90,27 87,2	329 327
2409	Warming	p path	Dothideomycetes sp. SH207406.06FU	52	A:EU035427 B:KC588625	*Fusicladium catenosporum* Fungi sp.	Pleosporales	98,31 99,4	178 176
2346	Warming	end	*Pseudogymnoascus roseus* SH236509.06FU	89	A:AJ608972 B:JX984721	*Pseudogymnoascus* sp. Fungi sp.	Inc sed, Leotiomycetes	97,59 96,8	249 249
3957	Warming	put root-as	Helotiales sp. SH209187.06FU	86	A:EF029237 B:GU083029	*Helicodendron luteoalbum* Helotiales sp.	Helotiales Helotiales	90,96 95,2	177 187
2572	Warming	root-as	Helotiales sp. SH209269.06FU	96	A:AF486132 B:JX987752	*Phialocephala virens* Fungi sp.	Helotiales	92,99 93,8	157 178
3712	Warming	sapr	Stictidaceae sp. SH212637.06FU	45	A:AY527308 B:AY527308	*Stictis radiata* *Stictis radiata*	Ostropales Ostropales	96,68 96,7	271 271
248	Warming	p path/ sapr	Dothideomycetes sp. SH207406.06FU	71	A:EU035446 B:HQ211781	*Venturia alpina* *Venturia* sp.	Pleosporales Pleosporales	98,48 99,6	263 263

5197	Warming	sapr	Dothideomycetes sp. SH207406.06FU	6	A:EU035466 B:HQ432962	*Venturia polygoni-vivipari* Ascomycota sp.	Pleosporales	97,47 97,8	277 275
Moist tundra type									
3355	Control	root-as	*Alatospora* sp. SH207334.06FU	100	A:AY204589 B:AY204589	*Alatospora acuminata* *Alatospora acuminata*	Leotiales Leotiales	98,9 98,5	272 273
1434	Control	soil	Leotiomycetes sp. SH217860.06FU	100	A:AY204587 B:EF433993	*Alatospora acuminata* Fungi sp.	Leotiales	85,66 96,1	279 279
2527	Control	soil	Leotiomycetes sp. SH217860.06FU	100	A:AY204590 B:EF433993	*Alatospora acuminata* Fungi sp.	Leotiales	86,33 96,4	278 279
4084	Control	soil	Leotiomycetes sp. SH217860.06FU	100	A:AY204587 B:EF433993	*Alatospora acuminata* Fungi sp.	Leotiales	84,4 94,6	218 223
4482	Control	soil	Leotiomycetes sp. SH217860.06FU	100	A:AY204587 B:EF433993	*Alatospora acuminata* Fungi sp.	Leotiales	84,23 94,8	241 248
4654	Control	soil	Leotiomycetes sp. SH217860.06FU	100	A:AY204589 B:EF433993	*Alatospora acuminata* Fungi sp.	Leotiales	84,55 95,6	246 250
6232	Control	soil	Leotiomycetes sp. SH217860.06FU	100	A:AY204588 B:EF433993	*Alatospora acuminata* Fungi sp.	Leotiales	82,33 91,7	215 218
846	Control	end/ sapr	*Aureobasidium* sp. SH206630.06FU	100	A:EU272483 B:JX984742	*Aureobasidium pullulans* Fungi sp.	Dothideales	99,64 99,7	276 290
4310	Control	put root-as	Ascomycota sp. SH197703.06FU	95	A:FJ839606 B:HQ446013	*Brycekendrickomyces acaciae* Fungi sp.	Chaetothyriales	90,65 96,7	107 271

1238	Control	put root-as	Dothideomycetes sp. SH227704.06FU	84	A:AF050261 B:EF434005	*Capronia villosa* Fungi sp.	Chaetothyriales	86,47 96,9	170 193
6542	Control	put root-as	Dothideomycetes sp. SH227703.06FU	47	A:AF050261 B:EF434102	*Capronia villosa* Fungi sp.	Chaetothyriales	83,33 93,9	216 212
4327	Control	lich	*Massaria aucupariae* SH240306.06FU	9	A:DQ534198 B:DQ534198	*Cladonia fimbriata* *Cladonia fimbriata*	Lecanorales Lecanorales	95,75 95,8	212 212
854	Control	soil	Helotiales sp. SH220876.06FU	46	A:EF029222 B:JX001616	*Clathrosphaerina zalewskii* Helotiales sp	Helotiales Helotiales	90,15 94,9	274 276
5316	Control	unk	*Ophiocordyceps rubiginosiperitheciata* SH227646.06FU	8	A:AJ509868 B:GQ153126	*Pseudogymnoascus* sp. Leotiomycetes sp.	Inc sed, Leotiomycetes Leotiomycetes	83,91 81,9	174 177
5210	Control	soil	Ascomycota sp. SH241580.06FU	3	A:AJ509868 B:JX317140	*Pseudogymnoascus* sp. *Meliniomyces sp.*	Inc sed, Leotiomycetes Inc sed, Leotiomycetes	86,88 83,3	282 281
3682	Control	root-as	Sordariomycetes sp. SH224631.06FU	13	A:EF029228 B:FJ237215	*Hemibeltrania mitrata* Fungi sp.	Inc sed, Pezizomycotina	82,05 95,8	273 284
1206	Control	unk	Onygenales sp. SH236516.06FU	11	A:DQ534472 B:EU489998	*Lecidea cancriformis* Ascomycota sp.	Lecideales	86,94 83,5	268 266
1291	Control	soil	Helotiales sp. SH218183.06FU	11	A:DQ534472 B:HQ211691	*Lecidea cancriformis* Leotiomycetes sp.	Lecideales	86,48 87,1	281 278
1324	Control	unk	Helotiales sp. SH218183.06FU	5	A:DQ534472 B:HQ211691	*Lecidea cancriformis* Leotiomycetes	Lecideales	84,82 83,6	257 262

2187	Control	soil	*Pannaria pallida* SH229354.06FU	2	A:DQ534472 B:HQ211691	*Lecidea cancriformis* Leotiomycetes	Lecideales	84,91 82,5	159 154
1411	Control	ecm	*Meliniomyces* sp. SH207190.06FU	45	A:EF093180 B:HQ211966	*Meliniomyces bicolor* *Meliniomyces bicolor*	Inc sed, Leotiomycetes Inc sed, Leotiomycetes	96,42 98,2	279 277
1176	Control	unk	Helotiales sp. SH196873.06FU	10	A:FJ839617 B:EF635666	*Microglossum viride* Helotiales sp.	Geoglossales Helotiales	83,52 81,3	267 257
321	Control	sapr	*Phaeococcomyces* sp. SH217607.06FU	100	A:AY843154 B:EU480260	*Phaeococcomyces nigricans* soil fungus	Chaetothyriales	96,25 96,2	240 290
1586	Control	sapr	*Herpotrichiellaceae* sp. SH241308.06FU	100	A:AF050278 B:EU480246	*Phaeococcomyces nigricans* soil fungus	Chaetothyriales	84 96,5	300 289
1056	Control	soil	Leotiomycetes sp. SH217860.06FU	94	A:GU934582 B:EF433993	*Pseudeurotium bakeri* Fungi sp.	Inc sed, Dothideomycetes	88,27 86,6	162 194
1271	Control	soil	Leotiomycetes sp. SH217860.06FU	100	A:GU934582 B:EF433993	*Pseudeurotium bakeri* Fungi sp.	Inc sed, Dothideomycetes	88,89 94,4	162 195
4851	Control	soil	Leotiomycetes sp. SH217860.06FU	91	A:GU934582 B:EF433993	*Pseudeurotium bakeri* Fungi sp.	Inc sed, Dothideomycetes	88,27 92,8	162 167
6588	Control	soil	Leotiomycetes sp. SH217860.06FU	100	A:GU934582 B:EF433993	*Pseudeurotium bakeri* Fungi sp.	Inc sed, Dothideomycetes	87,58 93,8	161 192
2877	Control	unk	Leotiomycetes sp. SH217860.06FU	67	A:GU934582 B:KC007335	*Pseudeurotium bakeri* *Meliniomyces sp.*	Inc sed, Dothideomycetes Inc sed, Leotiomycetes	87,96 84,3	274 267

264	Control	end	Dothideomycetes sp. SH234498.06FU	98	A:AF297232 B:JQ759476	*Ramulispora sorghi* Dothideomycetes sp.	Capnodiales	86,07 96,8	280 277
4564	Control	soil	Leotiomycetes sp. SH217860.06FU	100	A:AF384677 B:EF433993	*Rhynchosporium secalis* Fungi sp.	Helotiales	82,2 95,7	264 253
96	Control	soil	*Pleomassariaceae* sp. SH194663.06FU	36	A:AY265337 B:HQ432975	*Tumularia aquatica* Ascomycota	Inc sed, Pezizomycotina	93,33 93,7	285 284
768	Warming	sapr	Ascomycota sp. SH194662.06FU	100	A:GQ152143 B:HQ211540	*Clavariopsis aquatica* Sordariomycetes sp.	Pleosporales	95,39 99,6	282 282
3365	Warming	soil	Helotiales sp. SH209317.06FU	43	A:AY245636 B:AM260896	*Elaphocordyceps ophioglossoides* Fungi sp.	Hypocreales	92,23 96	283 276
1955	Warming	in path	Helotiales sp. SH215692.06FU	100	A:AY245636 B:HQ211516	*Elaphocordyceps ophioglossoides* Leotiomycetes sp.	Hypocreales Leotiomycetes	89,45 95,9	218 222
739	Warming	sapr	Hypocreales sp. SH230367.06FU	100	A:EF110618 B:HQ211823	*Eucasphaeria capensis* Hypocreales	Inc sed, Sordariomycetes	88,51 100	174 189
1606	Warming	sapr	*Exophiala moniliae* SH214882.06FU	100	A:AB114131 B:HE605213	*Fonsecaea pedrosoi* *Exophiala moniliae*	Chaetothyriales Chaetothyriales	86,27 99,7	335 330
760	Warming	unk	*Libertella* sp. SH241107.06FU	39	A:FJ438389 B:KC588656	*Geastrumia polystigmatis* Fungi sp.	Inc sed, Pezizomycotina	86,91 90,6	275 276
1623	Warming	sapr	*Pseudogymnoascus appendiculatus* SH236515.06FU	30	A:EF540755 B:JX131373	*Pseudogymnoascus* sp. *Pseudogymnoascus* sp.	Inc sed, Leotiomycetes Inc sed, Leotiomycetes	95,15 93,2	165 161

1683	Warming	sapr	*Pseudogymnoascus roseus*	44	A:EF540755	*Pseudogymnoascus* sp.	Inc sed, Leotiomycetes	96,41	167
			SH236509.06FU		B:JX131373	*Pseudogymnoascus* sp.	Inc sed, Leotiomycetes	93,4	166
1730	Warming	sapr	*Pseudogymnoascus roseus*	57	A:EF540755	*Pseudogymnoascus* sp.	Inc sed, Leotiomycetes	96,95	164
			SH236509.06FU		B:JX131373	*Pseudogymnoascus* sp.	Inc sed, Leotiomycetes	94,5	163
3752	Warming	sapr	*Pseudogymnoascus roseus*	50	A:EF540755	*Pseudogymnoascus* sp.	Inc sed, Leotiomycetes	98,77	162
			SH236509.06FU		B:JX131373	*Pseudogymnoascus* sp.	Inc sed, Leotiomycetes	95,9	172
4837	Warming	sapr	*Pseudogymnoascus appendiculatus*	36	A:EF540755	*Pseudogymnoascus* sp.	Inc sed, Leotiomycetes	94,64	168
			SH236515.06FU		B:JX131373	*Pseudogymnoascus* sp.	Inc sed, Leotiomycetes	94,6	166
4913	Warming	sapr	*Pseudogymnoascus roseus*	93	A:EF540755	*Pseudogymnoascus* sp.	Inc sed, Leotiomycetes	98,18	165
			SH236509.06FU		B:JX131373	*Pseudogymnoascus* sp.	Inc sed, Leotiomycetes	97,6	166
4917	Warming	sapr	*Pseudogymnoascus roseus*	17	A:EF540755	*Pseudogymnoascus* sp.	Inc sed, Leotiomycetes	96,79	156
			SH236509.06FU		B:JX131373	*Pseudogymnoascus* sp.	Inc sed, Leotiomycetes	94,3	158
4946	Warming	unk	*Pseudogymnoascus appendiculatus*	45	A:EF540755	*Pseudogymnoascus* sp.	Inc sed, Leotiomycetes	93,6	172
			SH236515.06FU		B:JX131373	*Pseudogymnoascus* sp.	Inc sed, Leotiomycetes	90,5	169
4954	Warming	sapr	*Pseudogymnoascus roseus*	62	A:EF540755	*Pseudogymnoascus* sp.	Inc sed, Leotiomycetes	97,6	167
			SH236509.06FU		B:JX131373	*Pseudogymnoascus* sp.	Inc sed, Leotiomycetes	97,6	167

4963	Warming	sapr	*Pseudogymnoascus roseus*	37	A:EF540755	*Pseudogymnoascus* sp.	Inc sed, Leotiomycetes	95,78	166
			SH236509.06FU		B:JX131373	*Pseudogymnoascus* sp.	Inc sed, Leotiomycetes	94,5	165
5078	Warming	sapr	*Pseudogymnoascus appendiculatus*	32	A:EF540755	*Pseudogymnoascus* sp.	Inc sed, Leotiomycetes	97,04	169
			SH236515.06FU		B:JX131373	*Pseudogymnoascus* sp.	Inc sed, Leotiomycetes	97	169
5241	Warming	sapr	*Pseudogymnoascus appendiculatus*	29	A:EF540755	*Pseudogymnoascus* sp.	Inc sed, Leotiomycetes	96,09	179
			SH236515.06FU		B:JX131373	*Pseudogymnoascus* sp.	Inc sed, Leotiomycetes	93,9	179
5259	Warming	sapr	*Pseudogymnoascus roseus*	73	A:EF540755	*Pseudogymnoascus* sp.	Inc sed, Leotiomycetes	97,66	171
			SH236509.06FU		B:JX131373	*Pseudogymnoascus* sp.	Inc sed, Leotiomycetes	94,2	173
5502	Warming	sapr	*Pseudogymnoascus roseus*	49	A:EF540755	*Pseudogymnoascus* sp.	Inc sed, Leotiomycetes	98,66	149
			SH236509.06FU		B:JX131373	*Pseudogymnoascus* sp.	Inc sed, Leotiomycetes	96,7	153
5838	Warming	sapr	*Pseudogymnoascus roseus*	39	A:EF540755	*Pseudogymnoascus* sp.	Inc sed, Leotiomycetes	96,3	162
			SH236509.06FU		B:JX131373	*Pseudogymnoascus* sp.	Inc sed, Leotiomycetes	93,3	163
5937	Warming	sapr	*Pseudogymnoascus appendiculatus*	25	A:EF540755	*Pseudogymnoascus* sp.	Inc sed, Leotiomycetes	95,76	165
			SH236515.06FU		B:JX131373	*Pseudogymnoascus* sp.	Inc sed, Leotiomycetes	93,9	164
6010	Warming	sapr	*Pseudogymnoascus roseus*	58	A:EF540755	*Pseudogymnoascus* sp.	Inc sed, Leotiomycetes	98,05	154
			SH236509.06FU		B:JX131373	*Pseudogymnoascus* sp.	Inc sed, Leotiomycetes	93,3	164

6142	Warming	sapr	*Pseudogymnoascus roseus* SH236509.06FU	59	A:EF540755 B:JX131373	*Pseudogymnoascus* sp. *Pseudogymnoascus* sp.	Inc sed, Leotiomycetes Inc sed, Leotiomycetes	98,74 96,9	159 161
6854	Warming	unk	*Pseudogymnoascus appendiculatus* SH236515.06FU	26	A:EF540755 B:JX131373	*Pseudogymnoascus* sp. *Pseudogymnoascus* sp.	Inc sed, Leotiomycetes Inc sed, Leotiomycetes	94,41 90,3	179 185
5938	Warming	soil	*Pseudogymnoascus roseus* SH236509.06FU	57	A:AY873965 B:HQ211674	*Pseudogymnoascus* sp. Leotiomycetes sp.	Inc sed, Leotiomycetes Leotiomycetes	98,05 95,7	154 162
4935	Warming	sapr	*Pseudogymnoascus roseus* SH236509.06FU	28	A:EF540755 B:GU083197	*Pseudogymnoascus* sp. soil fungus	Inc sed, Leotiomycetes	96,43 95,2	168 166
5025	Warming	sapr	*Pseudogymnoascus roseus* SH236509.06FU	77	A:EF540755 B:GU083185	*Pseudogymnoascus* sp. soil fungus	Inc sed, Leotiomycetes	96,51 94,7	172 171
5144	Warming	sapr	*Pseudogymnoascus roseus* SH236509.06FU	63	A:EF540755 B:GU083185	*Pseudogymnoascus* sp. soil fungus	Inc sed, Leotiomycetes	96,65 94,4	179 179
5823	Warming	sapr	*Pseudeurotiaceae* sp. SH209202.06FU	45	A:EF540755 B:GU083185	*Pseudogymnoascus* sp. soil fungus	Inc sed, Leotiomycetes	96,93 96,3	163 163
6137	Warming	sapr	*Pseudogymnoascus roseus* SH236509.06FU	52	A:EF540755 B:GU083185	*Pseudogymnoascus* sp. soil fungus	Inc sed, Leotiomycetes	95,18 94,5	166 165
6141	Warming	sapr	*Pseudogymnoascus roseus* SH236509.06FU	39	A:EF540755 B:JQ666376	*Pseudogymnoascus* sp. soil fungus	Inc sed, Leotiomycetes	95,18 93,4	166 167

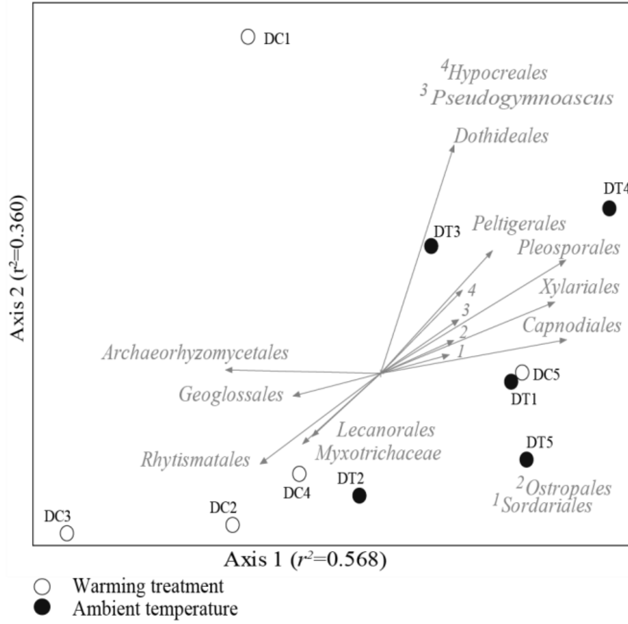
2346	Warming	end	*Pseudogymnoascus roseus*	89	A:AJ608972	*Pseudogymnoascus* sp.	Inc sed, Leotiomycetes	97,59	249
			SH236509.06FU		B:JX984721	Fungi sp.		96,8	249
2592	Warming	root-as	*Lachnum* sp.	95	A:AB267648	*Lachnum papyraceum*	Helotiales	96,4	222
			SH189775.06FU		B:HQ212064	*Lachnum* sp.	Helotiales	98,7	229
1073	Warming	sapr	*Lecanicillium* sp.	70	A:AB378519	*Lecanicillium psalliotae*	Hypocreales	98,63	291
			SH196298.06FU		B:FJ490755	*Lecanicillium* sp	Hypocreales	99,3	293
5601	Warming	put root as	*Meliniomyces* sp.	99	A:EF093178	*Meliniomyces variabilis*	Inc sed, Leotiomycetes	91,55	284
			SH207201.06FU		B:GU998155	*Meliniomyces vraolstadiae*	Inc sed, Leotiomycetes	96,4	280
270	Warming	put p path	Dothideomycetes sp.	65	A:DQ459079	*Mycosphaerella areola*	Capnodiales	94,62	279
			SH240141.06FU		B:FR773398	Capnodiales	Capnodiales	99,5	219
1217	Warming	sapr	*Oidiodendron tenuissimum*	64	A:AF062808	*Oidiodendron tenuissimum*	Inc sed, Dothideomycetes	98,13	268
			SH217752.06FU		B:KC588613	Fungi sp.		97,1	275
785	Warming	sapr	*Penicillium angulare*	100	A:AF125937	*Penicillium angulare*	Eurotiales	99,33	297
			SH213212.06FU		B:KC773828	*Penicillium angulare*	Eurotiales	99,3	297
6069	Warming	sapr	*Penicillium bialowiezense*	100	A:AB479306	*Penicillium brevicompactum*	Eurotiales	98,3	294
			SH193630.06FU		B:FJ386891	Fungi sp.		98,6	294
358	Warming	ecm	*Meliniomyces* sp.	92	A:AY394907	*Rhizoscyphus ericae*	Helotiales	95,32	278
			SH207168.06FU		B:HQ211522	Sordariomycetes sp.		99,3	276
3375	Warming	end	Dothideomycetes sp.	97	A:AF455415	*Saccharicola bicolor*	Pleosporales	98,5	266
			SH196053.06FU		B:AM260897	Fungi sp.		99,2	266

Author Contributions:

JG conceived the research idea, and JG and LNM carried out the soil sampling for this paper. TAS extracted the DNA, carried out PCR and prepared the samples for the Ion Torrent run. JG, LNM and TAS conducted the bioinformatic and statistical analyses and wrote the manuscript with input from JMW, MDW and ES. All authors read and approved the final manuscript.

Dr. Luis N Morgado and Dr. József Geml on the way to the sampling plots

Dry tundra



Moist tundra

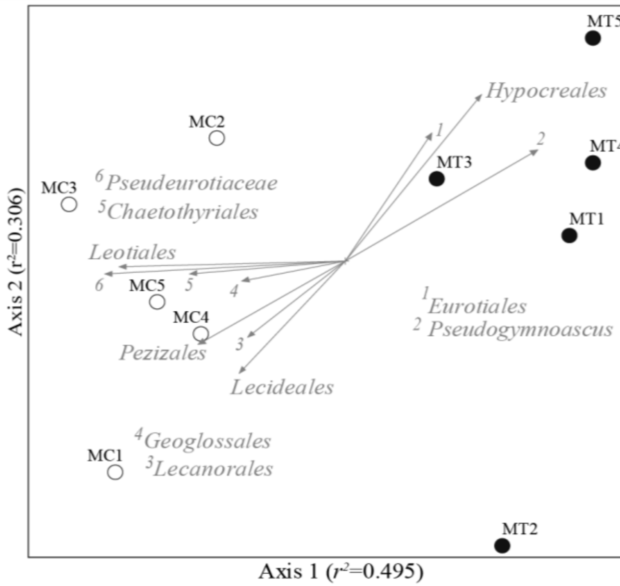


Figure 2.S3. *(previous page)* Non-metric multidimensional scaling (NMDS) ordination plot for ascomycete communities of the dry and moist tundra types based on OTU abundance data. *Vectors are shown for variables correlated with ordination axes at |R|>0.5 Dry tundra: Archaeorhyzomycetales R= -0.756, Rhytismatales R= -0.665, Geoglossales R= -0.567, Myxotrichaceae R= -0.535; Lecanorales R= -0.502, Sordariales R=0.501, Ostropales R=0.518, Dothideales R=0.519, Pseudogymnoascus R=0.535, Hypocreales R=0.547, Peltigerales R=0.641, Xylariales R=0.801, Pleosporales R=0.825, Capnodiales R=0.827. Final stress value for the ordination plot =4.79; MRPP: A=0.0324, p=0.0585. MANOVA: F=1.63, P=0.053. Treatment explained 11% of the variation. Moist tundra: Pseudeurotiaceae R= -0.949, Leotiales R= -0.921, Chaetothyriales R= -0.764, Pezizales R= -0.742, Lecideales R= -0.629, Geoglossales R= -0.621, Lecanorales R= -0.604, Eurotiales R=0.569, Hypocreales R=0.715, Pseudogymnoascus R=0.848.Final stress value for the ordination plot =7.46; MRPP: A=0.145, P=0.035. MANOVA: F=4.11, P=0.008. Treatment explained 38.4% of the variation*

Data Accessibility: *Representative sequences of all OTUs in this paper were submitted to Genbank: KJ826608-KJ828710. The raw (fastq) sequence data is submitted to Dryad (doi:10.5061/dryad.2fc32).*

Open-top chambers at Toolik Lake research field station, Alaska

Chapter 3

CHANGES IN COMPOSITION AND ABUNDANCE OF FUNCTIONAL GROUPS OF ARCTIC FUNGI IN RESPONSE TO LONG-TERM WARMING

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Abstract

We characterized fungal communities in dry and moist tundra and investigated the effect of long-term experimental summer warming on three aspects of functional groups of arctic fungi: richness, community composition, and species abundance. Warming had profound effects on community composition, abundance, and, to a lesser extent, on richness of fungal functional groups. In addition, our data show that even within functional groups, the direction and extent of response to warming tend to be species-specific and we recommend that studies on the role of fungal communities in nutrient cycling take into account species-level responses.

Keywords: climate change, fungal ecology, metabarcoding, tundra

Introduction

The arctic tundra is considered a maritime biome (Walker et al, 2005) and as a result of the retreating sea ice, arctic land surface temperatures are increasing, causing major changes in terrestrial ecosystems (Pearson et al, 2013; Post et al, 2013). In response to warming temperatures, shifts in land surface vegetation and

ecosystem C cycling have already been observed in terrestrial arctic ecosystems (Pearson et al, 2013; Leffler et al, 2016). However, the responses of belowground communities, such as soil microbes, are less certain (Schaeffer et al, 2013).

Fungi play a central role in 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). Given their intimate relationships with plants in a wide range of symbioses, fungi are expected to play an important role in arctic vegetation change. In this study, we compared fungal communities across plots with ambient and experimentally increased summer air and near-surface soil temperature to reveal (1) how community composition and abundance of functional groups of fungi change in response to long-term increase in summer temperature; and (2) whether these responses are similar in dry and moist tundra.

Materials and Methods

Data generation

The study was conducted at the Toolik Field Station in Alaska, USA, where the main vegetation types are dry acidic heath and moist acidic tussock tundra (Walker et al, 1999; Welker et al, 2000). Open top chambers (OTCs), with 1 m^2 area and 0.4 m height, were established in 1994 in both tundra types to increase summer air and upper soil temperature by ca. 2 °C, leading to shifts in edaphic factors and vegetation (Walker et al, 1999; Welker et al, 2000; Walker et al, 2006). We sampled 100 soil cores across 20 plots: five replicate plots in the OTC and control plots in each tundra type, with five soil cores of 2 cm diameter and 20 cm depth per plot that were mixed and lyophilized. We extracted DNA using Macherey-Nagel NucleoSpin-Soil kit. PCR and sequencing of the ITS2 rDNA were done with primers fITS7 and ITS4, labelled with sample-specific tags, as described earlier (Morgado et al, 2015; Semenova et al, 2015; Geml et al, 2015). We generated 4 047 811 reads using Ion 318™ Chip (doi:10.5061/dryad.2fc32).

Bioinformatics

Primers and adapters were removed and poor-quality ends were trimmed off using 0.02 error probability limit in Geneious Pro 5.6.1. Sequences were truncated to 200 bp and sequences with expected error > 1 were discarded using USEARCH v.8.0

(Edgar, 2010). The remaining 1 632 682 sequences were collapsed into unique sequence types on a per-sample basis while preserving read counts. Singletons were discarded and the resulting 1 092 238 high-quality sequences were grouped into 4069 operational taxonomic units (OTUs) with USEARCH at 97% sequence similarity, while excluding 9026 (0.3%) chimeras. We identified 3501 OTUs based on the UNITE fungal database, discarding OTUs with < 70% similarity to any fungal sequence. We assigned ecological functions to 1655 OTUs following (Tedersoo et al, 2014): arbuscular mycorrhizal (5 OTUs), animal parasitic (18), ectomycorrhizal (417), lichenicolous (9), lichenized (156), mycoparasitic (39), plant pathogenic (134), and saprotrophic (877) fungi. Because of low richness, arbuscular mycorrhizal fungi were excluded, while animal- and mycoparasites were combined, as were lichens and lichenicolous fungi.

Statistical analyses

For each functional group, OTU richness (S), Shannon's and Simpson's diversity indices were calculated in PC-ORD v. 6.0 (McCune and Grace, 2002) and were compared using two-way ANOVA to test for effects of warming, tundra type, and their interaction. We visualized changes in community composition of functional groups with non-metric multidimensional scaling (NMDS) based on presence-absence data with Bray-Curtis distance and 500 iterations in PC-ORD. We tested for statistical difference in fungal community composition among tundra types and treatments using multi-response permutation procedure (MRPP).

We assessed the effect of warming on abundance on a per-OTU basis by comparing DNA sequence counts (Hedges' *D*) and calculating the mean effect size with 95% confidence intervals using METAWIN v. 2.0 (Rosenberg et al, 1999). Using sequence read counts as a proxy for abundance (biomass) is constrained due to interspecific differences in copy number and length of ITS (Amend et al, 2010). However, for individual OTUs, changes in sequence counts can indicate relative changes in abundance (biomass) (Amend et al, 2010). We compared per-OTU mean read counts across the control and warmed plots to calculate size effects with variance and calculated mean effect size with 95% confidence interval for each functional group. This approach allowed us to depict the variation in responses of individual OTUs to warming and evaluate the overall responses of functional groups.

Results

Diversity measures

Tundra type had the strongest effect on lichens, where all diversity measures were significantly higher in the dry tundra. Similarly, in the animal- and mycoparasitic fungi, both Shannon's and Simpson's diversity indices were higher in the dry tundra, even though differences in richness were insignificant. Warming only affected richness in ectomycorrhizal fungi, with a strong decrease in the moist tundra, although Shannon's and Simpson's diversity indices were not significantly affected. A similar, but somewhat weaker trend was seen in lichens. Shannon's diversity decreased in saprotrophic fungi, even though neither richness nor Simpson's diversity were strongly affected. The interaction of warming and tundra type showed significant decrease in richness in ectomycorrhizal and saprotrophic fungi, and only in saprotrophs regarding Shannon's and Simpson's diversity (Table 3.1).

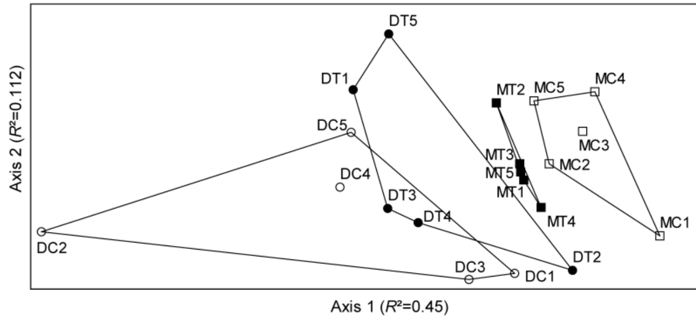
Community composition

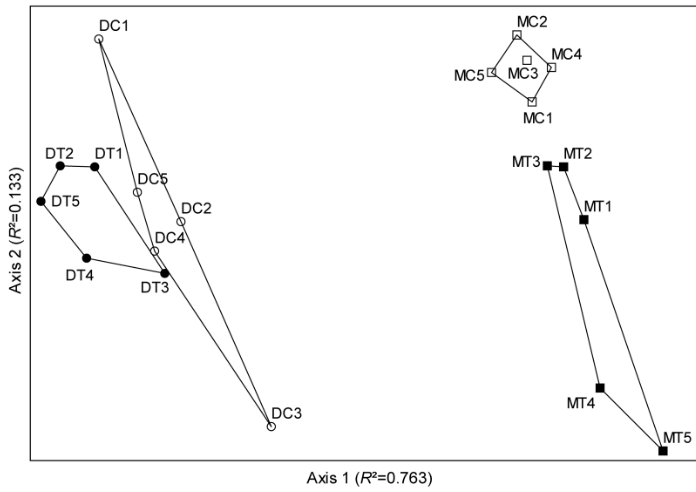
NMDS analyses resulted in 2-dimensional solutions with final stress values of 0.11101 (animal and mycoparasites), 0.09244 (ectomycorrhizal fungi), 0.05238 (lichens and lichenicolous fungi), 0.12336 (plant pathogens), and 0.07267 (saprotrophs), with final instability values < 0.00001. The NMDS plots revealed strong structuring in all functional groups with tundra type being the most influential variable (Table 3.2, Fig. 3.1). Warming had a strong effect on the fungal community in the moist tundra, where community composition was significantly different between treatment and control in all functional groups. However, in the dry tundra, only plant pathogens showed a significant treatment effect on composition (Table 3.2).

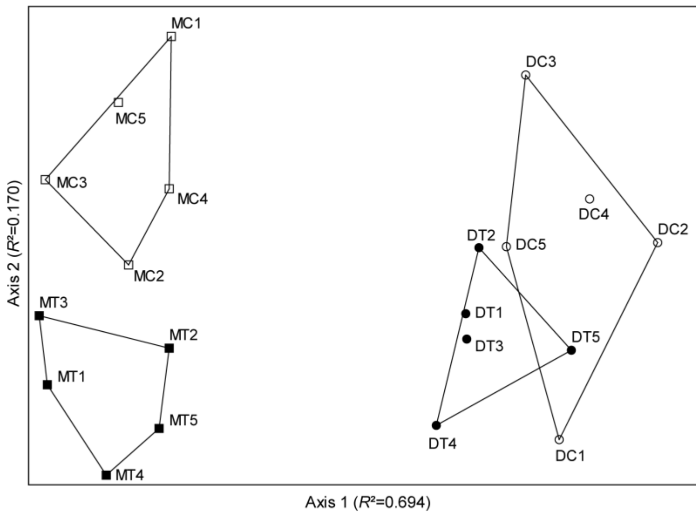
Abundance at the species-level

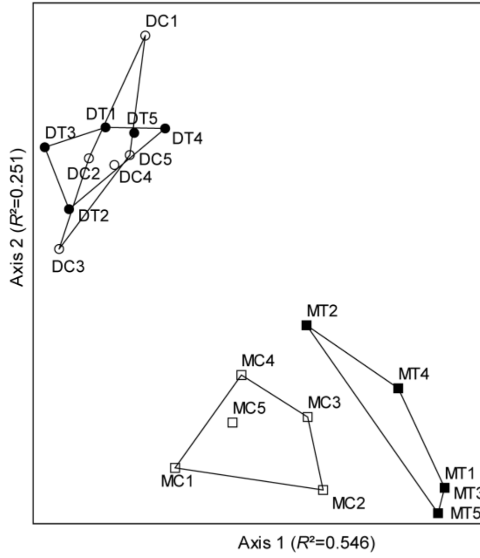
Sequence read counts (a proxy for abundance or biomass) of most OTUs differed between the control and treatment as indicated by non-zero effect values and their variance intervals (Fig. 3.2a).

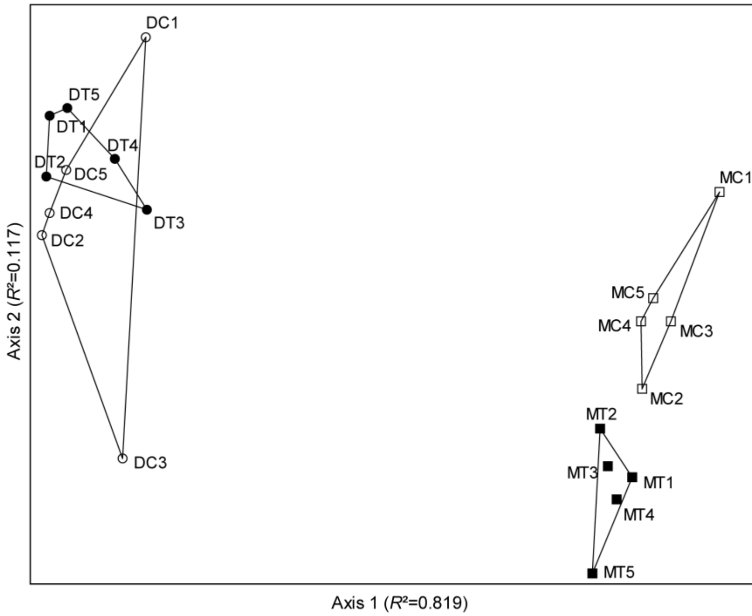
Figure 3.1. (next two pages) *Non-metric multidimensional scaling (NMDS) ordination plots for functional groups of arctic fungal communities in the warmed and control plots in the dry and moist tundra types based on presence-absence. M = moist tundra, D = dry tundra, C = control, T = warming.*


Animal- and mycoparasites
DT5
DT1
MT2
MC5
MC4
DC5
MC3
MT3
MT5
MC2
DC4
MT1
DT3
DT4
MT4
MC1
DC2
DT2
DC3
DC1
Axis 2 (R^2=0.112)
Axis 1 (R^2=0.45)


Ectomycorrhizal fungi
DC1
MC2
MC4
MC5
MC3
MC1
DT2
DT1
MT3
MT2
DC5
DT5
DC2
MT1
DC4
DT4
DT3
MT4
DC3
MT5
Axis 2 (R^2=0.133)
Axis 1 (R^2=0.763)


Plant pathogens
MC1
DC3
MC5
MC3
MC4
DC4
DC2
DT2
DC5
MC2
MT3
DT1
DT3
MT2
DT5
MT1
MT5
DT4
DC1
MT4
Axis 2 (R^2=0.170)
Axis 1 (R^2=0.694)


Lichens and lichenicolous fungi
DC1
DT1
DT5
DT3
DT4
DC5
DC2
DC4
DT2
DC3
MT2
MC4
MT4
MC5
MC3
MT1
MC1
MT3
MC2
MT5
Axis 2 (R^2=0.251)
Axis 1 (R^2=0.546)


Saprotrophs
DC1
DT5
DT1
DT4
DT2
DC5
DT3
DC4
DC2
MC1
MC5
MC4
MC3
MC2
MT2
DC3
MT3
MT1
MT4
MT5
Axis 2 (R^2=0.117)
Axis 1 (R^2=0.819)

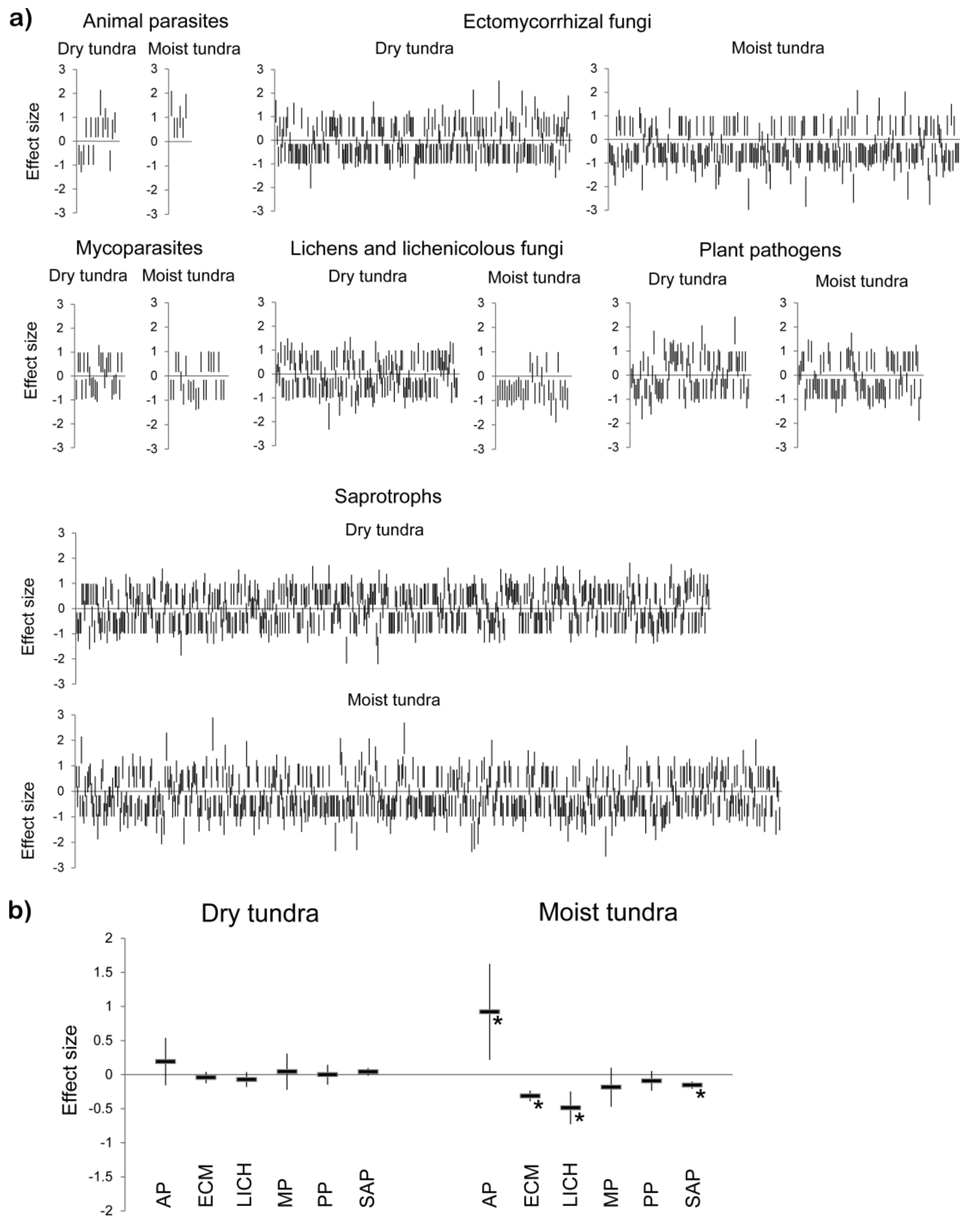


Figure 3.2. a) *Responses of individual OTUs in the functional groups to warming. Each vertical line represent the effect of warming on mean DNA sequence read count with variance for a fungal OTU. Positive and negative effects indicate increased and decreased abundance in the warmed plots, respectively. b) Summarized responses of functional groups of arctic fungi to warming. The values represent the mean effect size and 95% confidence interval from meta-analyses of all OTUs in the functional group in question. Functional group abbreviations are given in Table 3.1.*

Table 3.1. (next page, upper table) *The results of two-way ANOVA on OTU richness, Shannon's and Simpson's diversity indices calculated for functional groups of fungi. Significant p-values are indicated in bold. Abbreviations: ECM = ectomycorrhizal fungi, AP = animal parasites, MP = mycoparasites, LIC = lichens and lichenicolous fungi, PP = plant pathogens, SAP = saprotrophs.*

Table 3.2. (next page, lower table) *Effects of tundra type the warming on community composition of functional groups of fungi as calculated using Multi-Response Permutation Procedure. Significant P-values are indicated in bold.*

Index	Effects	ECM	AP+MP	LIC	PP	SAP
Richness (S)	treatment (warming)	**0.0168**	0.3932	0.069	0.6171	0.2476
	tundra type (dry vs. moist)	0.2692	0.604	**<0.0001**	0.531	0.5854
	treatment × tundra type	**0.0176**	1	0.5795	0.4854	**0.0477**
Shannon's diversity (H)	treatment (warming)	0.2623	0.0881	0.0782	0.494	**0.0324**
	tundra type (dry vs. moist)	0.1237	**0.0309**	**<0.0001**	**0.036**	0.2213
	treatment × tundra type	0.8647	0.7132	0.844	0.4612	**0.0023**
Simpson's diversity ('D)	treatment (warming)	0.373	0.0541	0.2935	0.6529	1
	tundra type (dry vs. moist)	0.1313	0.0368	0.0001	0.0693	1
	treatment × tundra type	1	1	0.5028	0.6529	0.001

Functional groups	Tundra type		Warming in dry tundra		Warming in moist tundra	
	effect (A)	***P***	**effect (A)**	***P***	**effect (A)**	***P***
Ectomycorrhizal	0.15236	< 0.00001	0.0219	0.07663	0.10865	0.00197
Animal parasites and mycoparasites	0.1153	0.00002	0.01459	0.69563	0.14281	0.00196
Lichens and lichenicolous fungi	0.21142	< 0.00001	0.00677	0.28502	0.15166	0.01258
Plant pathogens	0.18262	< 0.00001	0.04895	0.03357	0.09515	0.00308
Saprotrophs	0.19335	< 0.00001	0.01331	0.16814	0.08925	0.00389

Meta-analyses of trends of the individual OTUs per functional groups indicated significant changes only in the moist tundra, where there was a significant decline in ectomycorrhizal, lichenized, and saprotrophic fungi, as well as a significant increase in animal pathogens, while mycoparasites and plant pathogens showed a non-significant decline (Fig. 3.2b).

Discussion

Tundra type greatly affected fungal communities with shifts in composition and OTU abundance in response to warming being stronger in the moist as opposed to the dry tundra. Because most fungal symbiotic plants occur in both vegetation types, the profound fungal compositional differences between moist and dry tundra are likely caused by differences in fundamental abiotic attributes, such as snow cover, active layer depth, soil moisture, nutrients, and temperature (Walker et al, 1999). These findings and the accumulating evidence (Welker et al, 2000; Welker et al, 1997) suggest that warming responses of microbial and plant communities likely are predicated on soil water conditions and resulting differences in productivity among tundra types.

Changes in communities of arctic fungal functional groups have been scarcely documented, except in ectomycorrhizal fungi (Morgado et al, 2015). The compositional differences between the warmed and control plots in all functional groups indicate that even in groups without major changes in richness, the turnover is substantial. Although such compositional shifts are particularly evident in the moist tundra, animal- and mycoparasites, ectomycorrhizal fungi, and plant pathogens also display clearly visible changes in the dry tundra in response to warming (Fig. 3.1, Table 3.2).

The high proportion of OTUs with marked changes in abundance was a striking result (Fig. 3.2a). Even in the dry tundra, where the overall effect size of warming was not significant, most OTUs showed a clear trend, with only a small fraction of OTUs seemingly unaffected by warming. This indicates that response to warming likely is species-specific within these broad ecological groups. The importance of species-specific response has not been emphasized in other Arctic system studies of climate change and may be influenced by fine-scale changes in soil traits and species interactions.

Overall trends were more profound in the moist tundra, where significant changes were observed in most functional groups (Fig. 3.2b). The only increase was in

animal parasites that is in agreement with observed warming-induced increases in insect abundance (Hasle, 2013). All OTUs of animal parasites in the moist tundra were positively affected by warming and even in the dry tundra this group showed the largest, although not significant, increase. Abundance decrease in ectomycorrhizal fungi may have functional implications and the fact that several ectomycorrhizal fungi showed positive response to warming, while most were negatively affected, indicates substantial shift in the community. The strong decrease in lichen abundance was in agreement with formerly reported decrease in lichen cover due to increased shading by shrubs in the warmed moist tundra (Welker et al, 2000). In the dry tundra, where shading is minimal, several lichens benefited from warming (Fig. 3.2a). The decrease in saprotrophs is surprising in light of non-significant changes in richness (above) and previous findings on warming-induced increase in litter accumulation (Welker et al, 2000) and in microbial decomposition rates (Sistla et al, 2013). However, distinct species-specific responses to warming were revealed in saprotrophic taxa as well.

In this paper, we provide evidence that long-term experimental summer warming has profound effects on community composition and abundance of functional groups of arctic fungi. We also emphasize that, while there are similarities within functional groups, changes in occurrence and abundance in response to warming tend to be 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. Finally, we advocate the integration of taxonomic and functional data into climatic models to better understand the influence of climate on soil microbial community structure and function and their contributions to climate-linked processes.

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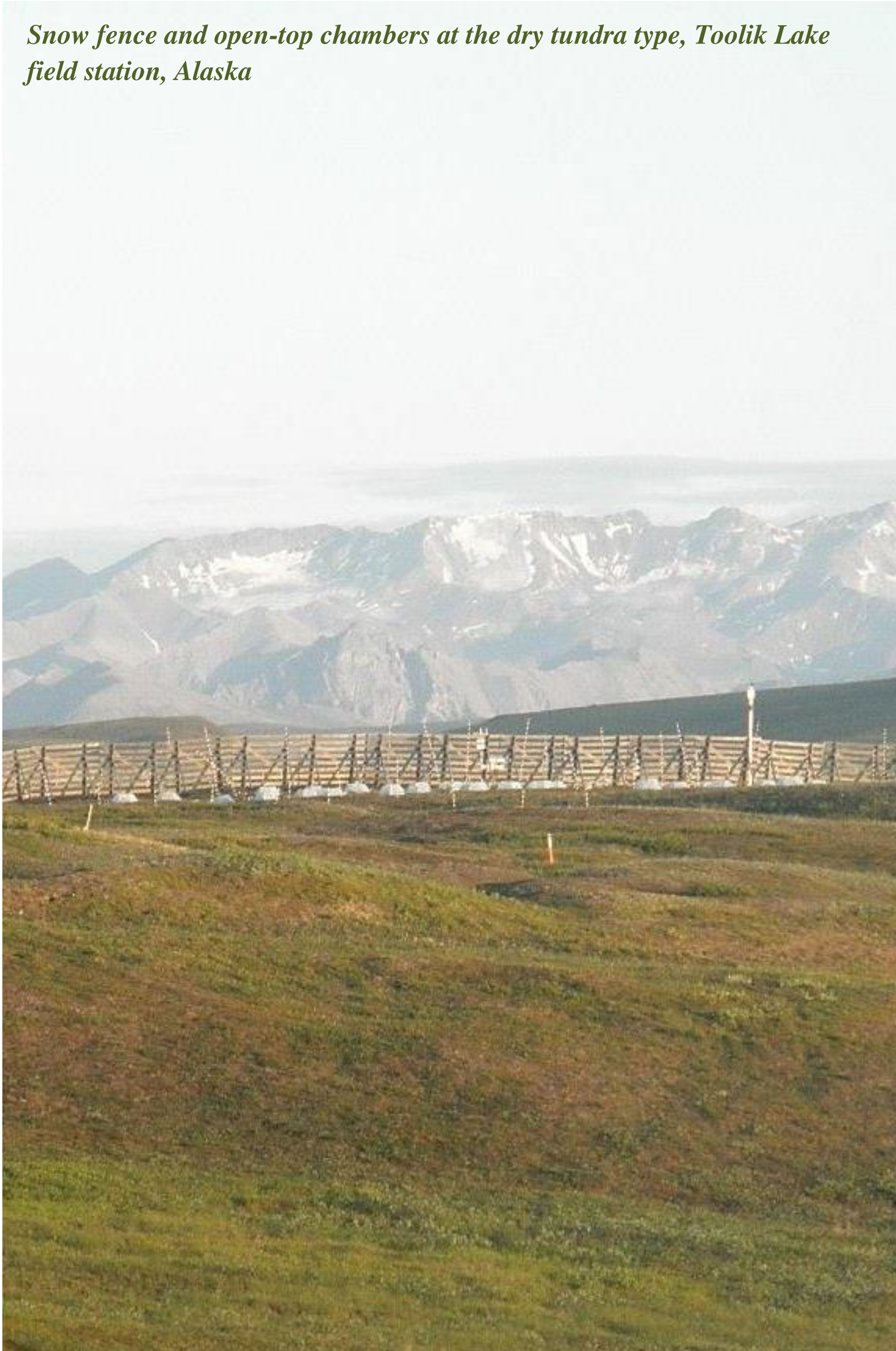

Snow fence and open-top chambers at the dry tundra type, Toolik Lake field station, Alaska

Chapter 4

COMPOSITIONAL AND FUNCTIONAL SHIFTS IN ARCTIC FUNGAL COMMUNITIES IN RESPONSE TO EXPERIMENTALLY INCREASED SNOW DEPTH

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Abstract

Climate warming leads to more intensive evaporation from the Arctic sea resulting in increased precipitation in the low Arctic, e.g., higher snowfall during winter. Deeper snow keeps the arctic soils warmer and alters soil attributes and vegetation, e.g., increase in nitrogen availability, expansion of shrubs and decline in shade-intolerant lichens and bryophytes. Saprotrophic and plant-symbiotic fungi play key roles in these processes, but the effects of increased snow depth on their community composition remain unknown. In the present work, we utilized DNA metabarcoding to study the effects of long-term experimental manipulations of snow depth on soil fungal communities in dry heath and moist tussock tundra in Arctic Alaska. We report strong changes in fungal community compositions in the two tundra types, with pronounced declines observed in the majority of fungal functional guilds, including ectomycorrhizal, lichenized, plant pathogenic, saprotrophic and bryophyte-associated species. The observed changes in lichenized and bryophyte-associated fungi are in agreement with previously published above-ground changes, i.e. decrease of lichen and bryophyte cover and diversity. However, the majority of observed trends, including the decline of ectomycorrhizal fungi (that were anticipated to benefit from the expansion of their host plants), suggest that changes in fungal communities do not entirely correspond to and are not primarily driven by shifts in vegetation. Instead, arctic fungal communities

appear to exhibit faster turnover that may be influenced by dynamic interactions with numerous biotic and abiotic factors, e.g., soil nutrient cycling and community dynamics in other groups of soil microorganisms. We highlight the importance of "below-ground studies" in assessing ecosystem responses to climatic changes, because faster turnover of microbial communities may be applicable for monitoring early-stage alterations caused by climatic changes.

Introduction

Arctic ecosystems are among the biomes with the greatest rate of climate warming (IPCC 2007; Kaufman et al, 2009; Serreze and Bary, 2011; Bintaja and Selten, 2014). Annual temperature increases reported over the last three decades for the arctic region approached 0.1°C (Anisimov et al, 2007), which is exceeding considerably the global average value of 0.017°C (Comiso and Hall, 2014). Rising temperatures intensify local surface evaporation of the Arctic sea, as well as enhance moisture inflow from lower latitudes (Bintaja and Selten, 2014), leading to increased precipitation in the Arctic region (Kattsov and Walsh, 2000; Stocker et al, 2013; Bintaja and Selten, 2014). According to models, arctic precipitation may increase more than 30 to 50 percent by the end of twenty-first century (Stocker et al, 2013; Bintaja and Selten, 2014). In the arctic tundra, where winter can be up to 9 months long and winter temperatures are well below the freezing point, most of the precipitation falls as snow resulting in deeper snow cover in winter (Derksen et al, 2015). Increased snow depth is expected based on forecasted precipitation increases (Bintaja and Selten, 2014) and due to expansion of shrubs that increase snow depth by 10-25% due to increased wind shelter, i.e. snow trapping effect (Larsen et al, 2007; Sturm et al, 2001).

Deeper snow, in turn, insulates soils and prevents soils from becoming excessively cold leading to a suite of consequences during winter and during the subsequent summers (Jones et al, 1998; Schimel et al, 2004; Screen and Simmonds, 2012; Collins et al, 2013). These consequences include alterations in microbial N and C mineralization (Schimel et al, 2004; Sturm et al, 2005; Welker et al, 2005), increases in plant leaf N (Welker et al, 2005), greater C fixation (Pattison and Welker, 2014), shifts in nutrient cycling and changes in plant community composition (Sweet et al, 2014). For instance, in the moist tussock tundra of Arctic Alaska, experimental snow addition (ca 1-1.2 m) resulted in significant increases in 1) soil temperatures from December to April and greater monthly thaw depth (Natali et al, 2011), 2) soil moisture likely resulting from the surface subsidence

(Natali et al, 2012), 3) ecosystem respiration during the growing season and a 2-fold increase in annual CO_2 loss to the atmosphere, 4) NH_4^+ levels in soils through the fall and winter (Schimel et al, 2004), 5) foliar and litter N mass (Natali et al, 2012), 6) mean N availability (Pattison and Welker, 2014), 7) overall plant growth and canopy height, 8) cover of deciduous shrubs *Betula nana* and *Salix pulchra* 9) cover of a graminoid *Eriophorum vaginatum*, and in 10) accumulation of litter (Mercado-Diaz, 2011). Simultaneously, the cover of shade-intolerant lichens and bryophytes has decreased (Wahren et al, 2005; Wipf and Rixen, 2010; Mercado-Diaz, 2011; Loranty and Goetz, 2012; Pattison and Welker, 2014). In the adjoining dry heath tundra deeper snow resulted in 1) a nearly 4-fold increase in winter NH_4^+ levels, 2) increased cover of shrubs *Dryas octopetala*, *Arctostaphylus alpina*, *Vaccinium vitis-idea*, *Loiseleuria procumbens*, 3) decline of lichens, and in 4) increased accumulation of litter (Schimel et al, 2004; Fahnestock et al, 2000; Wahren et al, 2005; Mercado-Diaz, 2011).

Alterations in the arctic plant communities are generally expected to be coupled with the shifts in soil fungal communities (Dahlberg and Bultman, 2013), especially, in mycorrhizal and root-associated fungi, due to tight associations between fungi and plants in nutrient-poor tundra soils (Hobbie and Hobbie, 2006; Buckeridge and Grogan, 2008; Hobbie et al, 2009). Such a correspondence between changes in vegetation coverage, soil temperature and fungal community composition has been observed in summer warming experiments in the Arctic (Deslippe et al, 2011; Geml et al, 2015; Morgado et al, 2015; Semenova et al, 2015). In Alaska, an 18-year summer warming experiment (+2°C, ca 2 months per year) resulted in reorganization of soil fungal communities, particularly in the moist tussock tundra: ectomycorrhizal basidiomycetes declined in richness and their community composition shifted according to their functional traits (e.g., mycelial characteristics) (Morgado et al, 2015), while the richness of ascomycetes did not change, and their communities shifted in accordance with the availability of hosts/ -substrates for different ecological groups (Semenova et al, 2015).

Besides preventing soils from cooling fast (i.e. keeping them warm), deeper snow protects the soils and vegetation from the wind and frost disturbances (Blok et al, 2015), alters soil moisture, and shortens the vegetation season due to the delayed snowmelt (Wahren et al, 2005; Blok et al, 2015). The collective effects of deeper snow on arctic soil fungal communities are unknown, although there is some evidence that deeper snow increases the potential of pathogenic fungi to cause

disease outbreaks in arctic and alpine ecosystems (Oloffson et al, 2011; Natali and Mack, 2011; Barbeito et al, 2013).

In this study we investigated the responses of soil fungi to increased snow depth in arctic tundra of Northern Alaska. We compared fungal community compositions across the plots with ambient and experimentally increased snow depth in two main tundra types found throughout the region: dry heath and moist tussock tundra. By analyzing these four experimental treatments, we aimed to answer 1) how richness and community composition of fungi change in response to long-term increase in snow depth; 2) whether these responses are similar in dry and moist tundra; 3) how taxonomic and ecological groups of fungi alter in response to long-term winter warming.

Materials and methods

Study sites

The experimental sites were located at Toolik Lake area, situated at the northern foothills of the Brooks Range, Alaska (68°38´N, 149°34´W). Two main vegetation types are found throughout the region: dry heath and moist tussock tundra. The dry site is represented by the dwarf-shrub and fruticose-lichen tundra, and is characterized by *Dryas octopetala*, *Salix polaris* and *Vaccinium spp.* The moist site vegetation is tussock-sedge dwarf-shrub tundra, characterized by *Betula nana*, *Salix pulchra* and *Eriophorum vaginatum* (Walker et al, 1999). The detailed description of the both sites could be found at arcticatlas.org and in Walker et al. (1999) and Kade et al. (2005). The annual precipitation in the region ranges from 200 mm to 400 mm, and ca. 50% of the precipitation falls as snow. The average snow depth approaches ca. 50 cm (DeMarco et al, 2011). As a part of the International Tundra Experiment (ITEX) program initiated in 1994 (Henry and Molau, 1997; Welker et al, 1997; Jones et al, 1998; Welker et al, 2000), the snow fence experiments were established in both dry and moist tundra. Snow fences are wooden fences, 2.8 m tall and 60 m long, and they are set on east-west axes to accumulate snow (Fig 4.1a) brought over by the predominant winds and storms blown from the Brooks Range to the south (Walker et al, 1999). Fences create a leeward drift of approximately 60 m long, with three zones: a deep zone with the snow depth of 2-3 m, moderate zone (0.5-2 m snow depth) and a shallow zone (< 0.5 m snow depth) (Fig. 4.1a) (Walker et al, 1999; Pattison and Welker, 2014). Snow accumulation behind the fence causes more consistent and largely higher winter soil temperatures; for instance, at the 2 cm depth, the average soil

temperatures in snow fence treatment approached -2.9°C versus -4.7°C in the control soils (Pattison and Welker, 2014). The lowest soil temperatures reported for the snow fence treatment were ca -7°C versus ca. -35°C observed across the control plots (Walker et al, 1999; Schimel et al, 2004).

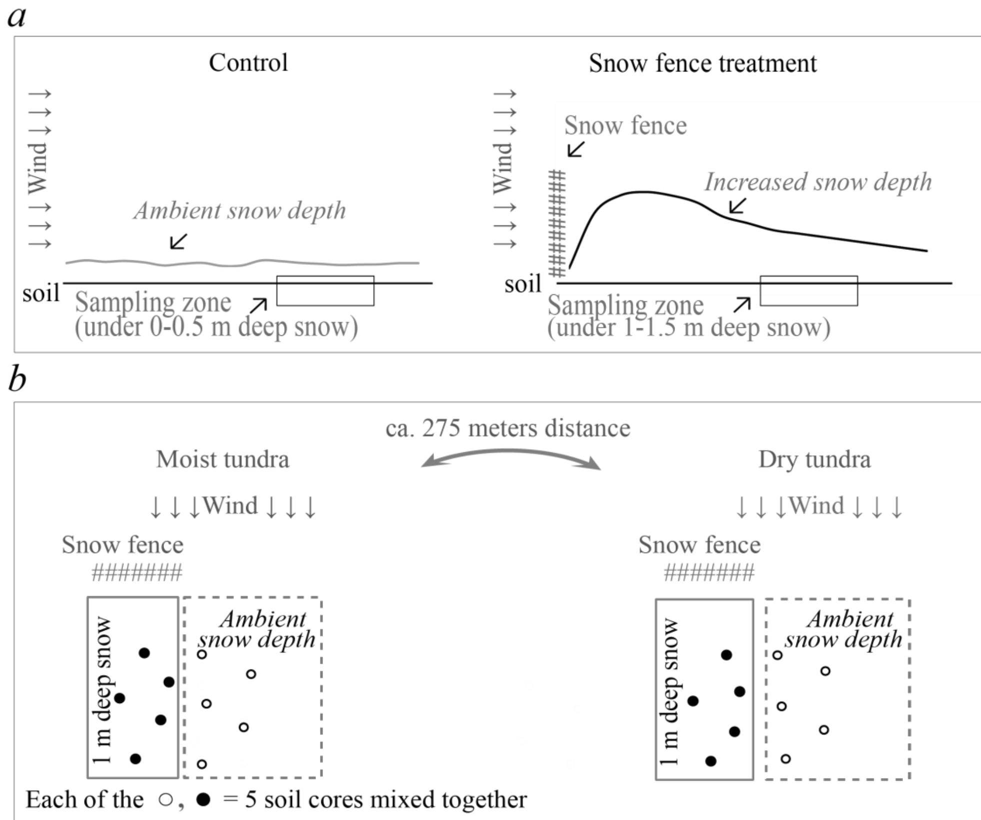


Figure 4.1. *Experimental setup. **(a)** A methodological set up to experimentally increase the snow depth by a snow fence. In ambient (control) conditions (left part of the figure) snow depth does not exceed 0.5 m. The snow fence (right part of the figure) leads to snow accumulation behind the fence by creating a leeward drift with a snow depth of ca. 2 m (deep zone), 1.5-1 m (medium zone) and 0.5 m (shallow zone). In dry and moist tundra types, soils were sampled from 1) control plots and 2) medium zone snow fence plots, where the snow was ca 1-1.5 m deep. **(b)** Location of the sampling plots in dry and moist tundra types. In each of the tundra types we sampled 5 plots in control conditions (shown as ○) and 5 plots in experimental treatment (1-1.5 m deep snow, shown with ●). Each of the 20 plots was a composite sample of five soil cores mixed together.*

A suite of physical alterations in the ecosystem caused by the snow fence treatment led to significant shifts in the plant communities, described in details in Walker et al (1999), Wahren et al (2005), Welker et al (2005), Mercado-Diaz (2011) and Pattison and Welker (2014). Because these studies indicated the strongest responses of the plant communities in the zone where the snow was ca 1-1.5 m deep, we sampled soils from that particular zone (Fig 4.1a).

Soil sampling

In July 2012, we collected soils from moist and dry tundra, from the zones where the snow depth was ambient (control, i.e. < 0.5 m) and experimentally increased to ca 1-1.5 m (snow fence treatment). Five experimental replicates were taken from each of the four experimental treatments, i.e. dry and moist tundra types, ambient and increased snow depth (Fig. 4.1b). Each of the experimental replicates was a composite sample of five soil cores taken from the area of ca 1 m^2 by the soil corer of ca 2 cm in diameter and ca. 20 cm deep (Fig. 4.1b). In total, we sampled 100 soil cores and combined them in 20 samples. Coarse particles (litter, aboveground parts of the plants etc.) were removed from the samples, although some fine particles (e.g. plant roots) could still be present in the sample. The samples were kept frozen until lyophilisation that was carried out the same day as the soil sampling.

Molecular work

Prior to downstream applications the lyophilized soil samples were thoroughly mixed. DNA extraction was carried out using Macherey-Nagel NucleoSpin Soil kit (Macherey-Nagel Gmbh and Co., Düren, Germany), using SL2 lysis buffer. The volume of elution buffer was set to 30 µl. For each of the twenty samples the DNA extraction was carried out twice resulting in ca. 0.4-1 g of the soil used for DNA extraction per sample. (ca. 0.2-0.5 g of the soil could be processed in one extraction).

The forward primer fITS7-trP1 (Ihrmark et al, 2012) and reverse sample-specific primer ITS4 (White et al, 1990) were used to amplify the ITS2 r DNA region of ca. 250 bp. The ITS4 primer was labelled with sample-specific Multiplex Identification DNA-tags. For each of the 20 samples, the following PCR protocol was used for three positive and a one negative reactions: one cycle of 95°C for 5 min, then 25 cycles of 95°C for 20 sec, 56°C for 30 sec, and 72°C for 1.5 min, ending with one cycle of 72°C for 7 min. Negative PCR reactions were made for each primer pair and contained elution buffer instead of DNA. PCR products were

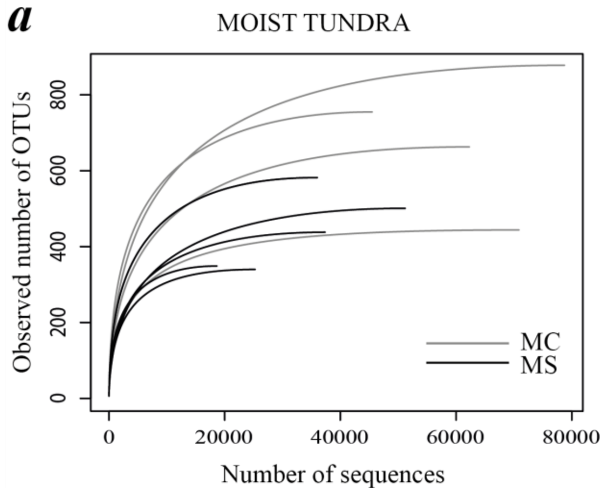
checked for DNA concentrations using QIAxcel Advanced System (QIAGEN). Emulsion PCR and Ion Torrent sequencing was carried out at the Naturalis Biodiversity Center. We used the sequencing Ion 318^{TM} Chip to allow for highest possible sequencing coverage. Ion Torrent sequencing resulted in 3 960 925 reads with the median sequence length of 268 bp.

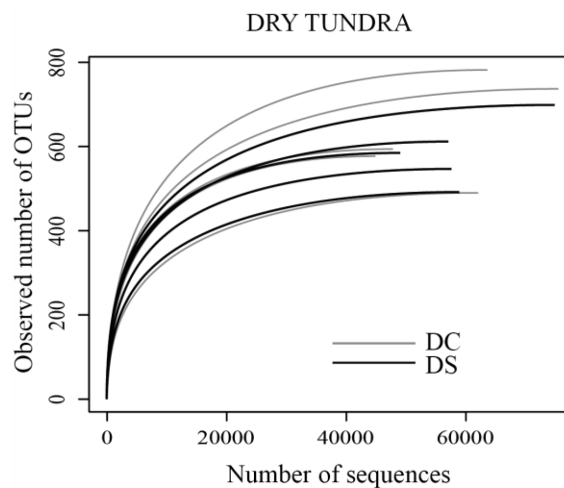
Bioinformatic analyses

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 adapters (identification tags) were removed. The poor-quality ends were trimmed off based on 0.02 error probability limit in Geneious Pro 5.6.1 (BioMatters, New Zealand). Subsequently, sequences were filtered using USEARCH v.8.0 (Edgar, 2010) based on the following settings: all sequences were truncated to 200 bp and sequences with expected error > 1 were discarded. The resulting 1,971748 high-quality sequences were grouped into 5169 operational taxonomic units (OTUs) by UPARSE algorithm in USEARCH at 97% sequence similarity, following other fungal metabarcoding studies (e.g., Bjorbækmo et al, 2010; Geml et al, 2010; Bellemain et al, 2013; Tedersoo et al, 2014), while simultaneously excluding 14067 putative chimeric sequences. We assigned sequences to taxonomic groups based on pairwise similarity searches against the curated UNITE fungal ITS sequence database containing identified fungal sequences with assignments to Species Hypothesis groups (Kõljalg et al, 2013). After discarding global singletons and OTUs that did not have at least 80% similarity to any fungal sequence in UNITE, the final dataset contained 3550 OTUs. The ecological functions for the OTUs were determined using FUNGuild software (Nguyen et al, 2015) and the dataset of Tedersoo et al. (2014), resulting in 1174 OTUs with identified functional guilds. For the same OTUs we independently assigned ecological functions based on the isolation source for the closest reference sequences in UNITE. The two approaches resulted in largely similar (ca. 81%) datasets. The inconsistence was observed in fungal genera that have been generally considered saprotrophic but were isolated from surface-sterilized or ectomycorrhizal roots.

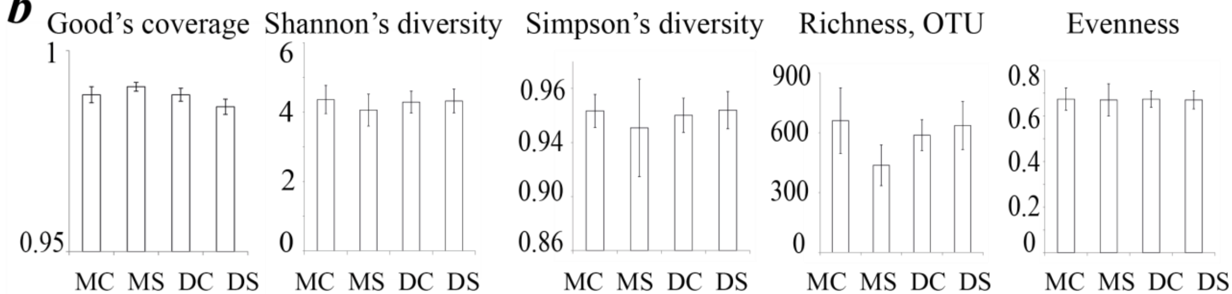
Figure 4.S1. (next page) *Community richness and diversity estimators in control and deep snow plots in dry and moist tundra. **(a)** rarefaction curves obtained for total number of fungal OTUs in the four experimental treatments, M - moist tundra, D - dry tundra, C - control, S - deeper snow, **(b)** Good's coverage, observed OTU richness, evenness, Simpson's and Shannon's diversity indexes. All estimators are shown ± SD.*

a





b



For example, species of *Penicillium* - *P. swiecickii* SH279517.07FU (Min et al, 2014), *P. spinulosum* SH207148.07FU (Summerbell, 1989), *P. neocrassum* SH107619.07FU, *P. soppii* SH199403.07FU (Summerbell et al, 2005), *P. angulare* SH182512.07FU, *P. thomii* SH407691.07FU (Ghen et al, 2008) were assigned to root-associated or endophytic fungi. Root-associated lifestyle was assigned to *Mycena metata* SH220724.07FU (Toju et al, 2013) and *M. cinerella* SH220744.07FU. A guild of dark septated endophytes involved *Meliniomyces variabilis* SH181078.07FU, *Cadophora finlandica* SH214265.07FU, *Leptodontidium* sp SH205736.07FU, *Phialocephala fortinii* SH204986.07FU and *Acephala* sp SH020838.07FU (Addy et al, 2005; Newsham, 2011). The ecological assignment based on the isolation source for the reference sequence (Species Hypothesis in UNITE) was selected for further analysis as more accurate.

Representative sequences of fungal OTUs were submitted to GenBank with the accession numbers KM673298-KM675060.

Statistical analyses

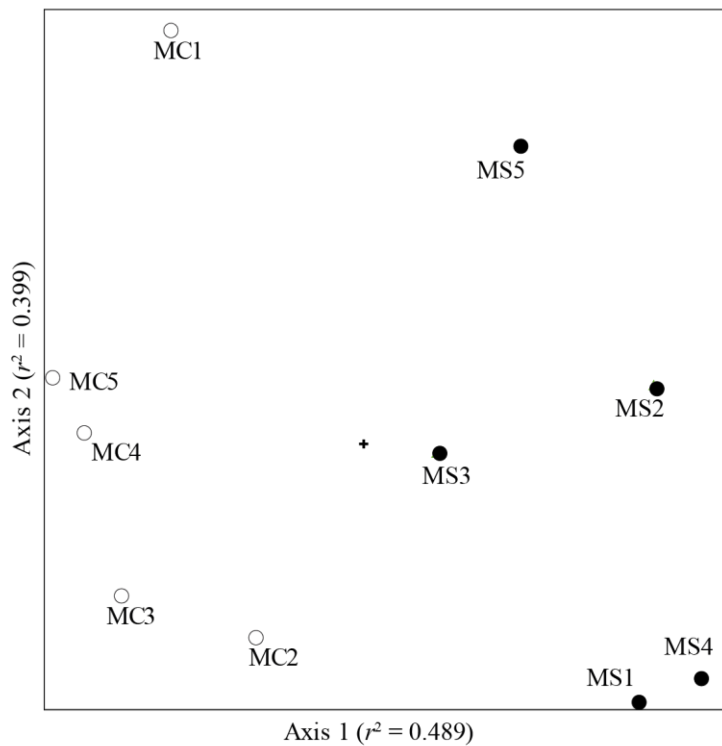
Depth of sequencing coverage was quantified by rarefaction curve and coverage estimators. Rarefaction analysis, Good's coverage, Shannon's (H) and Simpson's diversity indexes, OTU richness (S) and evenness (H/ln S) was carried out/ calculated using "rarefy" function in Vegan package (Oksanen et al, 2012) in R software for statistical computing (R Core team, 2013). The rarefaction curves reached plateau suggesting that we sequenced almost all fungal species in the sampled plots (Fig.4.S1a). High Good's coverage estimators (98.9±0.2%) indicated equally deep OTU recovery across the treatments (Fig 4.S1b). There was no significant difference in richness, evenness, and diversity estimators across the treatments (Fig 4.S1b).

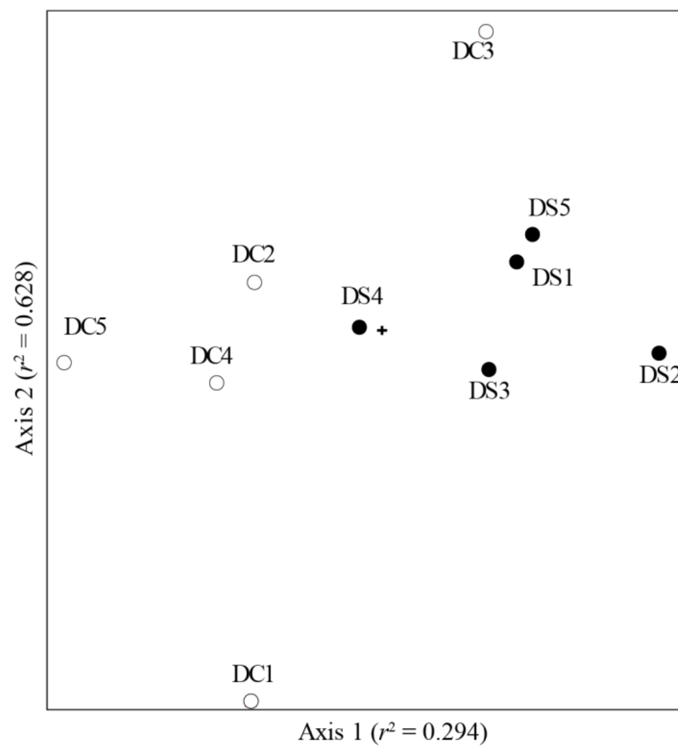
The effect of increased snow depth on fungal community composition was estimated using PC-ORD v. 5.32 (McCune and Grace, 2002) separately for dry and moist tundra, in order to eliminate all the factors unrelated to the treatment but contributing to the variation in fungal community compositions. The presence-absence data (for abundance-based data see Supplementary material for the paper, Fig 4.S2) was used to estimate shifts in richness of fungal taxa and ecological groups by non-metric multidimensional scaling (NMDS) in PC-ORD. Following recommendations of Lindahl et al. (2013), presence was set as >4 sequences on a per sample basis. The primary matrix contained of experimental plots by OTUs

(i.e. fungal community composition). The secondary matrix contained of plots by richness of fungal taxa (i.e. number of OTUs belonging to specific taxa) and richness in ecological groups containing at least 8 OTUs per group in interest. For the moist tundra, this final dataset contained of 1363 fungal OTUs, and for dry tundra – of 1328 OTUs. The dataset was subjected to 500 iterations per run using the Sørensen similarity (Bray-Curtis index) and a random starting number. The resulting NMDS solution with the lowest final stress was rotated to maximize the correlation between the snow fence treatment and the axis 1 (i.e. left part of the axis indicated ambient snow depth and right part – increased snow depth). Although, Pearson's correlation coefficient $R^2 > 0.2$ could be used as indication of correlation (as in McCune and Grace, (2002), we present the results for $|R| > 0.5$ that are indicated by PC-ORD as strong correlations and that are important for characterizing shifts in fungal communities (Rogers et al, 2009). To test whether fungal community compositions in ambient and deep snow zones plots were statistically different, we used a multi-response permutation procedure (MRPP) and permutation-based nonparametric MANOVA (Anderson, 2001), also in PC-ORD. In addition, this software was used to examine if specific fungal OTUs were characteristic for any of the experimental treatments using indicator species analysis (Dufrêne and Legendre, 1997).

To test for statistical difference in read counts between the control and treatment, we carried out a two-group analysis in Comprehensive Meta-Analysis software (Smith, 2014) on a per-OTU basis. Using sequence read abundance as proxy for fungal biomass is constrained due to interspecific differences in copy numbers and length of ITS regions as well as other species-specific biological factors. However, for a particular OTU, changes in sequence counts in different samples can be considered indicative of relative changes and trends with respect to abundance (or biomass) (Amend et al, 2010). The input matrix contained the mean read abundance, standard deviation and sample size (5 replicates) across the control and deep snow plots. For each OTU we compared the mean read counts across the ambient and deep snow plots to calculate size effect and 95% confidence range.

Figure 4.2. (next page) *The effect of increased snow depth on fungal community compositions in moist and dry tundra. D – dry tundra, M – moist tundra, S – snow fence treatment, C – ambient snow depth. The communities of the plots with ambient and increased snow depth are shown with ○ and ●, respectively.*


MOIST TUNDRA
MC1
MS5
MC5
MS2
MC4
MS3
MC3
MC2
MS4
MS1
Axis 1 ($r^2 = 0.489$)
Axis 2 ($r^2 = 0.399$)


DRY TUNDRA
DC3
DS5
DC2
DS1
DS4
DC5
DC4
DS2
DS3
DC1
Axis 1 ($r^2 = 0.294$)
Axis 2 ($r^2 = 0.628$)

The analyses were run for 10 functional groups containing 10 OTUs at very least, including animal pathogenic, bryophyte-associated, dark septated endophytes, endolichenic, lichenized, mycoparasitic, root-associated, ectomycorrhizal, plant pathogenic and saprotrophic fungi. In addition, we run an analysis for the overall fungal community. This approach allowed us to depict the variation in responses of individual OTUs to increased snow depth as well as to evaluate the overall response of the functional groups.

Results

Increased snow depth alters fungal community composition

Tests of community differences revealed that fungal assemblages changed significantly under the deeper snow in both tundra types. For the moist tundra, NMDS analysis resulted in a two-dimensional solution with a final stress of 6.53 and final instability < 0.00001 (Fig 4.2). Correlation coefficients for ordination axes were axis 1: r^2=0.489 and axis 2 : r^2=0.399. Strong effect of deep snow on fungal community compositions was revealed by both MRPP (P=0.0027; A=0.076) and MANOVA (P = 0.0074; F=2.56) analyses. According to the MANOVA, snow fence treatment explained 23.7% of the variation in fungal community compositions. The effect of increased snow depth on richness in fungal taxa and ecological groups is presented in Tables 4.1 and 4.2. We observed a decline in seventeen fungal taxa and functional groups including bryophyte-associated, dark septated endophytic, ectomycorrhizal, lichenized, plant pathogenic and saprotrophic fungi, although no taxonomic or ecological groups increased in richness under deep snow. In the dry tundra, NMDS resulted in a two-dimensional solution with a final stress of 5.78 and final instability < 0.00001. Correlation coefficients for ordination axes were: axes 1: r^2=0.294, axis 2: r^2=0.628, with orthogonality 81.8%. Significant changes in fungal community compositions were shown by both MRPP (P=0.015; A=0.052) and MANOVA (P = 0.022; F=1.90) analyses. Snow fence treatment explained 15.2% of the variation in fungal community composition, as revealed by MANOVA statistics. Shifts in richness of fungal taxa and ecological groups are presented in Tables 4.1 and 4.2. Increased snow depth resulted in declines in four fungal orders (Capnodiales, Lecanorales, Peltigerales and Thelephorales), although three taxa increased in richness across the treatment plots (Archaeorrhizomycetales, Venturiales and *Clavaria*). Among the ecological groups, ectomycorrhizal and lichenized species declined, and root-associated increased in richness (Table 4.2).

Table 4.1. *Effect of increased snow depth on richness of fungal taxonomic groups in dry and moist tundra, as revealed by PC-ORD. Correlation values are shown for fungal taxonomic groups that correlated with ordination axes at |R| > 0.5. Negative and positive values indicate higher richness of the group in interest across the control and treatment plots, respectively.*

MOIST TUNDRA			**DRY TUNDRA**		
Taxonomic group	No of OTUs	Correlation	Taxonomic group	No of OTUs	Correlation
Agaricales	132	*R*= - 0,758	Capnodiales	48	*R* = - 0,492
Chaetothyriales	104	*R*= - 0,755	Lecanorales	54	*R* = - 0,724
Helotiales	241	*R*= - 0,781	Peltigerales	11	*R* = - 0,642
Hypocreales	26	*R* = - 0,644	Thelephorales	28	*R* = - 0,887
Lecanorales	14	*R* = - 0,667			
Pezizales	15	*R* = - 0,824	Archaeorhizomycetales	22	*R* = 0,716
Pleosporales	28	*R* = - 0,704	*Clavaria*	15	*R* = 0,581
Russulales	17	*R* = - 0,504	Venturiales	8	*R* = 0,654
Sebacinales	86	*R* = - 0,558			
Sporidiobolales	14	*R* = - 0,712			
Thelephorales	32	*R* = - 0,704			
Trechisporales	9	*R* = - 0,535			
Tremellales	39	*R* = - 0,732			
Clavaria	8	*R* = - 0,844			
Inocybe	10	*R* = - 0,710			
Cryptococcus	25	*R* = - 0,631			
Mycena	10	*R* = - 0,575			

Table 4.2. (next page) *Effect of increased snow depth on richness and abundance of fungal functional groups in dry and moist tundra. Shifts in fungal abundance were analysed by comprehensive meta-analysis as written in Fig. 3 caption. The effect of snow depth on richness was analysed by non-metric multidimensional scaling in PC-ORD. Correlations |R| > 0.5 are shown in bold. For convenience, significant changes are shown with* ⬆ *for increase and* ⬇ *for decline in richness/abundance of the group in interest.*

Functional group	**Moist tundra**, effect on:					**Dry tundra**, effect on:				
		abundance		**richness**			**abundance**		**richness**	
	No of OTUs	size effect	*P value*	*R*	effect	*No of OTUs*	size effect	*P value*	*R*	effect
animal pathogens	*6*					*14*	-0.09	0.599	-0.242	
bryophyte-associated	*10*	-0.57 ⬇	**0.006** **	**-0.596**	⬇	*6*				
dse	*23*	-0.12	0.379	**-0.617**	⬇	*32*	0.07	0.535	0.143	
ectomycorrhizal	*93*	-0.24 ⬇	**<0.001** ***	**-0.675**	⬇	*95*	0.269 ⬆	**<0.001***	**-0.749**	⬇
endolichenic	*7*					*16*	-0.234	0.145	-0.321	
endophytic	*30*	-0.07	0.577	0.214		*25*	0.043	0.736	-0.08	
lichenized	*30*	-0.503 ⬇	**<0.001** ***	**-0.797**	⬇	*107*	-0.318 ⬇	**<0.001***	**-0.866**	⬇
mycoparasitic	*18*	-0.235	0.122	-0.292		*21*	-0.004	0.98	-0.004	
plant pathogenic	*52*	-0.169	0.059	**-0.643**	⬇	*46*	0.137	0.152	0.215	
root-associated	*42*	0.076	0.807	-0.026		*49*	-0.014	0.88	**0.662**	⬆
saprotrophic	*365*	-0.106	**0.002** **	**-0.653**	⬇	*322*	0.018	0.62	-0.091	
Total Fungi		-0.15 ⬇	**<0.001***	**-0.876**	⬇		0.046	0.67	-0.382	

Indicator species analysis

In moist tundra, we observed 30 OTUs that were indicators (all $P<0.05$) for either control or snow fence treatment. The complete list of indicator OTUs, their distribution across the sampling plots, taxonomic affinities, putative ecological functions and *P*-values is presented in Table 4.S1. Among the 21 OTUs that were indicators of the control habitats, we observed 11 ascomycete and 7 basidiomycete species, with ectomycorrhizal, root-associated or saprotrophic lifestyles. For example, we observed the root-associated Sebaciales (SH201961.07FU and SH083523.07FU), dark septate endophytic *Phialocephala* sp (SH218122.07FU) and ectomycorrhizal *Inocybe leiocephala* (SH219800.07FU), as indicators of control conditions. Increased snow depth was favourable for 9 OTUs only. Among those that were identified to genus, we observed the saprotrophic *Capronia* (SH180465.07FU) and the ectomycorrhizal *Tomentella lapida* (SH189354.07FU).

In dry tundra, the indicator species analysis revealed an opposite trend: there were many more OTUs characteristic for the snow fence treatment (16 OTUs) compared to the control conditions (5 OTUs). Possibly the observed tendency was due to increased moisture in the snow fence treatment plots, that is a limiting factor for growth of many fungal species in dry tundra (Jones et al, 1998). There was no clear pattern for any of the ecological groups, although we anticipated more lichenized fungi to be indicators of the control conditions. For example, the lichenized *Pertusaria* sp (SH206383.07FU) and the plant pathogenic *Ilyonectria mors-panacis* (SH202967.07FU) were characteristic of the control plots, while the saprotrophic *Guehomyces pullulans* (SH212824.07FU) and *Phaeomoniella* sp (SH015552.07FU) and the root-associated *Archaeorhizomyces* (SH004487.07FU) were indicators of the deep snow plots.

Snow fence treatment alters abundance of fungal ecological groups

Comprehensive meta-analysis revealed significant changes in abundance of several fungal ecological groups (Table 4.2, Fig. 4.3). In moist tundra, we observed a decline in bryophyte-associated ($P=0.006$), ectomycorrhizal ($P<0.001$), lichenized ($P<0.001$) and saprotrophic ($P=0.002$) species, as well as a decline in total fungal abundance ($P<0.001$) under the deeper snow. These results were largely concordant with the trends observed for the richness: both analyses showed a decline in bryophyte-associated, ectomycorrhizal and lichenized fungi. We did not observe conflicting patterns in richness and abundance in any of the groups tested.

In dry tundra, snow fence treatment resulted in increase of ectomycorrhizal ($P<0.001$) fungi and a decline in lichenized ($P<0.001$) species (Table 4.2, Fig. 4.3). For lichenized species, this aligned with the decline in species richness revealed previously by NMDS. For ectomycorrhizal fungi an increase in abundance coupled with a decline in species richness across the treatment plots suggested that a relatively small subset of taxa could benefit from deeper snow, while the majority of ectomycorrhizal species petered out under the increased snow depth. Total fungal abundance in dry tundra was not affected significantly ($P=0.67$).

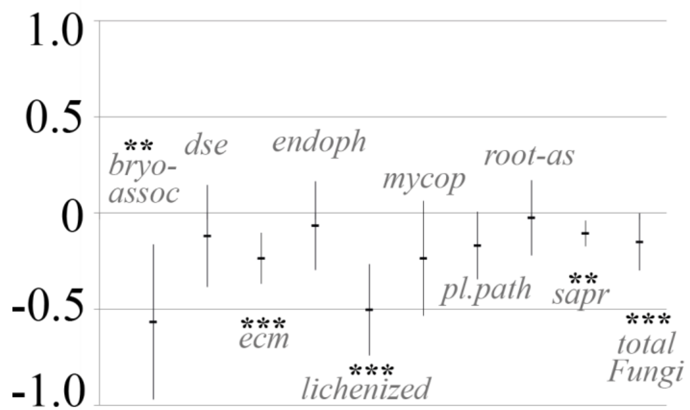
Discussion

Changes in fungal community composition

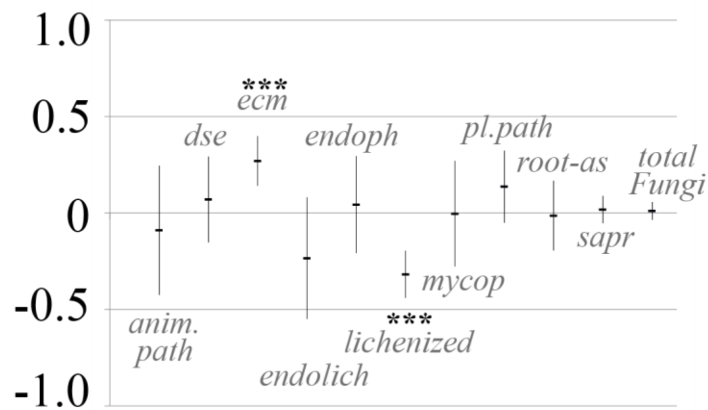
In both moist tussock and dry heath tundra, community composition of arctic soil fungi in tundra sites subjected to 18 years of increased snow depth differed significantly from communities in the control sites. The changes in both tundra communities were different from those observed in response to experimental summer warming, where only fungal communities in moist tundra exhibited significant compositional changes (Geml et al, 2015; Morgado et al; 2015; Semenova et al, 2015). The more pronounced fungal community response to deep snow compared to summer warming in dry heath tundra likely relates to the fact that higher snowpack not only maintains winter soil temperature higher, but also contributes to an enhanced summer soil moisture regime associated with additional snow melt water (Pattison and Welker, 2014), and soil moisture is known to be the main factor limiting fungal growth in general (Griffin, 1963). Another possible factor contributing to the strong fungal community response is that winters last much longer in the Arctic than summers and, therefore, for any given year the communities are exposed longer to experimentally increased snow depth than to summer warming (Jones et al, 1998; Welker et al, 2000).

Figure 4.3. (next page) Responses of fungal functional groups to deeper snow, an abundance-based comprehensive meta-analysis.*The graph shows the size effect with a 95% confidence interval calculated for various functional groups of fungi. Positive and negative values indicate increase and decrease in abundance in the deep snow plots, respectively. Significant correlations are shown with ** for $P < 0.01$ and *** - for $P < 0.001$. anim. path - animal pathogenic fungi, dse – dark septated endophytes, bryo-assoc – bryophyte-associated, ecm – ectomycorrhizal, endolich – endolichenic, root-as – root-associated, sapr – saprotrophic, mycop – mycoparaistic, pl.path. – plant pathogenic species of fungi.*

Moist tundra



Dry tundra



Our data suggest marked changes in fungal community compositions associated with this snow depth, although it was difficult to compare our results to any similar previous studies. For instance, Penton et al. (2013) found almost no effect of 1 year of snow fence treatment (1.2 m deep snow) on fungal richness and community composition in boreal Alaskan discontinuous permafrost. Their analysis of OTU abundance revealed a decline in only one ascomycete family – Helotiaceae, and increases in basidiomycete genera *Russula*, *Lactarius* and *Cortinarius*, which did not coincide with our results. Within the Canadian low Arctic, Buckeridge and Grogan (2008) found no compositional changes in fungal biomass and hyphal length in birch hummock tundra after 3 years of deeper snow (ca. 1 m in the treatment vs ca. 0.3 m in the control), and no information was provided on the taxonomic composition of that community. We believe that effects of snow depth may require several years to become noticeable in the composition of soil fungi, and may be influenced by associated changes in vegetation and particularly soil attributes, and therefore, may not be comparable in different ecosystems (Wahren et al, 2005; Mercado-Diaz, 2011). In both dry and moist tundra, we observed a decline in richness of ectomycorrhizal fungi under deep snow, which coincides with the trends observed for ectomycorrhizal basidiomycetes in long-term summer warming (Morgado et al, 2015) and snow addition experiments (Morgado et al, 2016) in the same area at Toolik Lake, Alaska.

Changes in taxonomic and functional groups

Increased snow depth resulted in declines in richness in many fungal taxonomic and functional groups. In dry tundra, the total species richness was not affected, likely because the loss of species in one fungal lineage was coupled with increased richness in another fungal taxa. In moist tundra, total fungal richness decreased in the deep snow treatment, as suggested by the NMDS Pearson correlation value, although the difference was not significant in the *t*-test, likely because of the high standard deviation values possibly caused by the large spatial heterogeneity, i.e. “patchiness” of fungal species (Blaalid et al, 2012). All taxonomic and functional groups with strong correlation values had lower richness and/or abundance in the deep snow plots. It is possible that trend would be significant if more samples were collected per plot. Unfortunately, given the destructive nature of soil sampling, we could not take more than 5 soil cores per plot that are also used for a variety of long-term research projects. In general, loss of species richness implies greater fluctuations in ecosystem functioning (e.g., productivity, rates of decomposition or nutrient cycling), as well as reduced robustness towards fluctuations in abiotic

factors, e.g., extreme temperatures or water regimes (Naeem et al, 1999). However, the negative consequences for the moist tundra ecosystems caused by the decline in fungal species richness are difficult to predict due to functional redundancy of the soil microbial community and numerous ways in which this decline may be compensated by other groups of fungi and bacteria (Coleman and Whitman, 2005 and references therein).

Patterns of richness and read abundance in our study revealed partly concordant results with previously reported changes in plant communities (Wahren et al, 2005; Wipf and Rixen, 2010; Mercado-Diaz, 2011; Loranty and Goetz, 2012; Pattison and Welker, 2014) in response to higher snowpack levels in arctic tundra. In all analyses in both the dry and the moist tundra, we observed a significant decline of lichenized fungi, implying, therefore, strong decrease in richness as well as abundance. The decline in richness and abundance of bryophyte-associated species observed in the moist tundra was in agreement with the previously reported losses of bryophytes caused by increased snow depth (Wahren et al, 2005; Mercado Diaz, 2011). A similar pattern was observed in response to long-term experimental summer warming (Geml et al, 2015; Semenova et al, 2015).

Somewhat unexpectedly, ericoid-, ectomycorrhizal and endophytic fungi either decreased or showed no significant change in richness and abundance under increased snow depth, despite higher shrub density and biomass reported in the deep snow areas in both tundra types (Mercado-Diaz, 2011; Pattison and Welker, 2014). Greater aboveground biomass and shrub density were assumed to reflect increased root biomass (Sullivan et al, 2007), which, in turn, would broaden niches for mentioned above fungal species. For instance, such increase in ectomycorrhizal fungi associated with *Betula nana* was reported in summer warming greenhouse experiments (Deslippe et al, 2011). However, our data clearly demonstrate a strong decline in ectomycorrhizal species in both richness and abundance in dry and moist tundra, while an increase was only observed for richness of root-associated fungi in dry tundra. We assume that in the moist tundra this decline could be due to decreased aeration caused by excess moisture following snow melt in the spring, as in general these fungi tend to avoid overly flooded habitats (Wal et al, 2013). In the dry tundra, ectomycorrhizal fungi may be influenced by various factors in addition to moisture, e.g., rising NH_4^+ concentrations under the deep snow (Boxman et al, 1986) or acidification of rhizosphere that follows higher uptake of NH_4^+ by the plants (Zhang and Bai, 2003).

It is currently unclear whether the sensitive ectomycorrhizal fungi decline as a direct consequence of altered environmental conditions or as a result of changes in competition dynamics affected by the increased snow depth. Morgado et al. (2015) and Geml et al. (2015) report a strong decline in ectomycorrhizal basidiomycetes in arctic tundra under summer warming, including the genera *Inocybe* and *Sebacina*, that strongly correlated with the control sites. Therefore, declines in ectomycorrhizal fungi observed in our study may not be related to altered moisture or NH_4^+ concentrations but instead may be caused by the effects of temperature increase on fungal metabolism (i.e. production of extracellular enzymes) and fungus-plant and fungus-fungus interactions (Morgado et al, 2015).

Similarly, we anticipated an increase in saprotrophic fungi across the deep snow plots, that was not supported by our data. An increase in richness of saprotrophic ascomycetes was reported in our summer warming experiments, likely as a consequence of accumulation of leaf litter across the warmed plots (Semenova et al, 2015). Because litter accumulation occurs also in the deep snow plots (Mercado-Diaz, 2011), we anticipated higher richness of saprotrophs in the snow fence experiments as well. However, no significant response was observed in dry tundra and strong declines in richness and abundance of saprotrophic fungi was observed in moist tundra. Because of their key roles in decomposition processes, saprotrophs are essential for nutrient turnover and soil C storage. In microcosm experiments (Hunt et al, 1987) deletion of saprotrophic fungi or bacteria led to extinction of other groups of organisms, although the system was still functioning when mycorrhizal fungi were removed. On the other hand, possible consequences of ca. 10 percent loss in saprotroph abundance observed in moist tundra are difficult to predict given high functional redundancy of microbial communities (Coleman and Whitman, 2005).

We did not observe any changes in abundance of plant pathogenic fungi, while richness in this guild declined in moist tundra. More intensive growth of shrubs and higher winter temperatures in deeper snow areas were anticipated to favour plant pathogens, similar to what was known from former studies (Oloffson et al, 2011; Natali et al, 2012). For example, a six year snow fence experiment (increased snow depth by 0.6-0.8 m) in northern Sweden resulted in outbreaks of the plant pathogenic ascomycete *Arwidssonia empetri* that caused shoot mortality and reduced the coverage of the dominant dwarf-shrub *Empetrum hermaphoditum* by 70% (Oloffson et al, 2011; Natali et al, 2012). Possibly, an observed decline in richness of plant pathogenic fungi in moist tundra was related to increased plant

fitness due to lesser frost cleft and higher nitrogen availability under deep snow, or other factors that overweighed the benefits of winter warming for plant parasitic fungi. In addition, *Arwidssonia empetri* (or syn. *Heterosphaeria* spp.) was not among the species detected in our dataset, despite the fact that *Empetrum* is present in both the dry and the moist plots. Therefore, it remains unknown if disease outbreaks similar to the ones reported from the Swedish snow fence experiment may occur in the arctic tundra.

Plant- versus fungal community responses to deep snow

Lichenized and bryophyte-associated fungi in our dataset responded to deeper snow in agreement with formerly reported shifts in plant communities across the same sampling plots, however shifts in many fungal lineages, i.e. ectomycorrhizal, dark septated endophytes, plant pathogenic and saprotrophic species appear counterintuitive in light of observed vegetation change and temperature records. A correspondence between plant and fungal responses is generally anticipated due to known tight associations between fungi and plants in nutrient-poor soils of arctic tundra (Hobbie et al, 2009), suggesting that fungal communities would change in their assemblages following the trends known for their hosts (Dahlberg and Bultman, 2013). On the other hand, plant and fungal responses to climate change involve a variety of strategies, including shifts in population ranges, symbiotic partners or timing of phenological events that provide a high potential for mismatches between interacting plants and their symbiotic microbes (Classen et al, 2015). Belowground communities in general have a much faster turnover compared to aboveground ones, and both may be strongly structured by different direct and indirect environmental drivers (Fierer and Jackson, 2006; Kardol et al, 2010; Classen et al, 2015). For example, bacteria-to-fungal ratio may increase (De Angelis et al, 2015) or decrease (Deslippe et al, 2012) in warmer soils, with a short-term impact on fungal assemblages but a retarded effect on climax arctic plant communities. Because of faster turnover, the state of present microbial communities may correspond to the ongoing climatic changes that will be reflected in plant community composition in future years. We, therefore, highlight the importance of studies in soil biology because monitoring the below-ground communities may be suitable for predicting and managing ecosystem disturbances on earlier stages.

Acknowledgements

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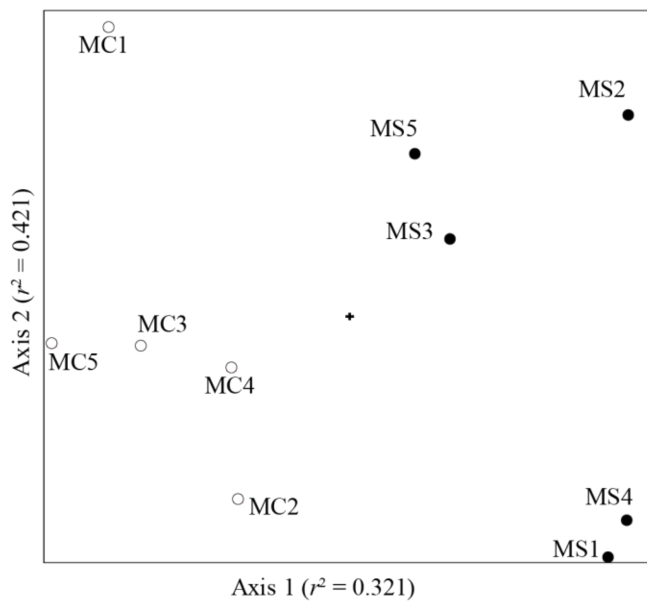
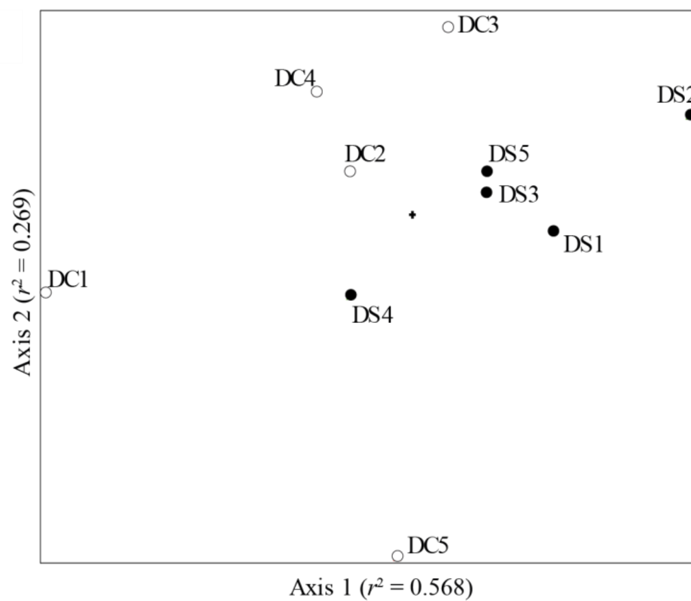
Table S2.1 *is too large to include in this document. A digital version is available at http://www.sciencedirect.com/science/article/pii/S003807171630102X*

Figure 4.S2. (next page) *The effect of increased snow depth on fungal community compositions in moist and dry tundra, an abundance-based analysis. D – dry tundra, M – moist tundra, S – snow fence treatment, C – ambient snow depth. The communities of the plots with ambient and increased snow depth are shown with ○ and ●, respectively. The results of statistical tests are presented in the table below the graph. Correlation values are shown for fungal taxonomic groups that correlated with ordination axes at |R| > 0.5. Negative and positive values indicate higher richness of the group in interest across the control and treatment plots, respectively.*


MOIST TUNDRA
MC1
MS2
MS5
MS3
MC3
MC5
MC4
MC2
MS4
MS1
Axis 2 ($r^2 = 0.421$)
Axis 1 ($r^2 = 0.321$)


DRY TUNDRA
DC3
DC4
DS2
DC2
DS5
DS3
DS1
DC1
DS4
DC5
Axis 2 ($r^2 = 0.269$)
Axis 1 ($r^2 = 0.568$)

Correlation values

MOIST TUNDRA

Final stress for 3-dimentional solution = 4.02. MANOVA: treatment explained 15.9% of the variation in fungal community composition; F=1.95; *P*=0.007; MRPP: A=0.05; *P*=0.005.

Fungal groups that correlated with control (ambient snow depth)

Agaricales *R* = - 0.679
Chaetothyriales *R* = - 0.754
Helotiales *R* = - 0.684
Hypocreales *R* = - 0.648
Lecanorales *R* = - 0.581
Pezizales *R* = - 0.785
Pleosporales *R* = - 0.715
Sebacinales *R* = - 0.558
Sporidiobolales *R* = - 0.614
Thelephorales *R* = - 0.697
Tremellales *R* = - 0.720
Clavaria *R* = - 0.844
Cryptococcus *R* = - 0.602
Inocybe *R* = - 0.573
bryophyte-associated *R*= - 0.655
dark septated endophytes *R* = - 0.637
ectomycorrhizal *R* = - 0.575
lichenized *R* = - 0.714
plant pathogens *R* = - 0.636
saprotrophic *R* = - 0.528

No fungal groups strongly correlated with increased snow depth

DRY TUNDRA

Final stress for 2-dimentional solution = 11.16. MANOVA: treatment explained 9.9% of the variation in fungal community composition; F=1.55; *P*=0.016; MRPP: A=0.03; *P*=0.021.

Fungal groups that correlated with control (ambient snow depth)

Thelephorales *R* = - 0.749
Peltigerales *R* = - 0.743
Cadophora *R* = - 0.504
animal pathogens *R* = - 0.577
endolichenic *R* = - 0.571
ectomycorrhizal *R* = - 0.585
lichenized *R* = - 0.702

Fungal groups that correlated with the treatment (increased snow depth)

Venturiales *R* = 0.759
bryophyte-associated *R* = 0.701

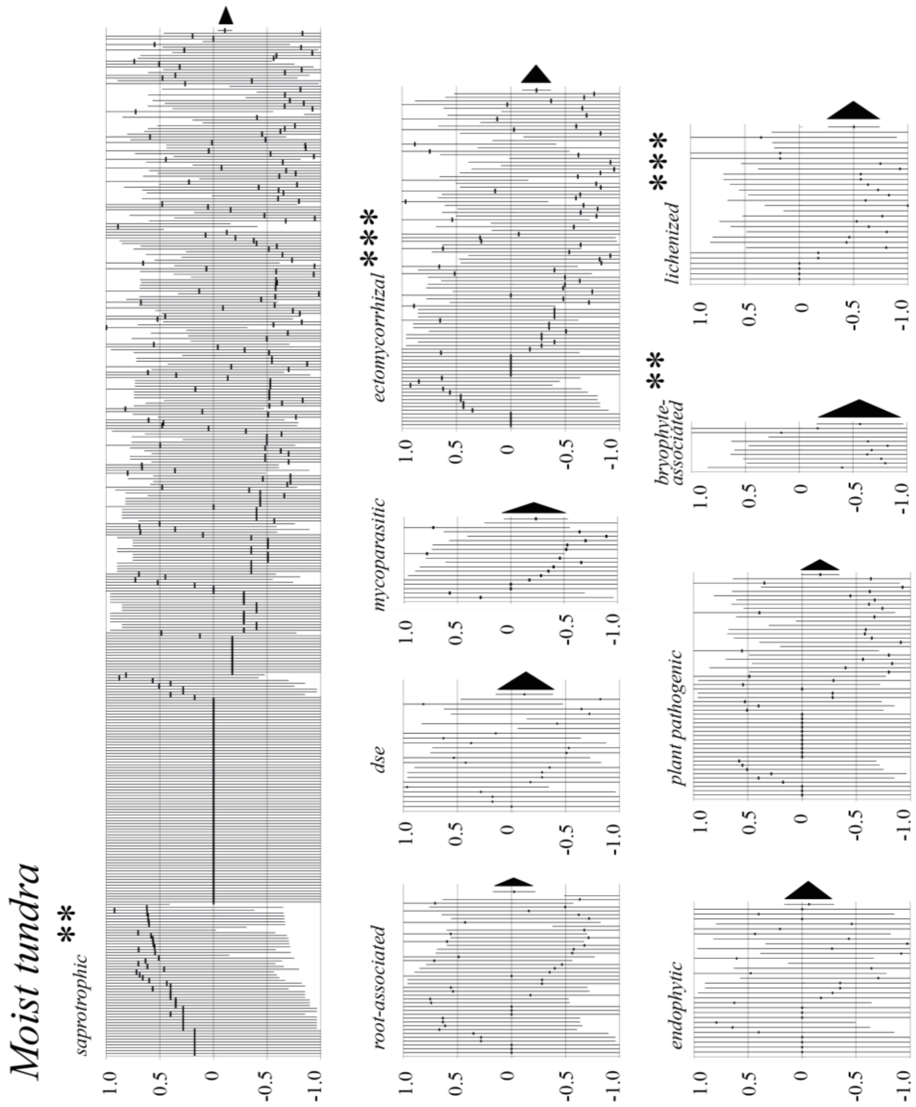


Figure 4.S3. The effect of increased snow depth on abundance of fungal functional guilds in moist tundra, as revealed by comprehensive meta-analysis. *Each of the vertical lines on the graph represents a size effect (a black dot in the middle of the line) with a 95% confidence interval for one OTU. The last line on the graph shows the overall size effect and 95% confidence interval (highlighted with a black triangle) for the fungal group in interest.*

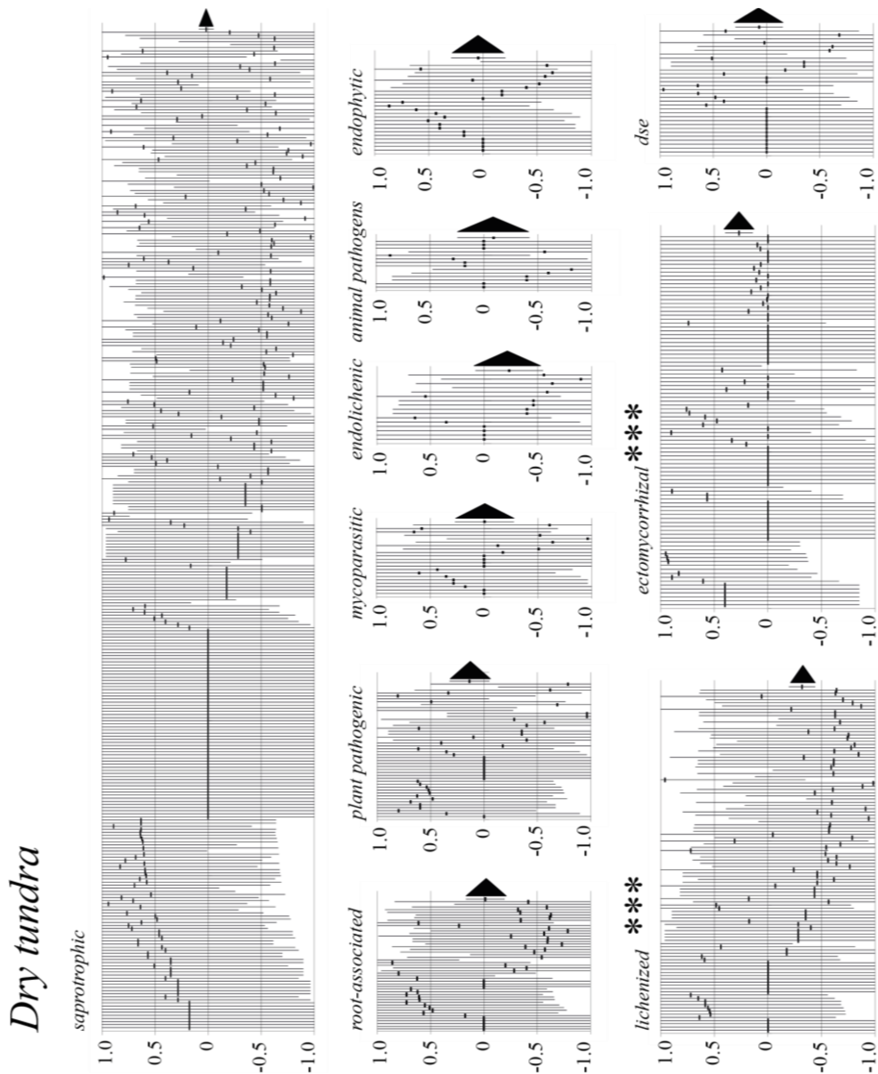
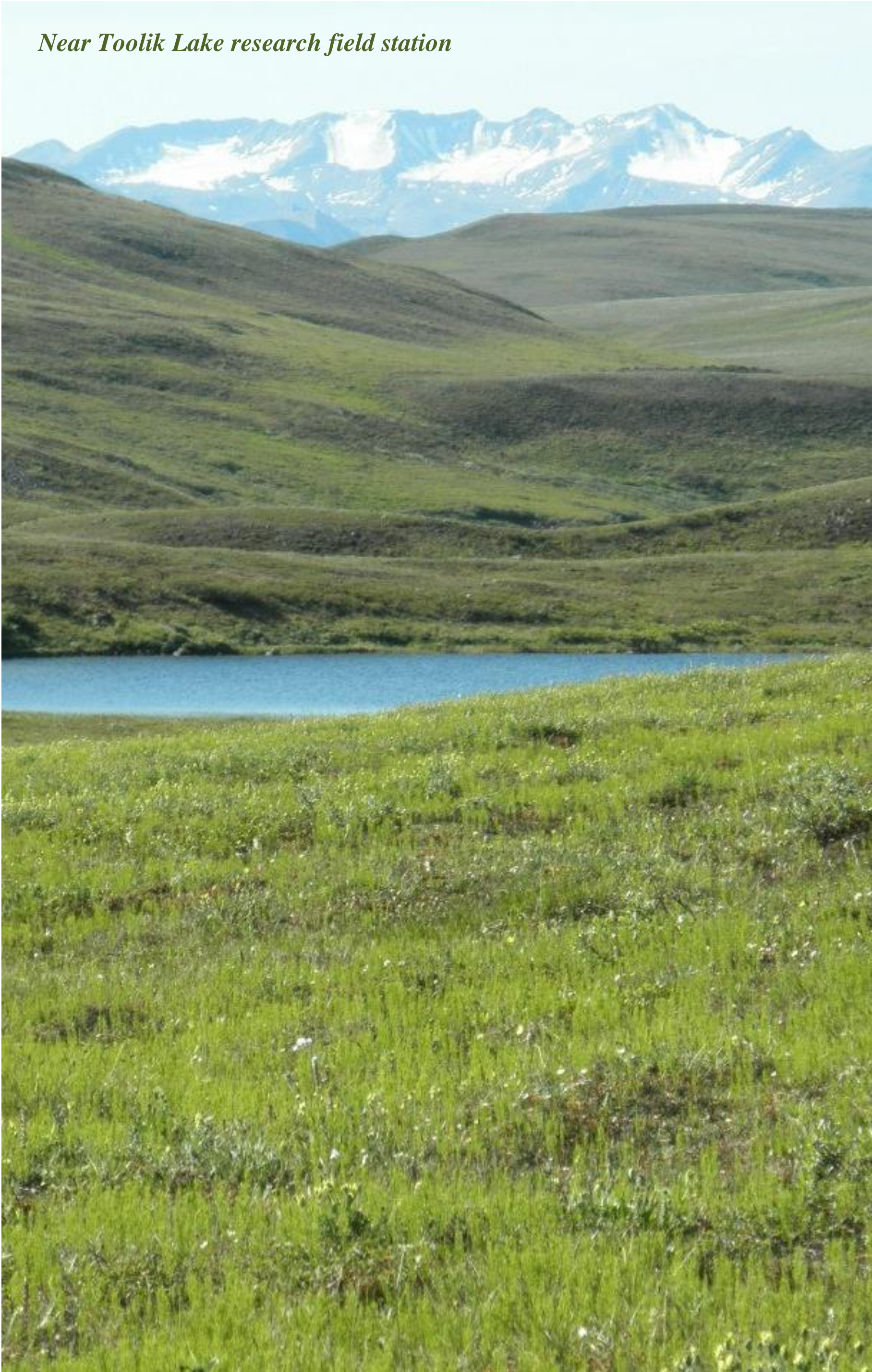


Figure 4.S4. The effect of increased snow depth on abundance of fungal functional guilds in dry tundra, as revealed by comprehensive meta-analysis. *Each of the vertical lines on the graph represents a size effect (a black dot in the middle of the line) with a 95% confidence interval for one OTU. The last line on the graph shows the overall size effect and 95% confidence interval (highlighted with a black triangle) for the fungal group in interest.*



Near Toolik Lake research field station

Chapter 5

GENERAL CONCLUSIONS AND DISCUSSION

Data presented in this thesis suggest strong shifts in fungal community composition, as well as in richness of their taxonomic and functional groups, caused by the long-term experimental climate manipulations in low arctic tundra of Northern Alaska. In addition, these data provide unprecedented insight into hidden fungal diversity in the Arctic. Approximately 6 million reads of fungal ITS2 rDNA region obtained in this work were deposited into a publicly available database (DRYAD: *doi.org/10.5061/dryad.cq2rb* & *doi:10.5061/dryad.2fc32*). These sequencing data contribute to our current knowledge of arctic fungal diversity, and may serve as a starting point for DNA-based monitoring of arctic fungal communities in the following decades. In addition to submitting the raw sequencing data, we deposited fungal operational taxonomic units (OTUs) in Genbank (KX401620-KX404870 & KJ826608-KJ828710). These OTUs were obtained by clustering high-quality sequences of identical length with a 97% sequence similarity. It is likely that a different approach, taking into account variation of the ITS2 region corresponding to well-delimited species in different lineages of Fungi, will be used in the future. Accordingly, the above DNA sequence data may be re-clustered using these different sequence similarity levels optimized for the specific fungal taxa. A vast majority of OTUs in our dataset were identified only to the level of Fungal order, while species names were assigned to ca. 20% of the OTUs. Future research will likely provide taxonomic affinities for unidentified OTUs, as well as information on their ecological functions in arctic soils. With the future development of arctic mycology, the analyses carried out in this work may be repeated with more complete sets of taxonomic and ecological fungal data. Future research may affect some of the data interpretations presented in this thesis, and below the various responses of arctic fungi to climate change are summarized using the most up-to-date knowledge on fungal taxonomy and ecology.

Fungal diversity revealed by NGS

Throughout this thesis, a conservative approach was used to only include sequences that likely were fungal, i.e. had at least 70-80% similarity to known fungal sequence in reference databases. This approach may be considered arbitrary, as deeply divergent fungal lineages that are not presented in publicly available databases would be excluded from the data analyses. On the other hand, considering that the arctic tundra is a young biome, that first appeared between 2 and 3 Mya, it is unlikely to find deeply divergent lineages of any organism type (including fungi) to be endemic to the Arctic. Because closely related taxa of all arctic species are bound to occur in boreal and temperate biomes as well, and fungi in general have a good potential to disperse, the majority of fungi in our samples were expected to be more than 70% similar to previously published sequences, e.g., from other arctic studies and from boreal forest soils. At the same time, such a conservative approach was expected to exclude the vast majority of erroneous reads in the dataset. The 6-6.5 million high-quality fungal sequences that we obtained were clustered in ca. 3500 - 4000 OTUs, a proxy for fungal species richness in the dominant tundra types in the Toolik Lake region. Fungal richness in one gram of soil that was used in our experimental replicates, lay in the range of 220-570 OTUs, and was not significantly different in the dry and moist tundra types. The majority of the identified OTUs (64%) belonged to the phylum Ascomycota, and 31.5% were identified as species of Basidiomycota. The species of Chytridiomycota, Glomeromycota and Zygomycota represented 0.6%, 0.2% and 2.2% of the OTUs, respectively. In addition, 1.4% of the OTUs were identified as Rozellomycota – a group of endoparasites of algae and water molds, branching at or near the phylogenetic root of the Fungal Kingdom and, therefore, considered either a lineage of Fungi or Choanozoa (Corsaro et al, 2014). Among the taxonomic orders of Ascomycota, Helotiales and Chaetothyriales had the highest species richness. In Basidiomycota, the order Agaricales was the most diverse, followed by Sebacinales.

Due to the dearth of knowledge of fungal identities and ecological functions in tundra soils, obtaining ecological assessments for the majority of fungal OTUs in our dataset was a complex process. Assigning ecological functions to ca. 50% of fungal OTUs was, therefore, considered a more than satisfactory result. Of the assigned OTUs in both the dry and moist tundra, saprotrophic species dominated (50-55%), followed by the ectomycorrhizal guild (16-19%). Richness of parasitic fungi was relatively low; ca. 4% of the OTUs were assigned mycoparasitic or

animal parasitic lifestyle, and approximately 4-5% were plant pathogens. In addition, in the dry tundra, lichens made a substantial contribution (14%) to fungal diversity. Because of their morphology, lichenized fungi are available in the vegetation description lists from Toolik Lake. In our datasets, 100-120 OTUs were identified as lichens. In total, 28 genera of lichens were identified by NGS, of which 12 genera were also found in morphological studies (http://www.arctic-atlas.org/). Sixteen genera detected by NGS only, included *Umbilicaria*, *Physcia*, *Lepraria*, *Japewia*, *Micarea* etc.

Fungal responses to experimental summer warming

Our data suggested strong responses in fungal communities of the moist tundra to experimental temperature increase, although no significant response was found for the overall community composition in dry tundra. Because plant communities also responded strongly to experimental summer temperature increase in moist tundra (which was shown in the former studies, e.g., Welker et al, 1997; Arft et al, 1999; Wahren et al, 2005; Walker et al, 2006), it was assumed that more pronounced fungal responses in this tundra type may correlate with the similar trend observed for the plants, although it remains unknown to what extent changes in fungal communities drive or are driven by shifts in vegetation. Another possible explanation for stronger fungal responses to warming in the moist tundra relates to natural fluctuations of temperature and water content in the dry and moist tundra types. In the moist tundra, soils are likely to experience lesser variation in temperatures due to higher water content, and established vegetation that buffer changes in environmental parameters. In dry tundra, where vegetation cover does not exceed 50% and water content is low, temperature and water stresses are likely to occur more often. Subsequently, plants and fungi in the dry tundra have evolved adaptations to strong variation in environmental parameters. The additional 2°C warming during a 2-month period per year, implemented by the experimental treatment in dry tundra, may be a negligible change given the high variation in ambient temperatures.

Summer warming alters richness in fungal taxonomic groups

To quantify the responses of fungal taxonomic groups to warming, a conservative approach was used with regards to initial OTU richness of the group of interest (not less than 8 OTUs) and strength of Pearson's correlation ($|R|>0.5$). Considering fungal groups of at least 8 OTUs aimed to avoid strong correlations coefficients

that could be obtained due to small sampling sizes. Although correlation coefficients R^2>0.2 could be used as identification of correlation in similar comparative studies as ours, focusing on strong correlations was considered more important for characterizing shifts in fungal community compositions. In moist tundra, the majority of fungal taxa had higher richness in the control plots, suggesting a negative effect of the warming treatment on fungi. Among the few taxa that benefited from warming, were saprotrophic molds (Eurotiales), saprotrophic or ericoid mycorrhizal genus *Pseudogymnoascus*, and insect pathogenic Hypocreales. In dry tundra, an opposite trend was found – the majority of fungal lineages seemed to benefit from warming in terms of OTU richness, although the trends were not significant. Similarly, significant decline in richness of the majority of fungal lineages in moist tundra and non-significant increase in dry tundra were shown for ECM basidiomycetes (Morgado et al, 2015). It is, therefore, important to refer to a specific arctic ecosystem in experimental studies on arctic fungi and their responses to climatic changes. Because microbial community composition not only responds to climate warming but is also governed by tundra type, various ecological consequences of the climate change may be revealed across different sampling sites. Whenever possible, a relatively fine taxonomic scaling should be utilized to quantify changes in fungal richness under climate manipulations. Because specific fungal lineages may increase or decline in richness under the altered conditions, operating with total fungal richness as a measure of fungal response to climate change may not be informative.

Responses of fungal ecological groups to summer warming

It was a challenge to assign fungal OTUs to specific functional guilds. The majority (app. 80%) of the fungal OTUs in the dataset were identified to major taxonomic levels only, e.g., orders or classes, and therefore, assigning ecological roles to them in a reliable manner required extra effort, e.g., by checking isolation source information for not fully identified reference sequences and by comparing the OTUs to sequences generated from 250 morphologically different fungal strains isolated from surface-sterilized roots of arctic plants from the research area (unpublished data). This approach allowed to unravel numerous root-associated species that otherwise would be considered saprotrophic. The same approach was used for those OTUs that were identified to a species level, but where the possibility for multi-functionality existed, e.g., when a fungus was known to have a predominantly saprotrophic lifestyle, but was a facultative symbiont of plants or animals. Ecological functions for the OTUs in obtained dataset were assigned using

two independent approaches; primarily, the published ecological information (e.g., Tedersoo et al, 2014) of the taxon in question was considered. Secondly, the source information of the closest reference sequence in publicly available databases (UNITE) was taken into account. Although these two approaches resulted in largely similar assignments, there still were different ecological functions set for numerous OTUs. The contradictions were solved using our culture-based dataset. For example, while *Penicillium* species generally are regarded as saprotrophic, several species of *Penicillium* were considered root-associated rather than saprotrophic in this study, according to the source of isolation for the closest reference sequence in UNITE, as well as obtained culture-based evidence for fungal root-endophytic lifestyle. This approach of assigning ecological functions to fungal OTUs was considered the most accurate given the currently available knowledge of arctic fungi.

Increased summer temperatures altered richness in many ascomycete functional groups. In both tundra types, there was a decline in lichenized and moss-associated species (orders Lecanorales and Geoglossales), which was in agreement with formerly reported decreases in lichen and bryophyte coverage and species diversity. Lichens and bryophytes most likely decline in response to increases in vascular plant biomass, as the declines in these groups did not occur in warming experiments where vascular plants were not present (Cornelissen et al, 2001). Expansion of shrubs across the warming plots and subsequent accumulation of plant leaf litter likely resulted in increases of ericoid mycorrhizal (Geml et al, 2015), endophytic, plant- and insect- pathogenic ascomycetes in moist tundra, and increase in saprotrophic ascomycetes in both the dry and the moist tundra. On the other hand, warming-induced changes in vegetation did not entirely correlate with shifts observed for fungi. For example, ECM and saprotrophic basidiomycetes strongly declined (Geml et al, 2015; Morgado et al, 2015), despite the expansion of ECM hosts and litter accumulation. While the current prevailing opinion is that altered plant community composition drives fungal community change in the Arctic, and possible mismatches in plant and fungal responses is due to retarded response in fungi (Dahlberg and Bultmann, 2013), shifts in fungal community composition are unlikely entirely driven by plants. The reported shifts in plant communities largely relate to changes in abundance of various functional groups rather than changes in plant richness or species identities (Wahren et al, 2005). In fungi, responses to warming are mostly due to differences in community compositions, i.e. presence of specific OTUs in one treatment and absence in the other. Possibly, different response mechanisms of plant and fungal communities

contribute to discrepancy in responses of plants and their associated fungi to warming. Supposedly, fungi are responding to altered conditions independently and faster than plants, and, therefore, may be useful for monitoring early ecosystem responses to environmental change.

In addition to richness estimations, it was important to quantify warming-induced changes in fungal functional groups with regard to species abundance. Total fungal biomass in soils can be measured using ergosterol and phospholipid fatty acid (e.g., 18:2ω6, Frostegård and Bååth, 1996) concentrations, however, these methods do not distinguish between different fungal ecological or taxonomic groups. Currently available methods do not directly quantify actual fungal biomass separately for species, genera or even the functional groups in question. Therefore, our aim was to estimate relative changes in abundance of different fungal ecological groups, assuming that higher fungal biomass would result in higher sequence read counts. In general, using sequence read abundance as proxy for biomass is constrained by interspecific differences in copy numbers and length of the ITS region as well as other species-specific biological factors. However, for particular species (or in this case, OTUs), changes in sequence counts in different samples may be considered indicative of relative changes and trends with respect to biomass (Amend et al, 2010). For each OTU assigned to an ecological group of interest, the mean read counts across the control and warming plots were compared to calculate size effect and 95% confidence interval. The mean of the size effect values (± standard deviation) was used as a measure of the overall response to warming of ecological group in question. This approach does not take into account interspecific differences in abundance, instead, it summarizes the trends observed in each individual OTU belonging to the same functional group, giving equal weight to every species. The abundance-based analyses revealed strong declines in ECM and lichenized fungi, and an increase in animal-pathogenic (including insect-pathogenic) species. These results were in agreement with richness trends observed for the same ecological groups. On the other hand, unexpectedly, saprotrophic fungi showed a strong decline in abundance across the warmed plots, despite the observed increase in leaf litter. Interestingly, very few OTUs in our dataset showed no response to warming, i.e., for the majority of the OTUs, either a strong increase or a decline in sequence counts induced by the warming treatment was observed. This indicates that within the broadly defined ecological or taxonomic groups, there are fungal species that grow poorly under the warming treatment, as well as species that benefit from the increased summer temperatures, irrespective of the overall group response. For instance, even among lichens that strongly declined in

both richness and abundance in the warmed plots as a group, few species (OTUs) clearly benefited from warming, i.e. increased in richness/abundance or were even indicators of the warming treatment.

Ecological implications of warming-induced shifts in fungal communities

Fungi play numerous ecological roles of key importance, e.g., in nutrient cycling (decomposers), as soil-forming organisms and pioneer species (lichenized fungi), facilitating plant growth (mycorrhizae, endophytes), regulating plant and animal populations (parasites), as components of food-webs (lichens and macrofungi), etc. In addition, fungi are intimately involved in a complex web of interactions with other organisms that are still poorly known. Therefore, warming-induced changes in fungal species composition, taxonomic richness, and abundance of functional groups observed in tundra soils are expected to have a wide range of consequences. For example, loss of lichens may have consequences for caribou and reindeer populations, because lichens (particularly, species of *Cladonia* and *Cetraria*) form a major part of the winter diet of these animals (Joly et al, 2009). Subsequently, populations of predators, i.e. wolves, bears and golden eagles, may be altered. Increased summer temperatures also result in rapid drying of fruticose lichens, which promotes frequent low-intensity fires and facilitates even greater loss of lichens (Joly et al, 2009). Declines in mycorrhizal fungi is expected to have a suite of consequences, including altered C and N cycling and storage, decreased nutrient availability for plants, and lower plant fitness as mycorrhizae influence the production of plant metabolites against herbivory (Vannette and Hunter, 2013). ECM species are known to compete with free-living decomposers for water and nutrients (Orwin et al, 2011), and a possible decline in ectomycorrhizal fungi may result in reduced C allocation to tundra soils, given that generally up to 50-70% of plant C uptake is transferred directly to ECM fungal mycelium (Clemmensen et al, 2013). Because ECM fungi also release nutrients from mineral particles of rocks by weathering, their decline may result in reduced availability of soil microelements (Orwin et al, 2011). Many species of mycorrhizal and free-living fungi produce relatively large fruiting bodies that are consumed by mammals and mushroom-feeding insects. Although there is no direct evidence in this regard, it is reasonable to assume that production of fruiting bodies may decrease with declining mycelial abundance, with possible dietary consequences for the above-mentioned animals. Future studies are needed to test the effects of winter and summer warming on fungal fructification in the Arctic. If altered, fungal fructification may have effects on a variety of natural processes in the Arctic, including plant herbivory, insect

food webs and decomposition. Activity of saprotrophic species is generally correlated with decomposition rates and flux of CO_2 between the terrestrial and atmospheric pools. Therefore, a decline in abundance of saprotrophic fungi may affect CO_2 emission. Loss of saprotrophic fungi may as well result in restrained nutrient cycling and reduced hydraulic redistribution of water, implying lower rates of C mineralization and reduced fungal enzymatic activity in soils (Guhr et al, 2015). Because numerous arctic invertebrates graze on fungal mycelium (Crowther et al, 2012), their populations may be altered in response to lower fungal availability, with consequences for decomposition processes. Another possible implication of warming-induced changes in arctic fungi relates to the production of melanin. Melanin is a stable compound produced by many ericoid mycorrhizal, dark septate endophytic and ECM species, and its accumulation in soils implies increased C storage. However, melanization occurs in different taxonomic and ecological groups of fungi that show various responses to warming, and therefore, it is difficult to speculate how levels of melanin may change in arctic soils under warmer conditions. In summary, changes in fungal community compositions are expected to have multiple consequences for ecosystem functioning, including decomposition and CO_2 flux, as well as alterations in species interactions in arctic tundra. These changes will be particularly prominent in moist tundra where the effect of summer warming on soil fungi was strong.

Fungal responses to increased snow depth

Experimentally increased snow depth strongly altered fungal community compositions in both the dry and moist tundra. Strong fungal community response in the moist tundra was somewhat expected, because fungi of this tundra type seemed to be highly sensitive to adjusted environmental conditions, e.g., changed significantly in summer warming experiments. In dry tundra, fungal communities could be better adapted to variation in soil temperatures, which explains why no significant changes in community compositions were observed under increased summer temperatures. Countrary to these above expectations, a strong (comparable to the observed in the moist tundra) effect of snow depth on fungal richness and community composition was found in the dry tundra. There are two main reasons that may explain why increased snow depth had a stronger effect on fungal communities in dry tundra than did summer temperatures. Primarily, winters in arctic tundra last up to 9 months, and for any given year fungal communities were exposed to deeper snow approximately 5 times longer than to summer warming. Secondly, deeper snowpack likely affected soil moisture by the additional snow

melt water in spring. Moisture is known to be an important factor influencing fungal growth in dry tundra (Jones et al, 1998). The combination of both increased moisture and temperature resulting from deeper snow cover likely contributed to strong shifts in soil fungi in dry tundra, although it is difficult to quantify the impacts of these two factors individually.

Responses of fungal taxonomic groups to increased snow depth

In moist tundra, we observed strong declines in seventeen fungal taxa, although no lineages increased in richness under the deep snow. Such a decline in at least three taxa, i.e. Eurotiales, Hypocreales and *Pseudogymnoascus*, is likely attributed to altered moisture, as higher temperatures resulted in their increased richness in our summer warming experiments. Possibly, soil moisture in deep snow plots becomes excessive in the time of spring snow melt, and negatively affects numerous fungal species of the moist tundra. In dry tundra, four fungal lineages declined in richness across the deep snow plots, however richness increased in the orders Archaeorhizomycetales and Venturiales, and a genus *Clavaria*. The genus *Clavaria* was particularly interesting because it showed strong responses in richness in both dry and moist tundra types and in both summer and winter experiments. Except for the snow addition experiment in dry tundra, this genus declined in richness, suggesting importance of high soil moisture and ambient (i.e. not increased) temperatures for growth. Given such strong responses to climate change, species richness in *Clavaria* may possibly serve an indicator of global warming in arctic tundra. In our warming experiments numerous fungal taxa either increased or decreased in richness in response to treatment. However, most of the observed trends were difficult to explain, given a variety of possible ecological responses in fungi. Even in the case of parasitic lineages, richness could increase either in line with expansion of the hosts, or due to a decrease in the host coverage attributed to a decline in the host fitness. For example, in dry tundra, increase in Archaeorhizomycetales that are parasites of mycorrhizal and root-associated species (Rosling et al, 2011), was in agreement with the decline in ECM fungi in deep snow plots, implying an increase in parasites related to the lower fitness of their hosts. Similarly, the increase in lichenicolous (lichen parasitic) species could be explained by decreased lichen fitness. On the other hand, increase in Venturiales, many of which are plant pathogenic, could relate to expansion of their hosts (shrubs and graminoids). Because the experimental setup did not allow to verify any specific ecological concept that could potentially explain changes in

fungal community structure, possible ecological explanations were omitted from this thesis.

Summer warming and increased snow depth treatments resulted in opposite responses in few fungal lineages. For instance, in dry tundra, the orders Capnodiales and Peltigerales were favored by summer warming, but declined in snow addition experiments. In the moist tundra, the order Hypocreales showed opposite responses to summer and winter climate change simulations. These examples show that various processes related to climate change, such as shifts in temperature and precipitation patterns, may have different effect even on the same fungal taxa. Therefore, studies addressing microbial responses to global warming should not exclusively focus on the effects of rising temperatures, but instead address a combination of multiple consequences of the warming climate, including winter precipitation.

Shifts in fungal ecological groups under increased snow depth

In moist tundra, deeper snow resulted in strong declines in richness of six ecological groups of fungi. For four of them, i.e., bryophyte-associated, lichenized, saprotrophic and ECM fungi, strong decreases in abundance were observed as well. In addition, total fungal read counts were higher in control plots, suggesting an overall decline in fungal biomass caused by the increased snow depth. As described above, these changes in the moist tundra may result in altered decomposition rates and CO_2 emission due to a decline in saprotrophic fungi, lower rates for nutrient turnover associated with declines in saprotrophs and mycorrhizal species, and decreased C storage in soil due to a decline in melanized fungi. Directly or indirectly, such shifts in fungal communities may affect populations of soil invertebrates and bacteria, as well as aboveground vegetation. In dry tundra, only the decline in lichens was supported by both the richness and abundance tests, and no significant shifts were reported for the overall fungal richness or abundance. As opposed to the trend observed in moist tundra, abundance of ECM fungi increased. Increased ECM abundance coupled with the decreased richness in this functional group could imply more intensive growth of few well-adapted ECM species, e.g., *Cortinarius* (Morgado et al, 2016) in deep snow plots. In part, trends observed for fungal functional groups in response to increased snow depth could be explained by the vegetation shifts. However, most of these changes did not correlate with formerly reported shifts in plants. Similar to observations in summer warming experiments, fungal responses to increased snow depth likely outpaced

responses in plants, presumably due to higher rates of community turnover in fungi compared to plants. Turnover relates to an important aspect of species interactions in arctic soils. It is possible that specific fungal lineages in our dataset declined or increased under climate manipulations due to alterations in richness/abundance of other taxa with which they could have mutualistic or competitive interactions. Given the complexity of below-ground communities, it is difficult to estimate the relative impact of edaphic factors versus species interactions on microbial responses to climate manipulations. In general, very little is known about fungal-fungal interactions in soils. Although understanding fungal-fungal interactions was outside the scope of this project, the data obtained may be used for revealing positive and negative co-occurrence patterns of the OTUs. Patterns as such may include possible examples for mutualistic or antagonistic interactions to be tested in future studies. In addition, addressing fungal interactions with other soil organisms, e.g., bacteria, myxomycetes and soil invertebrates by a similar approach, may be another exciting area for future research on arctic biota and its responses to warming climate.

Future research

This thesis provides a baseline for understanding the fungal community responses to two processes associated with the warming climate - long-term increases in summer temperature and in winter snow depth. The study implementation was based on the long-term experimental setup simulating climatic changes. The experimental setup, however, was primarily designed to address changes in plant community compositions, implying few limitations that we had to face addressing changes in communities of soil fungi. For instance, none of the habitats studied in this work could have a true replicate due to the existing design of experiments that lasted for more than 20 years. Recently, some changes were made at the Toolik experiment to overcome some of these limitations. For example, the long 60 m snow fence was divided into several shorter sections, so that experimental and control plots are adjacent to each other. In addition, experiments that involve removal of the climate manipulation devices will show if changes in arctic ecosystems are reversible, and if so, how shifts to the ambient state occur.

Projects addressing combined effects of summer warming and increased snow depth will be carried out in the future, possibly using more advanced techniques (that do not require a PCR step and, therefore, provide more reliable information regarding the relative occurrence of different fungal taxa) to quantify shifts in

abundance of various fungal taxonomic and ecological groups. Open-top chambers and snow fences similar to the ones used in this project have also been established in other ecosystems, inside and outside the Arctic. A meta-analysis of these experiments could be helpful to study microbial responses to climatic changes at distant localities. Because microbial communities in different regions vary in species composition, the meta-analysis could show how the similar experimental manipulations may affect turnover in microbial communities under different environmental conditions, particularly with regard to taxonomic and functional groups. The list of fungal OTUs that were favored by the climate manipulations involved representatives of all fungal taxonomic and functional groups, as revelaed in this study. A similar trend was observed for the OTUs that declined in richness /abundance under the climate manipulations. Supposedly, both the taxonomic and ecological groups of fungi are too broadly defined to explain the mechanisms of the climate change effects on fungal communities. An additional approach targeting genes responsible for production of specific extracellular enzymes or compounds (e.g., melanin) could be used in the future to build predictions regarding functional implications of global warming in the Arctic.

This study is among the first addressing the effects of climate change on soil fungal communities in the arctic tundra. This work provided estimations for fungal species richness, taxonomic and functional diversity in the two main vegetation types found throughout Toolik area, i.e. dry and moist tundra. The data indicate strong changes in fungal community compositions under the two experimental treatments - summer warming and winter snow addition, and show strong differences in fungal community compositions of dry and moist tundra. Because fungi are embedded in a large network of interactions with other organisms, this work has a potential for developing further research projects, e.g., in mycology, lichenology, botany, soil sciences, entomology, arctic ecology, climatology and other sciences. In addition, it addresses gaps in current DNA-based methods for characterizing microbial communities, including uncomplete databases and poor knowledge of fungal diversity. Future technological advancement allowing accurate single molecule sequencing that does not require a PCR step, will be helpful to provide reliable estimations of fungal species abundance in soil. Due to a high community turnover, fungi could serve as good model organisms for monitoring changes in ecosystems under climatic stresses. Therefore, mycological studies are expected to complete the existing assessments of early-stage ecosystem disturbances of various kinds, including climate change.

References

Abarenkov K, Nilsson RH, Larsson KH, et al. 2010. The UNITE database for molecular identification of fungi – recent updates and future perspectives. *New Phytologist* **186**: 281–285.

Addy HD, Piercey MM, Currah RS. 2005. Microfungal endophytes in roots. *Canadian Journal of Botany* **83**:1–13.

Adler LS, de Valpine P, Harte J, Call J. 2007. Effects of long-term experimental warming on aphid density in the field. *Journal of the Kansas Entomological Society* **80(2)**: 156-168.

Alheit J, Hagen E. 1997. Long-term climate forcing of European herring and sardine populations. *Fish Oceanography* **6**:130–139.

Allison NL, Bindoff RA, Bindschadler PM, et al. 2009. The Copenhagen Diagnosis: Updating the World on the Latest Climate Science, The University of New South Wales Climate Change Research Centre (CCRC), Sydney, Australia, 60pp.

Allison SD, Treseder KK. 2008. Warming and drying suppress microbial activity and carbon cycling in boreal forest soils. *Global Change Biology* **14:** 2898-2909.

Amend AS, Seifert KA, Bruns TD. 2010. Quantifying microbial communities with 454 pyrosequencing: does read abundance count? *Molecular Ecology* **19**: 5555-5565.

Anderson IC, Drigo B, Keniry K, et al. 2013. Interactive effects of preindustrial, current and future atmospheric CO2 concentrations and temperature on soil fungi associated with two Eucalyptus species. *FEMS Microbiology Ecology* **83**:425-437.

Anderson MJ. 2001. A new method for non-parametric multivariate analysis of variance. *Austral Ecology* **26**: 32–46.

Anisimov OA, Vaughan DG, Callaghan TV, et al. 2007. Polar regions (Arctic and Antarctic). Climate Change 2007: Impacts, Adaptation and Vulnerability. Contribution of Working Group II to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change (Parry ML, Canziani OF, Palutikof JP, van der Linden PJ & Hanson CE, eds), pp. 653–685. Cambridge University Press, Cambridge, UK.

Arft AM, Walker MD, Gurevitch J, et al. 1999. Responses of tundra plants to experimental warming: meta-analysis of the international tundra experiment. *Ecological Monographs* **69(4):** 491-511.

Arnold AE, Miadlikowska J, Higgins KL, et al. 2009. A phylogenetic estimation of trophic transition networks for ascomycetous fungi: Are lichens cradles of symbiotrophic fungal diversification? *Systematic Biology* **58:**283–297.

Banfield AWF. 1972. Are arctic ecosystems really fragile? Int. Reindeer. Caribou Symp., 1st. Fairbanks, Alaska: *Institute of Arctic Biol*ogy. 1 6. Bank, T. P. 1958.

Barbeito I, Brücker RL, Rixen C, et al. 2013. Snow Fungi- Induced mortality of *Pinus cembra* at the alpine treeline: evidence from plantations. *Arctic, Antarctic and Alpine research* **45(4)**: 455-470.

Barnett DN, Brown SJ, Murphy JM, et al. 2006. Quantifying uncertainty in changes in extreme event frequency in response to doubled CO2 using a large ensemble of GCM simulations. *Climate Research* **26**: 511 – 889.

Beebee TJC. 1995. Amphibian breeding and climate change. *Nature* **374**: 219-220.

Bellard C, Bertelsmeier C, Leadley P, et al. 2012. Impacts of climate change on the future of biodiversity. *Ecology Letters* **15(4)**:365-377.

Bellemain E, Davey ML, Kauserud H, et al. 2013. Fungal paleodiversity revealed using high-throughput metabarcoding of ancient DNA from arctic permafrost. *Environmental Microbiology* **15(4)**: 1176-1189.

Bintaja R, Selten FM. 2014. Future increases in arctic precipitation linked to local evaporation and sea-ice retreat. *Nature* **509**: 479-482.

Bintanja R, Graversen RG, Hazeleger W. 2011. Arctic winter warming amplified by the thermal inversion and consequent low infrared cooling to space. *Nature Geoscience* **4**, doi:10.1038/NGEO1285.

Bjorbækmo MFM, Carlsen T, Brysting A, et al. 2010. High diversity of root associated fungi in both alpine and arctic Dryas octopetala. *BMC Plant Biology* **10**: 244.

Blaalid R, Carlsen T, Kumar S, et al. 2012. Changes in the root-associated fungal communities along a primarily succession gradient analysed by 454 pyrosequencing. *Molecular Ecology* **21(8)**: 1897 - 1908.

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

Bliss LC, Courtin GM, Pattie DL, et al. 1973. Arctic tundra ecosystems. *Annual Review of Ecology, Evolution, and Systematics* **4**: 359-399.

Blok D, Weijers S, Welker JM, Cooper E, Michelsen A, Elberling B. 2015. Deepened winter snow increases stem growth and alters stem 13C and 15N in evergreen dwarf shrub

Cassiope tetragona in high-arctic Svalbard tundra. *Ecological Research Letters* **10**: 044008.

Borner AP, Kielland K, Walker MD. 2008. Effects of simulated climate change on plant phenology and nitrogen mineralization in Alaskan arctic tundra. *Arctic, Antarctic and Alpine Research* **40**:27-38.

Boxman AW, Sinke RJ, Roelofs J.G.M. 1986. Effects of HN_4^+ on the growth and K^+ uptake of various ectomycorrhizal fungi in pure culture. *Water, Air and Soil pollution* **31**: 517-522.

Brown JL, Shou-Hsien L, Bhagabati N. 1999. Long-term trend toward earlier breeding in an American bird: A response to global warming? *Proceedings of the National Academy of Sciences* **96**: 5565.

Brown SP, Callaham MAJr, Oliver AK, et al. 2013. Deep Ion Torrent sequencing identifies soil fungal community shifts after prescribed fires in a south-eastern US forest ecosystem. *FEMS Microbiology Ecology* **86(3)**: 557-566.

Bruns TD, White TJ, Taylor JW. 1991. Fungal Molecular Systematics. *Annual Review of Ecology and Systematics* **22**: 525–564.

Buckeridge KM, Grogan P. 2008. Deepened snow alters soil microbial nutrient limitations in arctic birch hummock tundra. *Applied Soil Ecology* **39**:210-222.

Butler JH, Montzka SA. 2016. The NOAA annual greenhouse gas index (AGGI). Earth System Research Laboratory, *http://esrl.noaa.gov/.*

Butler MJ, Day AW. 1998. Fungal melanins: a review. *Canadian Journal of Microbiology* **44**:1115-1136.

Campbell BJ, Polson SW, Hanson TE, et al. 2010. The effect of nutrient deposition on bacterial communities in Arctic tundra soil. *Environmental Microbiology* **12**: 1842-1854.

Chapin FS. III, Shaver GR, Giblin AE, et al. 1995. Responses of Arctic tundra to experimental and observed changes in climate. *Ecology* **76**:694–711.

Classen AT, Sundqvist MK, Henning JA, et al. 2015. Direct and indirect effects of climate change on soil microbial and soil microbial-plant interactions: What lies ahead? *Ecosphere* **6(8)**: 130.

Clemmensen KE, Michelsen A, Jonasson S, Shaver GR. 2006. Increased ectomycorrhizal fungal abundance after long-term fertilization and warming of two arctic tundra ecosystems. *New Phytologist* **171(2)**: 391-404.

Clemmensen KE, Ovaskainen ABO, Dahlberg A, et al. 2013. Roots and associated fungi drive long-term carbon sequestration in boreal forest. *Science* **339**:1615.

Coleman DC, Whitman WB. 2005. Linking species richness, biodiversity and ecosystem function in soil systems. *Pedobiologia* **49**:479-497.

Collins M, Knutti R, Arblaster J, et al. 2013. Long-term climate change: Projections, commitments and irreversibility. In Climate Change 2013: The Physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change. Stocker, T.F., Qin, D., Plattner, G.-K., Tignor, M., Allen, S.K., Doschung, J. et al. (Eds.). Cambridge, UK, Cambridge University Press, pp. 1029-1136.

Comiso JC, Hall DK. 2014. Climate trends in the Arctic as observed from space. Wiley Interdiscip. *Reviews in Climate Change* **3**: 389-409.

Cornelissen JHC, Callaghan TV, Alatalo JM, et al. 2001. Global change and arctic ecosystems: is lichen decline a function of increases in vascular plant biomass? *Journal of Ecology* **89**:984-994.

Corsaro D, Walochnik J, Venditti D, Steinmann J, Müller KH, Michel R. 2014. Microsporidia-like parasites of amoebae belong to the early fungal lineage Rozellomycota. *Parasitology Research* **113**:1909–1918.

Cox PM, Betts RA, Jones CD, Spall SA, Totterdell IJ. 2000. Acceleration of global warming due to carbon cycle feedbacks in a coupled climate model. *Nature* **408**: 184-187.

Crick HQP, Dudley C, Glue DE, Thomson, DL. 1997. UK birds are laying eggs earlier. *Nature* **388**: 526.

Crowther TW, Boddy L, Jones TH. 2012. Functional and ecological consequences of saprotrophic fungus-grazer interactions. *ISMEJ* **6(11):** 1992-2001.

Dahlberg A, Bültmann H. 2013. Fungi. Chapter 10 in Arctic Biodiversity Assessment. Status and trends in Arctic Biodiversity. Conservation of Arctic Flora and Fauna (CAFF). Edited by Hans Meltofte. Narayana Press, Denmark, 676 pp.

DeAngelis KM, Pold G, Topcuoglu BD, et al. 2015. Long-term forest soil warming alters microbial communities in temperate forest soils. *Frontiers in Microbiology* **6**.

DeMarco J, Mack MC, Bret-Harte MS. 2011. The Effects of Snow, Soil Microenvironment, and Soil Organic Matter Quality on N Availability in Three Alaskan Arctic Plant Communities. *Ecosystems* **14**: 804–817.

Derksen C, Brown R, Mudryk L, et al. 2015. Terrestrial Snow Cover. *In Arctic Report Card:Update for 2015.* NOAA Press Release. http://www.arctic.noaa.gov/reportcard.

Deslippe JR, Hartmann M, Mohn WW, et al. 2011. Long-term experimental manipulation of climate alters the ectomycorrhizal community of *Betula nana* in Arctic tundra. *Global Change Biology* **17(4)**: 1625-1636.

Deslippe JR, Hartmann M, Simard SW, et al. 2012. Long-term warming alters the composition of Arctic soil microbial communities. *FEMS Microbiology Ecology* **82**:303–315.

Devi LS, Khaund P, Nongkhlaw FMW, Joshi SR. 2012. Diversity of culturable soil micro-fungi along altitudinal gradients of Eastern Himalayas. *Mycobiology* **40(3)**: 151-158.

Dlugokencky EJ, et al. 2005: Conversion of NOAA CMDL atmospheric dry air CH4 mole fractions to a gravimetrically prepared standard scale. *Journal of Geophysical Research* **110**, *D18306, doi:10.1029/2005JD006035*

Dollery R, Hodkinson ID, Jonsdottir IS. 2006. Impact of warming and timing of snow melt on soil microarthropod assemblages associated with Dryas-dominated plant communities on Svalbard. *Ecography* **29(1)**: 111-119.

Dufrêne M, Legendre P. 1997. Species assemblages and indicator species: the need for a flexible asymmetrical approach. *Ecological Monographs* **67**: 345-366.

Dunn PO, Cockburn A. 1999. Extrapair mate choice and honest signaling in cooperatively breeding superb fairy-wrens. *Evolution* **53**:938-946.

Edgar RC, 2010. Search and clustering orders of magnitude faster than BLAST. *Bioinformatics* **26(19)**: 2460-2461.

Edgar RC, Haas BJ, Clemente JC, Quince C, Knight R. 2011. UCHIME improves sensitivity and speed of chimera detection. *Bioinformatics* **27**: 2194–2200.

Etheridge DM, Steele LP, Francey RJ, Langenfields RL. 1998. Atmospheric methane between 1000 A.D. and present: Evidence of anthropogenic emissions and climatic variability. *Journal of Geophysical Research* **103**:15979-15993.

Fahnestock JT, Povirk K.A, Welker JM, 2000. Abiotic and biotic effects of increased litter accumulation in arctic tundra. *Ecography* **23**: 623-631.

Feely RA, et al. 2004. Impact of anthropogenic CO_2 on the $CaCO_3$ system in the oceans. *Science* **305**: 362– 366.

Fernandez CW, Koide RT. 2013. The function of melanin in the ectomycorrhizal fungus *Cenococcum geophillum* under water stress. *Fungal Ecology* **6**:479-486.

Fierer N, Jackson RB. 2006. The diversity and biogeography of soil bacterial communities. *Proceedings of the National Academy of Sciences USA* **103**: 626–631.

Ford JD, McDowell G, Jones J. 2014. The state of climate change adaptation in the Arctic. *Environmental Research Letters* **9**, 9 p. *doi:10.2105/AJPH.2010.300105*.

Frostegård A, Bååth E. 1996. The Use of Phospholipid Fatty Acid Analysis to Estimate Bacterial and Fungal Biomass in Soil. *Biology and fertility of soils* **22(1)**:59-65.

Gardes M, Dahlberg A. 1996. Mycorrhizal diversity in arctic and alpine tundra: An open question. *New Phytologist* **133**: 147–157.

Geml J, Gravendeel B, Nielen M, et al. 2014a. DNA metabarcoding reveals high fungal diversity and pH-correlated habitat partitioning in protected coastal *Salix repens* communities in the Netherlands. *PLOS ONE* **9(6)**:e99852.

Geml J, Laursen GA, Herriott IC, et al. 2010. Phylogenetic and ecological analyses of soil and sporocarp DNA sequences reveal high diversity and strong habitat partitioning in the boreal ectomycorrhizal genus *Russula* (Russulales; Basidiomycota). *New Phytologist* **187**: 494-507.

Geml J, Laursen GA, Taylor DL. 2008. Molecular diversity assessment of arctic and boreal *Agaricus* taxa. *Mycologia* 1**00**: 577–589.

Geml J, Laursen GA, Timling I, et al. 2009. Molecular phylogenetic biodiversity assessment of arctic and boreal ectomycorrhizal *Lactarius* Pers. (Russulales; Basidiomycota) in Alaska, based on soil and sporocarp DNA. *Molecular Ecology* **18**: 2213–27.

Geml J, Morgado LN, Semenova TA, et al. 2015. Long-term warming alters richness and composition of taxonomic and functional groups of arctic fungi. *FEMS Microbiology Ecology* **91**: 1-13.

Geml J, Pastor N, Fernandez L, et al. 2014. Large-scale fungal diversity assessment in the Andean Yungas forests reveals strong community turnover among forest types along an altitudinal gradient. *Molecular Ecology* **23(10)**:2452-2472.

Geml J, Timling I, Robinson CH, et al. 2012. An arctic community of symbiotic fungi assembled by long-distance dispersers: phylogenetic diversity of ectomycorrhizal basidiomycetes in Svalbard based on soil and sporocarp DNA. *Journal of Biogeography* **39**: 74–88.

Ghen G, Zhu Y, Wang HZ, et al. 2007. The metabolites of a mangrove endophytic fungus, *Penicillium thomii. Journal of Asian Natural Products Research.* **9(2)**: 159-164.

Gihring TM, Green SJ, Schadt CW. 2012. Massively parallel rRNA gene sequencing exacerbates the potential for biased community diversity comparisons due to variable library sizes. *Environmental Microbiology* 14:285–290.

Grabherr G, Gottfried M, Pauli H. 1994. Climate effects on mountain plants. *Nature* **369**: 448.

Grace J, Berninger F, Lazlo N. 2002. Impacts of climate change on the thee line. *Annals of Botany* **90(4)**: 537-544.

Graversen RG, Wang M. 2009. Polar amplification in a coupled climate model with locked albedo. *Climate Dynamics* **33**(5).

Graversen RG. 2006. Do changes in the midlalitude circulation have any impact on the arctic surface air temperature trend? *Journal of climate* **19**: 5422-5438.

Grelet GA, Johnson D, Vrålstad T, Alexander IJ, Anderson IC. 2010. New insights into the mycorrhizal *Rhizoscyphus ericae* aggregate: spatial structure and co-colonization of ectomycorrhizal and ericoid roots. *New Phytologist* **188(1)**: 210-222.

Griffin DM. 1963. Soil moisture and the ecology of soil fungi. *Biological reviews* **38(2)**: 141-166.

Grime JP. 2001. Plant strategies, vegetation processes, and ecosystem properties. Chichester, UK: John Wiley & Sons. 456 pp.

Guhr A, Borken W, Spohn M, Matzner E. 2015. Redistribution of soil water by a saprotrophic fungus enhances carbon mineralization. *PNAS* **112(47)**:14647-14651.

Gutknecht JLM, Field CB, Balser TC. 2012. Microbial communities and their responses to simulated global change fluctuate greatly over multiple years. *Global Change Biology* **18**:2256-2269.

Hambleton S, Sigler L. 2005. *Meliniomyces*, a new anamorph genus for root-associated fungi with phylogenetic affinities to *Rhizoscyphus ericae* (*Hymenoscyphus ericae*), Leotiomycetes. *Studies in Mycology* **53(1)**: 1-27.

Hasle TE. 2013. *The effect of experimental warming on insect herbivory in an alpine plant community*. M.Sc. Thesis. Norwegian University of Life Sciences.

Hawksworth D. 2001. The magnitude of fungal diversity: the 1.5 million species estimate revisited. *Mycological Research* **105(12)**: 1422-1432.

Hayden HL, Mele PM, Bougoure DS, et al. 2012. Changes in the microbial community structure of bacteria, archaea and fungi in response to elevated CO_2 and warming in an Australian native grassland soil. *Environmental Microbiology* **14:** 3081-3096.

Henry GHR, Molau U. 1997. Tundra plants and climate change: The International Tundra Experiment (ITEX). *Global Change Biology* **3**: 1–9.

Hersteinsson P, MacDonald DW. 1992. Interspecific competition and the geographical distribution of red and arctic foxes Vulpes vulpes and Alopex lagopus. *Oikos* **64**: 505–515.

Higgins KL, Arnold AE, Miadlikowska J, et al. 2007. Phylogenetic relationships, host affinity, and geographic structure of boreal and arctic endophytes from three major plant lineages. *Molecular Phylogentics and Evolution* **42**:543–555.

Hobbie JE, Hobbie EA, Drossman H, et al. 2009. Mycorrhizal fungi supply nitrogen to host plants in Arctic tundra and boreal forests: ^{15}N is the key signal[1]. *Canadian Journal of Microbiology* **55**: 84-94.

Hobbie JE, Hobbie EA. 2006. 15N in symbiotic fungi and plants estimates nitrogen and carbon flux rates in Arctic tundra. *Ecology* **87**: 816-822.

Hobbie JE. 2008. A mycorrhizal fungus, Cortinarius favrei, grows among associated plant species in the Alaskan tundra. *http://ecoed.net/browse/browseRecords/detail?recordId=486*

Holbrook SJ, Schmitt RJ, Stephens JSJr. 1997. Changes in an assemblage of temperate reef fishes associated with a climate shift. *Ecological Applications* **7**: 1299–1310.

Hollingsworth TN, Walker MD, Chapin FS III, Parsons AL. 2006. Scale-dependent environmental controls over species composition in Alaskan black spruce communities. *Canadian Journal of Forest Research*, **36**: 1781-1796

Hollister RD, Webber PJ, Bay C. 2005. Plant response to temperature in Northern Alaska: implications for predicting vegetation change. *Ecology* **86**:1562–1570.

Hulsen T, de Vlieg J, Alkema W. 2008. Bio-Venn- a web application for the comparison and visualization of biological lists using area-proportional Venn diagrams. *BMC Genomics* **9(1):** 488.

Hustad VP, Miller AN, Dentinger BTM, Cannon PF. 2013. Generic circumscriptions in *Geoglossomycetes*. *Persoonia* **31**: 101-111.

Ihrmark K, Bödeker ITM, Cruz-Martinez K, et al. 2012. New primers to amplify the fungal ITS2 region–evaluation by 454-sequencing of artificial and natural communities. *FEMS Microbiology Ecology* **82**: 666–677.

Inouye DW, Barr B, Armitage KB, Inouye BD. 2000. Climate change is affecting altitudinal migrants and hibernating species. *Proceedings of the National Academy of Sciences of the United States* **97**:1630–1633. doi:10.1073/pnas.97.4.1630.

IPCC, 1990. Climate Change. The IPCC Scientific Assessment. Houghton JT, Jenkins GJ, Ephraums JJ, eds. Cambridge university press, Cambridge, United Kingdom and New York, NY, USA.

IPCC, 2007. Climate change 2007, synthesis report. Contribution of Working Groups I, II and III to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change. Core Writing Team, Pachauri, R.K., Reisinger, A. (Eds). IPCC, Geneva, Switzerland, 104 pp.

IPCC. 2013. Stocker TF, Qin D, Plattner GK, Tignor M, Allen SK, Boschung J, Nauels A, Xia Y, Bex V, Midgley PM, eds. Climate change 2013: the physical science basis. Contribution of *Working Group I to the Fifth Assessment Report Panel of the Intergovernmental Panel on Climate Change* Cambridge, UK & New York, NY, USA: Cambridge University Press, 1535 pp.

Jägerbrand AK, Alatalo JM, Chrimes D, Molau U. 2009. Plant community responses to 5 years of simulated climate change in meadow and heath ecosystems at a subarctic-alpine site. *Oecologia* **161**:601-610.

Jeffries MO, Richter-Menge JA, Overland JE, eds. 2012. "Arctic Report Card 2012." National Oceanic and Atmospheric Administration. Retrieved online 2/22/2013 *from http://www.arctic.noaa.gov/reportcard.*

Joly K, Jandt RR, Klein DR. 2009. Decrease of lichens in Arctic ecosystems: the role of wildfire, caribou, reindeer, competition and climate in north-western Alaska. *Polar Research* **28**: 433-442.

Jones MH, Fahnestock JT, Walker DA, et al. 1998. Carbon dioxide fluxes in moist and dry arctic tundra during the snow-free season: responses to increases in summer temperature and winter snow accumulation. *Arctic and Alpine Research* **30**: 373-380.

Jumpponen A, Jones KL. 2013. Tallgrass prairie soil fungal communities are resilient to climate change. *Fungal Ecology* **XXX**:1-14.

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

Kade A., Walker DA, Raynolds MK. 2005. Plant communities and soils in cryoturbated tundra along a bioclimate gradient in the Low Arctic, Alaska. *Phytocoenologia* **35**: 761–820.

Kaplan E. 1996. Biomes of the World: Tundra. Hong Kong: Marshall Cavendish Corporation.

Kardol P, Jonathan R, Wardle DA. 2014. Local plant adaptation across a subarctic elevational gradient. *Royal Society Open Science* **1**:140-141.

Kattsov VM, Walsh JE. 2000. Twentieth-century trends of Arctic precipitation from observational data and a climate model simulation. *Journal of Climate* **13**: 1362–1370.

Kaufman DS, Schneider DP, McKay MP, et al. 2009. Recent warming reverses long-term Arctic cooling. *Science* **325**: 1236-1239.

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

Kennedy AD. 1995. Antarctic terrestrial ecosystem response to global environmental change. *Annual Review of Ecology, Evolution, and Systematics* **26**: 683–704.

Kharin VV, Zwiers FW. 2005. Estimating extremes in transient climate change simulations. *Journal of Climate* **18**: 1156–1173.

Kharuk VI, Ranson KJ, Sergey IT, Oskorbin PA, et al. 2013. Tree-line structure and dynamics at the northern limit of the Larch forest: Anabar Plateau, Siberia, Russia. *Arctic, Antarctic and Alpine Research* **45**:526-537.

Knudsen GR, Stack JP, Schuhmann SO, Orr K, LaPaglia C. 2006. Individual-Based Approach to Modeling Hyphal growth of a biocontrol fungus in soil. *Phytopathology* **96**:1108-1115.

Koenigk T, Brodeau L, Graversen RG, et al. 2013. Arctic climate change in 21st century CMIP5 simulations with EC-Earth. *Climate Dynamics* **40**:2719-2743.

Kohn LM, Stasovski E. 1990. The mycorrhizai status of plants at Alexandra Fiord, Ellesmere Island, Canada, a high arctic site. *Mycologia* **82**: 23-35.

Kõljalg U, Nilsson RH, Abarenkov K, et al. 2013. Towards a unified paradigm for sequence-based identification of Fungi. *Molecular Ecology* **22(21)**: 5271-5277.

Kug JS, Jeong JH, Jang YS, et al. 2015. Two distinct influences of Arctic warming on cold winters over North America and East Asia. *Nature Geoscience* **8**: 759-762.

Kullman L. 2001. 20th century climate warming and tree-limit rise in the southern Scandes of Sweden. *AMBIO: A Journal of the Human Environment - BioOne* **30(20)**:72-80.

Kytöviita MM, Ruotsalainen AL. 2007. Mycorrhizal benefit in two low Arctic herbs increases with increasing temperature. *American Journal of Botany* **94**: 1309-1315.

Larsen KS, Grogan P, Jonasson S, et al. 2007. Respiration and microbial dynamics in two subarctic ecosystems during winter and spring thaw: effects of increased snow depth. *Arctic, Antarctic and Alpine Research*, **39(2)**:9 pp.

Leffler JA, Klein ES, Oberbauer SF, Welker JM. 2016. Coupled long-term summer warming and deeper snow alters species composition and stimulates gross primary productivity in tussock tundra. *Oecologia* **181**: 287-297.

Lindahl BD, Nilsson RH, Tedersoo L, et al. 2013. Fungal community analysis by high-throughput sequencing of amplified markers–a user's guide. *New Phytologist* **199(1)**: 288–299.

Loranty MM, Goetz SJ. 2012. Shrub expansion and climate feedbacks in Arctic tundra. *Environmental Research Letters* **7**:011005.

Ludley KE, Robinson CH, 2008. Decomposer Basidiomycota in Arctic and Antarctic ecosystems. *Soil Biology and Biochemistry* **40**: 11e29.

Lüthy D, Floch M, Bereiter B, et al. 2008. High-resolution carbon dioxide concentration record 650,000-800,000 years before present. *Nature* **453**:379-382.

Mann ME, Jones PD. 2003. Global surface temperatures over the past two millennia. *Geophysical Research Letters* **30**: 15-18.

Margesin R, Schinner F. 1994. Properties of cold adapted microorganisms and their potential role in biotechnology. *Journal of Biotechnology* **33(1)**: 1-14.

Marion GM, Henry GHR, Freckman DW, et al. 1997. Open-top designs for manipulating field temperature in high-latitude ecosystems. *Global Change Biology* **3(1)**: 20-32.

McCune B, Grace JB. 2002. Analyses of Ecological Communities. *MjM Software, Glenden Beach, Oregon, USA*. 304 pp.

McMahon SK, Wallenstein MD, Schimel JP. 2009. Microbial growth in Arctic tundra soil at -2 deg C. *Environmental Microbiology Reports* **1**: 162–166.

Meehl GA, Arblaster JM, Tebaldi C. 2005. Understanding future patterns of increased precipitation intensity in climate model simulations. *Geophysical Research Letters* **32**:LI8719.

Menzel A, Estrella N. 2001. Plant phenological changes. In: *Fingerprints of Climate change – adapted behavior and shifting species ranges,* Walther GR, Burga CA, Edwards PJ, eds., Kluwer/Plenum, New York/ London, 123-137.

Mercado-Diaz J. 2011. Changes in composition and structure of plant communities in the Alaskan Arctic Tundra after 14 years of experimental warming and snow manipulation. MS Thesis, Chapter 3. University of Porto Rico.

Meshinev T, Apostolova I, Koleva E. 2000. Influence of warming on timberline rising: a case study on *Pinus peuce* Griseb. In Bulgaria. *Phytocoenologia* **30 (3-4)**:431-438.

Michaelson GL, Ping CL, Epstein H, Kimbe JM, Walker DA. 2008. Soils and forest boil ecosystems across the North American Arctic Transect. *Journal of Geophysical Research: Biogeosciences* **113**: G3.

Min YJ. Park MS, Fong JJ, et al. 2014. Diversity and Saline Resistance of Endophytic Fungi Associated with *Pinus thunbergii* in Coastal Shelterbelts of Korea. *Journal of Microbiology and Biotechnology.* **24(3)**: 324-333.

Minnis AM, Lindner DL. 2013. Phylogenetic evaluation of *Geomyces* and allies reveals no close relatives of *Pseudogymnoascus destructans*, comb. nov., in bat hibernacula of eastern North America. *Fungal Biology* **117(9)**: 638-49.

Montoya JM, Raffaelli D. 2010. Climate change, biotic interactions and ecosystem services. *Philosophical transactions of the Royal Society B* **365**: 201302018.

Morgado LN. 2016. Peeking into the future: Fungi of the greening Arctic. PhD thesis. GVO printers & designers. 159 pp.

Morgado LN, Semenova TA, Welker JM, et al. 2015. Summer temperature increase has distinct effect on the ectomycorrhizal fungal communities of moist tussock and dry tundra in Arctic Alaska. *Global Change Biology* **21(2)**: 959-72.

Morgado LN, Semenova TA, Welker JM, et al. 2016. Long-term increase in snow depth leads to compositional changes in arctic ectomycorrhizal fungal communities. *Global Change Biology* **22(9)**:3080-3096.

Morgado LN, Semenova TA, Welker JM, Walker MD, Smets E, Geml J. 2014. Summer temperature increase has distinct effect on the ectomycorrhizal fungal communities of moist tussock and dry tundra in Arctic Alaska. *Global Change Biology* **21(2)**: 959-972.

Nadelhoffer K, Shaver G, Fry B, Giblin A, Johnson L, McKane R. 1996. ^{15}N abundances and N use by tundra plants. *Oecologia* **107**: 386-394.

Naeem S, Chair FS, Costanza R, et al. 1999. Biodiversity and Ecosystem Functioning: Maintaining Natural Life Support Processes. *Issues in Ecology* **4**: 14 pp.

Natali SM, Mack CM. 2011. Fungal feedback to climate change. *Nature Climate Change* **1**: 192-193.

Natali SM, Schuur EAG, Rubin RL. 2012. Increased plant productivity in Alaskan tundra as a result of experimental warming of soil and permafrost. *Journal of Ecology* **100(2)**: 488-498.

Natali SM, Schuur EAG, Trucco C, et al. 2011. Effects of experimental warming of air, soil and permafrost on carbon balance in Alaskan tundra. *Global Change Biology* **17**:1394–1407.

Newsham KK. 2011. A meta-analysis of plant responses to dark septate root endophytes. *New Phytologist* **190(3)**: 783-793.

Newsham KK., Upson R, Read DJ. 2009. Mycorrhizas and dark septate root endophytes in polar regions. *Fungal Ecology* **2**:10–20.

Nguyen NH, Zewei S, Bates ST, et al. 2015. FUNGuild: An open annotation tool for parsing fungal community datasets by ecological guild. *Fungal Ecology*, **1**:8.

Nilsson RH, Abarenkov K, Larsson KH, et al. 2011. Molecular identification of fungi: rationale, philosophical concerns, and the UNITE database. *The Open Applied Informatics Journal* **5**: 81-86.

O'Brien H, Parrent JL, Jackson JA, et al. 2005. Fungal community analysis by large-scale sequencing of environmental samples. *Applied and Environmental Microbiology* **71**: 5544–5550.

Oechel WC, Laskowski CA, Burba G, Gioli B, Kalhori AAM. 2014. Annual patterns and budget of CO2 flux in an Arctic tussock tundra ecosystem. *Journal of Geophysical Research: Biogeosciences* **119**: doi: 10.1002/2013JG002431.

Oksanen J, Blanchett FG, Kindt R, et al. 2012. Vegan: community Ecology Package. R Package 2.0.3.

Olofsson J, Ericson L, Torp M, et al. 2011. Carbon balance of Arctic tundra under increased snow cover mediated by a plant pathogen. *Nature Climate Change* **1**: 220–223.

Olsson PA, Erikssen B, Dahlberg A. 2004. Colonization by arbuscular mycorrhizal and fine endophytic fungi in herbaceous vegetation in the Canadian High Arctic. *Canadian Journal of Botany* **82**:1547-1556.

Orwin KH, Kirschbaum MUF, St John MG, Dickie IA. 2011. Organic nutrient uptake by mycorrhizal fungi enhances ecosystem carbon storage: A model-based assessment, *Ecology Letters* **14(5)**: 493–502.

Papanikolaou N, Britton AJ, Helliwell RC, Johnson D. 2010. Nitrogen deposition, vegetation burning and climate warming act independently on microbial community structure and enzyme activity associated with decomposing litter in low-alpine heath. *Global Change Biology* **16(11)**: 3120-3132.

Parmesan C, Ryrholm N, Stefanescu C, et al. 1999. Poleward shifts in geographical ranges of butterfly species associated with regional warming. *Nature*. **399**:5790583.

Pattison RR, Welker JM. 2014. Differential ecophysiological response of deciduous shrubs and a graminoid to long-term experimental snow reduction and addition in moist tundra, Northern Alaska. *Oecologia* **174(2)**: 339-50.

Pearson RG, Phillips SJ, Loranty MM, et al. 2013. Shifts in arctic vegetation and associated feedbacks under climate change. *Nature Climate Change* **3**: 673-677.

Penton CR, StLouis D, Cole JR, et al. 2103. Fungal Diversity in Permafrost and Tallgrass Prairie Soils under Experimental Warming Conditions. *Applied Environmental Microbiology* **79(22)**: 7063–7072.

Peterson TC, et al. 2009. State of the Climate in 2008. *Special Supplement to the Bulletin of the American Meteorological Society* **90(8)**: S17-S18.

Post E, Bhatt US, Bitz CM, et al. 2013. Ecological consequences of sea-ice decline. *Science* **341**: 519-524.

Pounds AJ, Fogden MPL, Campbell JH. 1999. Biological response to climate change on a tropical mountain. *Nature* **398**: 611.

R Core team. 2013. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. IBSN 3-900051-07-0, URL http://www.R-project.org/.

Richter-Menge J, Jeffries MO, Overland JE, eds. 2011. Arctic Report Card 2011, *http://www.arctic.noaa.gov/reportcard.*

Robinson CH. 2001. Cold adaptation in Arctic and Antarctic fungi . *New Phytologist* **151(2)**:341–353.

Rodriguez RJ, Henson J, Volkenburgh EV, et al. 2008. Stress tolerance in plants via habitat-adapted symbiosis. *Multidisciplinary Journal of Microbiol Ecology* **B2**:404-416.

Rogers MC, Sullivan PF, Welker JM. 2011. Evidence of nonlinearity in the response of net ecosystem CO2 exchange to increasing levels of winter snow depth in the High Arctic of Northwest Greenland. Arctic, *Antarctic and Alpine Research* **43**: 95-106.

Rosenberg MS, Adams DC, Gurevitch J. 1999. *MetaWin: Statistical Software for Meta-Analysis.* Version 2.0. Sinauer Associates, Sunderland, Massachusetts.

Rosling A, Cox F, Cruz-Martinez K, et al. 2011. Archaeorhizomycetes: unearthing an ancient class of ubiquitous soil fungi. *Science* **333**:876–879.

Roy DB, Sparks TH. 2000. Phenology of British butterflies and climate change. *Global Change Biology* **6(4)**: 407-416.

Salzer MW, Bunn AG, Graham NE, Hughes MK, et al. 2014. Five millennia of palaeotemperature from tree-rings in the Great Basin, USA. *Climate Dynamics* **42**:1517-1526.

Savile D. 1982. Adaptations of fungi to arctic and subarctic conditions. In: Laursen GA, Ainmirati JF, eds. *Arctic and Alpine Mycology* **1**. Seattle: University of Washington Press, 357-370.

Schaeffer SM, Sharp E, Schimel J, Welker JM. 2013. Soil N processes in the High Arctic: Responses to a long-term multi-level warming and added summer water experiment. *Global Change Biology* **19**: 3529-2539.

Schimel JP, Bennett J. 2004. Nitrogen mineralization: Challenges of a changing paradigm. *Ecology* **85**: 591-602.

Schimel JP, Bilbrough C, Welker JM. 2004. Increased snow depth affects microbial activity and nitrogen mineralization in two arctic tundra communities. *Soil Biology and Biochemistry* **36**: 217-227.

Schloss PD, Westcott SL, Ryabin T, et al. 2009. Introducing mothur: open-source, platform-independent, community-supported software for describing and comparing microbial communities. *Applied Environmental Microbiology* **75(23)**: 7537-41.

Schmit JP, Mueller GM. 2007. An estimate of the lower limit of global fungal diversity. *Biodiversity and Conservation* **16**:99–111.

Screen JA, Simmonds I. 2010. The central role of diminishing sea ice in recent Arctic temperature amplification**.** *Nature*, **464(7293)**:1334-1337, *DOI:10.1038/nature09051*

Screen JA, Simmonds I. 2012. Declining summer snowfall in the Arctic: causes, impacts and feedbacks. *Climate Dynamics* **38**: 2243-2256.

Semenova TA, Morgado LN, Welker JM, et al. 2015. Long-term experimental warming alters community composition of ascomycetes in Alaskan moist and dry arctic tundra. *Moleclar Ecology* **24(2)**: 424-437.

Serreze M, Barrett AP, Stroeve J, Kindig DN, Holland MM. 2009. The emergence of surface-based Arctic amplification. *Cryosphere* **3**:11–19.

Serreze M, Barry RG. 2011. Processes and impacts of Arctic amplification: A research synthesis. *Global and Planetary Change* **77**: 85-96.

Sharkhuu A, Plante AF, Enkhmandal O, et al. 2013. Effects of open-top passive warming chambers on soil respiration in the semi-arid steppe to taiga forest transition zone in Northern Mongolia. *Biochemical Journal* **115**:333-348.

Shaver GR, Jonasson S. 1999. Response of Arctic ecosystems to climate change: results of long-term field experiments in Sweden and Alaska. *Polar Research* **18(2)**: 245-252.

Sistla SA, Moore JC, Simpson RT, et al. 2013. Long-term warming restructures Arctic tundra without changing net soil carbon storage. *Nature* **497**: 615–618.

Smith J. 2014. Comprehensive Meta-Analysis (Version 2) [Computer software]. Englewood, NJ: Biostat. Available from http://www.comprehensive.com

Southward AJ, Hawkins SJ, Burrows MT. 1995. 70 years observations of changes in distribution and abundance of zooplankton and intertidal organisms in the western English Channel in relation to rising sea temperature. *Journal of Thermal Biology* **20**: 127-155.

Stocker TF, Qin D, Plattner GK, et al. 2013. Climate Change 2013: The Physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report of the

Intergovernmental Panel on Climate Change. Cambridge, UK, Cambridge University Press, 1535 pp.

Sturm M, McFadden JP, Liston GE, et al. 2001. Shrub and snow interactions in arctic tundra: a hypothesis with climatic implications. *Journal of Climate* **14(3)**: 336 - 344.

Sturm M, Racine C, Tape K. 2001. Increasing shrub abundance in the Arctic. *Nature* **411**: 546-547.

Sturm M, Scimel J, Michaelson G, et al. 2005. Winter biological processes could help convert Arctic tundra to shrubland. *Bioscience* **55**: 17-26.

Sukyoung L. 2014. A theory for polar amplification from a general circulation perspective. *Asia-Pacific Journal of Atmospheric Sciences* **50**. doi:10.1007/s13143-014-0024-7.

Sullivan PF, Sommerkorn M, Rueth H, et al. 2007. Climate and species affect fine root production with long-term fertilization in acidic tussock tundra near Toolik Lake, Alaska. *Oecologia* **153**: 643-652.

Summerbell RC. 1989. Microfungi associated with the mycorrhizal mantle and adjacent microhabitats within the rhizosphere of black spruce. *Canadian Journal of Botany*, **67**: 1085–1095.

Summerbell RC. 2005. Root endophyte and mycorrhizosphere fungi of black spruce, Picea mariana, in a boreal forest habitat: influence of site factors on fungal distributions. *Studies in Mycolog*y **53**: 121–145.

Sweet SK, Gough L, Griffin KL, et al. 2014. Tall deciduous shrubs offset delayed start of growing season through rapid leaf development in the Alaskan arctic tundra. *Arctic, Antarctic and Alpine Research* **46**: 682-697.

Tape KD, Hallinger M, Welker JM, Ruess RW. 2012. Landscape Heterogeneity of Shrub Expansion in Arctic Alaska. *Ecosystems* **15**:711-724.

Tarnocai C, Canadell JG, Schuur EAG, et al. 2009. Soil organic carbon pools in the northern circumpolar permafrost region. *Global Biogeochemical Cycles* **23**:2.

Tedersoo L, Bahram M, Põlme S, et al. 2014. Global diversity and geography of soil fungi. *Science* **28(346)**: 6213.

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

Tedersoo L, Nilsson RH, Abarenkov K, et al. 2010b. 454 pyrosequencing and Sanger sequencing of tropical mycorrhizal fungi provide similar results but reveal substantial methodological biases. *New Phytologist* **188**: 291–301.

Timling I, Dahlberg A, Walker DA, et al. 2012. Distribution and drivers of ectomycorrhizal fungal communities across the North American Arctic. *Ecosphere* **3**:1-25.

Timling I, Walker DA, Nusbaum C, Lennon NJ, Taylor DL. 2014. Rich and cold: Diversity, distribution and drivers of fungal communities in patterned-ground ecosystem of the North American Arctic. *Molecular Ecology* **23(13)**:3258-72.

Toju H, Yamamoto S, Sato H, et al. 2013. Sharing of Diverse Mycorrhizal and Root-Endophytic Fungi among Plant Species in an Oak-Dominated Cool–Temperate Forest *PLoS One* **8(10)**: e78248

Treseder KK, Lennon JT. 2015. Fungal Traits That Drive Ecosystem Dynamics on Land. *Microbiol. Mol. Biol. Rev*. **79(2)**:243-262.

van der Wal A, Geydan TD, Kuyper TW, et al. 2013. A thready affair: linking fungal diversity and community dynamics to terrestrial decomposition processes. *FEMS Microbiology Reviews* **37(4)**: 477-494.

Vannette RL, Hunter MD. 2013. Mycorrhizal abundance affects the expression of plant resistance traits and herbivore performance. *Journal of Ecology* **101**: 1019–1029.

Väre H, Vestberg M, Eurola S. 1992. Mycorrhiza and root associated fungi in Spitsbergen. *Mycorrhiza* **1**: 93–104.

Vohnik M; Fendrych M; Albrechtova J; Vosatka M. 2007. Intracellular colonization of Rhododendron and Vaccinium roots by *Cenococcum geophilum, Pseudogymnoascus pannorum* and *Meliniomyces variabilis*. *Folia Microbiologica* **52(4)**:407-414.

Wahren CHA, Walker MD, Bret-Harte MS. 2005. Vegetation responses in Alaskan arctic tundra after 8 years of a summer warming and winter snow manipulation experiment. *Global Change Biology* **11**: 537-552.

Walker DA, Maier HA. 2008. Vegetation in the Vicinity of the Toolik Field Station, Alaska. *Biological Papers of the University of Alaska* **28**. Institute of Arctic Biology, Fairbanks, AK.

Walker DA, Raynolds MK, Daniëls FJA , et al. 2005. The Circumpolar Arctic vegetation map *Journal of Vegetation Science* **16**: 267–282.

Walker MD, Wahren HC, Hollister RD, et al. 2006. Plant community responses to experimental warming across the tundra biome. *PNAS* **103(5)**: 1342-1346.

Walker MD, Walker DA, Welker JM, et al. 1999. Long-term experimental manipulation of winter snow regime and summer temperature in arctic and alpine tundra. *Hydroogical Processes* **13**: 2315-2330.

Walther GR, Post E, Convey P, et al. 2002. Ecological responses to recent climate change. *Nature* **416**: 389-395.

Wardle P, Coleman MC. 1992. Evidence for rising upper limits of four native New Zealand forest trees. *New Zealand Journal of Botany* **30:** 303-314.

Welker JM, Brown KB, Fanhestock JT. 1999. CO_2 flux in arctic and alpine dry tundra: comparative field responses under ambient and experimentally warmed conditions. *Arctic, Antarctic and Alpine Research* **31**: 308-313.

Welker JM, Fahnestock JT, Henry GHR, O'Dea KW, Chimner RA. 2004. CO_2 exchange in three Canadian High Arctic ecosystems: response to long-term experimental warming. *Global Change Biology* **10**: 1981–1995.

Welker JM, Fahnestock JT, Jones MH. 2000. Annual CO_2 flux in dry and moist arctic tundra: field responses to increases in summer temperatures and winter snow depth. *Climatic Change* **44**: 139–150.

Welker JM, Fahnestock JT, Sillivan PF, Chimner RA. 2005. Leaf mineral nutrition of arctic plants in response to long-term warming and deeper snow in N. Alaska. *Oikos* **109**: 167-177.

Welker JM, Molau U, Parsons AN, et al. 1997. Response of *Dryas octopetala* to ITEX manipulations: a synthesis with circumpolar comparisons. *Global Change Biology* **3**: 61–73.

White JF, Bacon CW, Hywel-Jones NL, Spatafora JW. (Eds). 2003. Clavicipitalean Fungi: Evolutionary Biology, Chemistry, Biocontrol and Cultural Impacts. Marcel Dekker, New York.

White TJ, Bruns T, Lee S, et al. 1990. Amplification and direct sequencing of fungal ribosomal RNA for phylogenetics. In: PCR Protocols: A guide to methods and Applications (eds Innis, M.A., Gelfand, D.H., Sninsky, J.J., White, T.J.) , pp 315-321. Academic Press, San Diego.

Wilby RL, Wigley TML. 2002. Future changes in the distribution of daily precipitation totals across North America. *Geophysical Research Letters* **29(7)**: 391 – 394.

Wipf S, Rixen C. 2010. A review of snow manipulation experiments in Arctic and alpine tundra ecosystems. *Polar research* **29**: 95-109.

Wookey PA. 2007. Climate change and biodiversity in the Arctic – Nordic Perspectives. *Polar Research* **26**:96-103.

Zhang T, Yao YF. 2015. Endophytic fungal communities associated with vascular plants in the high Arctic zone are highly diverse and host-plant specific. *PLoS ONE* **10(6)**:e0130051. *doi:10.1371/journal.pone.0130051*

Zhang W, Miller PA, Smith B, et al. 2013. Tundra shrubification and tree-line advance amplify arctic climate warming: results from an individual-based dynamic vegetation model. *Environmental Research Letters* **8**:034023.

Zhang Y, Bai S. 2003. Effects of nitrogen forms on nutrient uptake and growth of trees. *The journal of applied ecology*, **14(11)**:2044-2048.

Zona D, Oechel WC, Kochendorfer JUKTP, et al. 2009. Methane fluxes during the initiation of a large-scale water table manipulation experiment in the Alaskan Arctic tundra. *Global Biogeochemistry* 23(2).

English summary

Over the last three decades, the rate of temperature increase in arctic ecosystems has largely exceeded the average rate of the Earth's climate warming. Besides the rising temperatures, precipitation is dramatically increasing in the Arctic as a result of enhanced evaporation from the Arctic ocean and increased moisture inflow from lower latitudes. Most of this precipitation falls as snow in cold arctic biomes, resulting in a deeper snow cover during the winter. Increased temperature and precipitation have strongly altered vegetation in low arctic tundra and have resulted in rapid expansion of deciduous shrubs and graminoids with the subsequent accumulation of their litter, and following decrease in shade-intolerant lichens and bryophytes. In this thesis, we addressed the effect of climate warming on soil fungal communities in low arctic tundra of Northern Alaska. We took advantage of the long-term ecological experiments being carried out at Toolik Lake Research Station, Alaska, USA. Samples were collected after 18 years of experimental treatments, such as simulated summer warming of air and near-surface soil temperature by open-top chambers, and increased snow depth by snow fences. Because two main vegetation types are found throughout arctic tundra (dry heath and moist tussock tundra), the research was carried out in both tundra types. Community composition of soil fungi was assessed by deep sequencing of the fungal ITS2 rDNA region from soil samples. In the summer warming experiments, soil fungi strongly shifted in their community compositions in the moist, but not in the dry tundra. Although total fungal richness was not affected significantly, strong changes in richness of several fungal taxonomic groups were observed. Among the functional groups, we observed declines in richness of ectomycorrhizal, ericoid mycorrhizal and lichenized species, and increases in saprotrophic, pathogenic and root-endophytic fungi. Abundance-based analyses revealed changes in moist tundra only, including declines in ectomycorrhizal, lichenized and saprotrophic fungi, as well as a significant increase in animal pathogens. In snow addition experiments, we observed strong shifts in fungal communities of both the dry and the moist tundra. Although an overall decline in fungal richness was not confirmed by the statistical tests, we observed decreased richness in numerous fungal groups. Ectomycorrhizal and lichenized fungi declined in their richness in both the dry and moist tundra, while responses in other functional groups were specific to tundra type. Abundance-based analysis revealed declines in bryophyte-associated,

ectomycorrhizal, saprotrophic and lichenized fungi, as well as an overall decrease of fungal abundance. In dry tundra, total fungal abundance was not altered, although a decline was observed for lichenized species, and an increase – for the ectomycorrhizal fungi. Given various roles that fungi play in arctic ecosystems (nutrient cycling, decomposition) and their tight interactions with plants and animals, we expect changes in fungal communities to result in a suite of consequences for nearly all arctic inhabitants, either directly or through alterations of abiotic processes. Changes in abundance of saprotrophic fungi will likely affect C and N storage, with potential feedback on climate change. A decline in ectomycorrhizal fungi is expected to affect water and nutrient availability for plants. Possibly, a decline in macrofungi may affect populations of mushroom-feeding insects, and loss of lichens may be of importance for caribou and reindeer populations. However, the extent to which we can predict changes in populations of arctic inhabitants based on the observed changes in soil fungi is difficult to estimate. Changes in fungal communities that we observed in our experiments only correlated in part with previously reported vegetation shifts, suggesting that different arctic organisms are characterized by various rates of community turnover. Therefore, shifts in the populations of other arctic organisms may not entirely correspond to alterations in fungal assemblages. Arctic fungal communities are more species rich and appear to be more dynamic than arctic plant communities. Consequently, soil fungi may be suitable for monitoring and predicting ecosystem disturbances at early stages.

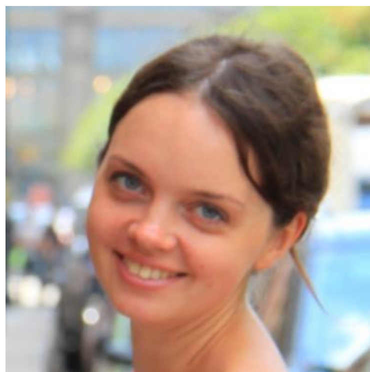
Nederlandse samenvatting

Kindly translated from English by Nieke T. J. Knoben

De temperatuurstijging in de arctische ecosystemen is de laatste dertig jaar sneller gegaan dan de gemiddelde opwarming van de aarde. Naast stijgende temperaturen is ook de neerslag enorm gestegen doordat er meer verdamping optreedt in de Arctische Oceaan en door een hogere instroom van vochtigheid van lagere breedtegraden. Deze neerslag valt voornamelijk als sneeuw in de koude arctische omgeving, wat resulteert in een dikkere sneeuwlaag in de winter. Hogere temperaturen en meer neerslag hebben de vegetatie in de lagere arctische toendra sterk veranderd en leiden tot een snelle uitbreiding van bladverliezende struiken en grassen met de daarbij horende toename van strooisel. Dat resulteert in een afname in schaduw-intolerante mossen en korstmossen. In dit proefschrift kijken we naar het effect van klimaatverandering op de bodemschimmelgemeenschappen in de lage arctische toendra van Noord Alaska. We hebben daarvoor gebruik gemaakt van een langlopend ecologisch experiment in Toolik Lake Research Station, Alaska. Monsters zijn verzameld nadat deze 18 jaar aan experimentele behandelingen zijn blootgesteld, zoals het toevoegen van extra warme lucht om langere zomers te simuleren en het verwarmen van de bodemtemperatuur door open-top kamers. Sneeuwhekken zijn gebruikt voor het testen van het effect van een dikkere sneeuwlaag. Het onderzoek is uitgevoerd in de twee meest voorkomende vegetatietypen in de arctische toendra's: droge en natte toendra. De samenstelling van de bodemschimmels werd bepaald door middel van deep sequencing van de ITS2 rDNA regio van de bodemmonsters. In de zomerwarmte experimenten zagen we dat bodemschimmels sterk veranderden in de samenstelling van de gemeenschappen in de natte toendra, maar niet in de droge toendra. Alhoewel de totale rijkdom van schimmels niet significant veranderde, werden er duidelijke veranderingen geconstateerd in de rijkdom van verschillende taxonomische groepen. Binnen deze functionele groepen zagen we een afname in rijkdom van ectomycorrhiza, ericoïde mycorrhiza en lichenvormende schimmels, terwijl er een toename van saprofytische, pathogene en endofytische wortelschimmels werd geconstateerd. Abundantie-analyses van soorten onthullen alleen veranderingen in de natte toendra, zoals een afname van ectomycorrhiza,

lichenvormende schimmels en saprofytische schimmels, maar ook een significante toename in dierlijke pathogenen. In het experiment waar extra sneeuw werd toegevoegd, hebben we duidelijke veranderingen geobserveerd in schimmelgemeenschappen in zowel de droge en de natte toendra. Hoewel een algemene afname van schimmelrijkdom niet statistisch kon worden vastgesteld, observeerden we dat verschillende groepen schimmels afnamen in rijkdom. Ectomycorrhiza en lichenvormende schimmels namen af in rijkdom in zowel de natte als de droge toendra, terwijl effecten bij andere functionele groepen specifiek waren voor één van de twee toendra typen. Abundantie-analyses lieten een daling zien in mos-bewonende, ectomycorrhiza, saprotrofysche en lichenvormende schimmels, naast een algehele daling van het voorkomen van schimmels. In de droge toendra was het totaal aantal schimmels niet veranderd, hoewel er een daling van de lichenvormende schimmels en een stijging van de ectomycorrhiza was. Gezien de verschillende rollen die schimmels spelen in Arctische ecosystemen (zoals de kringloop van nutriënten en decompositie) en de nauwe relaties met planten en dieren, verwachten we dat veranderingen in de schimmelgemeenschappen effect hebben op bijna alle bewoners van de arctische gebieden, zowel direct als indirect, als gevolg van veranderingen in de abiotische processen. Veranderingen in voorkomen van saprofytische schimmels zullen waarschijnlijk effect hebben op de C en N opslag, met mogelijk terugkoppeling naar klimaatverandering. Een afname van ectomychorriza-schimmels heeft waarschijnlijk effect op de beschikbaarheid van water en nutriënten voor planten. En een afname van macrofungi kan effect hebben op paddenstoelen etende insecten. Verlies van lichenvormende schimmels zou belangrijk kunnen zijn voor de populaties kariboes en rendieren. Desondanks is het erg lastig om daadwerkelijke effecten van veranderende bodemschimmels op andere arctische bewoners te voorspellen. Veranderingen in schimmelgemeenschappen komen slechts deels overeen met de eerder genoemde verschuivingen in vegetaties, wat er op wijst dat verschillende arctische organismen worden gekenmerkt door verschillende mate van verandering. Daarom kan het zo zijn dat veranderingen in populaties van arctische organismen niet helemaal overeenkomen met veranderingen in de schimmelgemeenschap. Arctische schimmelgemeenschappen zijn zeer divers aan soorten en lijken dynamischer dan arctische plantengemeenschappen. Dat maakt bodemschimmels wellicht zeer geschikt voor het monitoren en het vroeg voorspellen van verstoringen in het ecosysteem.

Curriculum vitae



Tatiana A. Semenova-Nelsen was born on August 7, 1985, in Kaliningrad Moscow region, Russia. She received her secondary education in Korolev Moscow region in July 2002,where she was awarded a gold medal for excellent studies at school. In September 2002, Tatiana entered the Biological Faculty of Moscow State University, (MSU), Russia. In June 2007, she graduated from MSU and received a Specialist diploma with honors in botany and mycology. Tatiana continued her education at MSU, and obtained a Candidate of Science degree in November 11, 2011. Her main project at that time dealt with insect pathogenic fungi of the genus *Cordyceps*. In 2008-2009, Tatiana worked and studied as an exchange ERASMUS student in the University of Copenhagen, Denmark, studying fungal secreted enzymes in the system of fungus gardens, where the ants rear their symbiotic fungi for food. In 2011-2012 Tatiana was awarded a CIMO fellowship to work on the diversity and enzyme profiles of polypore fungi at The University of Helsinki, Finland. In August 2012, Tatiana started her PhD study in Leiden University and Naturalis Biodiversity Center, the Netherlands, under the supervision of Dr. József Geml. The project was focused on shifts in arctic fungal communities in response to long-term experimental climate warming – i.e. increased summer temperature and winter snow depth. The results of this work were published in international peer-reviewed journals and presented at local and international conferences. After her graduation, Tatiana intends to continue research in fungal ecology, addressing evolutionary and ecological questions by methods of biochemistry and molecular biology.

Cordyceps militaris

Fungus garden

Publications IN PEER REVIEWED JOURNALS

Semenova TA, Morgado LN, Welker JM, Walker MD, Smets E, Geml J. (2016) Compositional and functional shifts in arctic fungal communities in response to experimentally increased snow depth. Soil Biology & Biochemistry, *DOI: 10.1016/j.soilbio.2016.06.001*

Morgado LN,·**Semenova TA**, Welker JM, Walker MD, Smets E, Geml J. (2016) Long-term increase in snow depth leads to compositional changes in arctic ectomycorrhizal fungal communities. Global Change Biology, *DOI: 10.1111/gcb.13294*.

Geml J, Morgado LN, **Semenova TA,** Welker JM, Walker MD, Smets E. (2015) Long-term warming alters richness and composition of taxonomic and functional groups of arctic fungi. FEMS Microbiology Ecology: 91(8).

Popova VV, Dunaevsky YE, Domash VI, **Semenova TA,** Beliakova GA, Belozersky MA. (2015) Some properties and possible biological role of peptidase inhibitors from the entomopathogenic fungus *Tolypocladium cylindrosporum*. Archives of Microbiology. 197(8);1001-1010.

Semenova TA, Morgado LN, Welker JM, Walker MD, Smets E, Geml J. (2015) Long-term experimental warming alters community composition of ascomycetes in Alaskan moist and dry arctic tundra. Molecular Ecology, 24:424-437.

Morgado LN, **Semenova TA,** Welker JM, Walker MD, Smets E, Geml J. (2014) Summer temperature increase has distinct effects on the ectomycorrhizal fungal communities of moist tussock and dry tundra in Arcti c Alaska. Global Change Biology: 1-14.

Geml J, Gravendeel B, Neilen M, Lammers Y, Raes N, **Semenova TA**, Noordeloos ME. (2014) The contribution of DNA metabarcoding to fungal conservation: diversity assessment, habitat partitioning and mapping red -listed fungi in protected coastal Salix repens communities in the Netherlands. PLOS ONE 9:e99852.

Geml J, Pastor N, Fernandez L, Pacheco S, **Semenova TA,** Becerra AG, Wicaksono CY, Nouhra ER. (2014) Large-scale fungal diversity assessment in the Andean Yungas forests reveals strong community turnover among forest types along an altitudinal gradient. Molecular Ecology, 23:2452-2472.

Dunaevsky YE, Popova VV, **Semenova TA,** Beliakova GA, Belozersky MA. (2014) Fungal inhibitors of proteolytic enzymes: classification, properties, possible biological roles, and perspecti ve for practical use. Biochimie 101:10-20

Semenova TA, Belozersky MA, Beljakova GA, Borisov BB, Semenova SA, Dunayevsky YaE. (2011) Secreted proteinase of entomopathogenic fungus Cordyceps militaris: enzymic properties and adsorption on insect cuticle. (in Russian). Mycology and Phytopathology (Russia) 45 (1): 64-69.

Semenova TA, Belozersky MA, Beljakova GA, Borisov BB, Semenova SA, Dunayevsky YaE. (2010) Secreted proteinase of entomopathogenic fungus Cordyceps militaris: optimization of fungal culture medium and enzyme purification. (in Russian). Mycology and Phytopathology (Russia) 44 (6): 535-541.

Semenova TA, Hughes DP, Schiott M, Boomsma JJ. (2011) Evolutionary patterns of proteinase activity in attine ant fungus gardens. BMC Microbiology 11:15.

CONFERENCE PRESENTATIONS (*2013-Present*)

Geml J, Morgado LN, **Semenova TA**, Pastor N, Nouhra ER (2016). A comparison of altitudinal distribution patterns of fungi in Borneo and in the Andes using DNA metabarcoding. Brazilian Mycological Congress, October 2016 (Oral presentation).

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)

Geml J, Morgado LN, **Semenova TA**, Smets E, Walker MD, Welker JM (2015). Peek into the future – long-term warming and increased snow depth alter richness and composition of taxonomic and functional groups of arctic fungi. Symposium Netherlands Polar Programme: Polar tipping points – identifying rapid changes in the polar regions.The Hague, the Netherlands. (Oral presentation)

Semenova TA, Morgado LN, Welker JM, Walker MD, Smets E, Geml J (2015). Climate warming increases arctic winter precipitation – how fungi respond to increased snow depth. Symposium Netherlands Polar Programme: Polar tipping points – identifying rapid changes in the polar regions. The Hague, the Netherlands. (Poster presentation)

Semenova TA, Morgado LN, Welker JM, Walker MD, Smets E, Geml J (2015). Ascomycete fungal communities reorganize in response to long-term summer and winter climate warming in moist and dry tundra of Arctic Alaska. XVII Congress of European Mycologists. Madeira, Portugal. (Oral presentation)

Geml J, Morgado LN, **Semenova TA**, Smets E, Walker MD, Welker JM (2015). Long-term warming and increased snow depth alter richness and composition of taxonomic and functional groups of arctic fungi. 21st International Tundra Experiment meeting: Integrating Arctic Plant and Microbial Ecology.Uppsala, Sweden. (Oral presentation)

Morgado LN, **Semenova TA**, Smets E, Walker MD, Welker JM, Geml J (2015). Compositional shifts in arctic ectomycorrhizal fungal community in response to long-term

increased snow depth in Northern Alaska. Ecology of soil microorganisms 2015 –microbes as important drivers of soil processes. Prague, Czech Republic. (Poster presentation)

Morgado LN, **Semenova TA**, Welker JM, Walker MD, Geml J (2015). Compositional shifts in ectomycorrhizal fungal community in response to long-term snow depth manipulations. Netherlands Annual Ecological Meeting. Lunteren, The Netherlands. (Oral presentation)

Semenova TA, Morgado LN, Welker JM, Walker MD, Smets E, Geml J (2015). Climate warming alters comunities of soil ascomycetes in arctic Alaskan tundra. Netherlands Annual Ecological Meeting. Lunteren, The Netherlands. (Oral presentation).

Semenova TA, Morgado LN, Welker JM, Walker MD, Smets E, Geml J (2015). Why does the dry arctic tundra remain unaffected by climate warming? Netherlands Annual Ecological Meeting. Lunteren, The Netherlands. (Poster presentation)

Morgado LN, **Semenova TA**, Welker JM, Walker MD, Smets E, Geml J (2014). The effect of long-term warming on arctic fungal communities. Netherlands Annual Ecological Meeting. Lunteren, The Netherlands. (Oral presentation)

Morgado LN, **Semenova TA**, Welker JM, Walker MD, Geml J. (2014). What can 1,000,000 sequences tell us about climatic changes and ectomycorrhizal (ECM) fungal communities? Netherlands Annual Ecological Meeting. Lunteren, The Netherlands. (Poster presentation)

Semenova TA, Morgado LN, Welker JM, Walker MD, Smets E, Geml J (2014). Global warming changes soil ascomycetous fungal communities in the arctic tundra. Netherlands Annual Ecological Meeting. Lunteren, The Netherlands. (Poster presentation)

Morgado LN, **Semenova TA**, Welker JM, Walker MD, Smets E, Geml J (2014). Long-term experimental warming have distinct effects in the ectomycorrhizal fungal communities of moist tussock and dry tundra in the Arctic Alaska. The 10th International Mycological Congress. Bangkok, Thailand. (Oral presentation)

Morgado LN, **Semenova TA**, Welker JM, Walker MD, Smets E, Geml J (2014). Linking local-scale diversity changes in ectomycorrhizal fungal communities with functional traits: a case study from long-term warming experiments in Arctic Alaska. The 10th International Mycological Congress. Bangkok, Thailand. (Poster presentation)

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)

Morgado LN, **Semenova TA**, Taylor DL, Geml J (2013). Biodiversity and habitat partitioning of arctic ectomycorrhizal fungi and their role in vegetation change due to climate change. Netherlands Annual Ecological Meeting. Lunteren, The Netherlands. (Poster presentation)

Morgado LN, **Semenova TA**, Welker JM, Geml J (2013). The effect of climate change on the composition of arctic soil fungal communities. Netherlands Annual Ecological Meeting. Lunteren, The Netherlands. (Oral presentation)

Geml J, Morgado LN, **Semenova TA** (2013). High-throughput DNA sequencing provides first insights into the fungal diversity of lowland rainforests and montane cloud forests in Borneo. 9th Flora Malesiana Symposium. Bogor, Indonesia. (Oral presentation)

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