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Peeking into the future : fungi in the greening Arctic

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Chapter 1

General introduction and thesis outline

Luis N. Morgado

General introduction

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

Climate change

Since 1884, the Earth's surface has warmed a total of 0.68 °C (<http://climate.nasa.gov/>) (Fig. 1.1). Additionally, proxies of global mean surface temperatures derived from tree rings, sediment layers, and ice cores revealed that temperatures during the past few decades exceeded those over the past four millennia (Mann *et al.*, 1999; Mann & Jones, 2003; Salzer *et al.*, 2014). Climate warming has accelerated since 1970 and the ten warmest years on record (131 years) occurred since the year 2000 (Post, 2013; <http://climate.nasa.gov/>) (Fig. 1.2). The global warming is largely due to increased concentration in atmospheric greenhouse gases. For example, according to comparisons of ice cores and detailed records, atmospheric concentration of CO₂ in 2015 is the highest in record of the last 800,000 years (Lüthi *et al.*, 2008) and reached

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

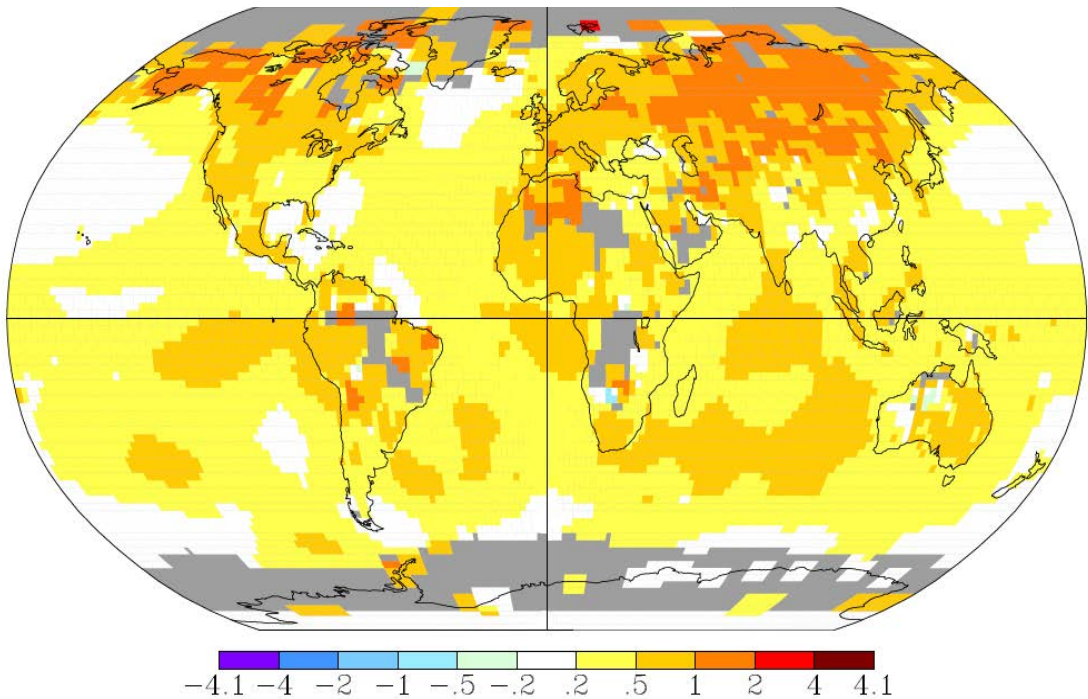


Figure 1.1. Map of annual average global temperature (°C) anomaly between 1981 and 2014 compared in relation to the period between 1950 and 1980. Adapted from <http://www.giss.nasa.gov/>.

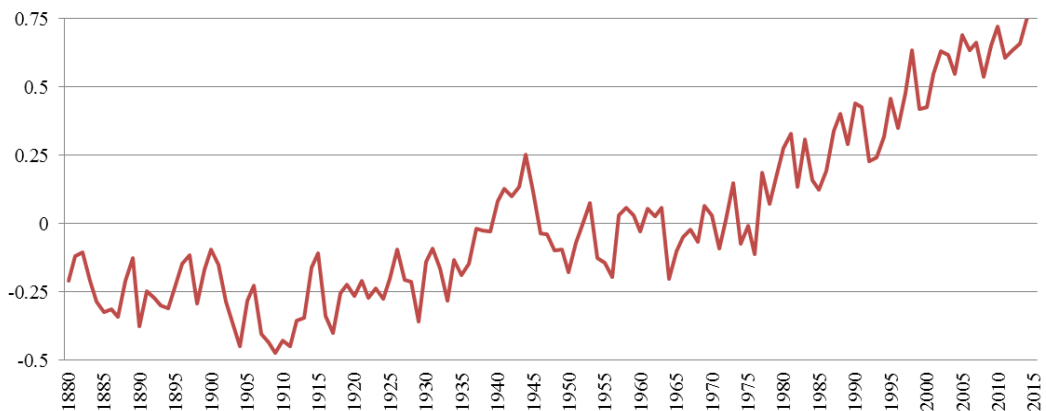


Figure 1.2. Change in global surface temperature relative to 1951-1980 average temperatures (°C). Data source NASA's Goddard Institute for Space Studies (GISS).

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

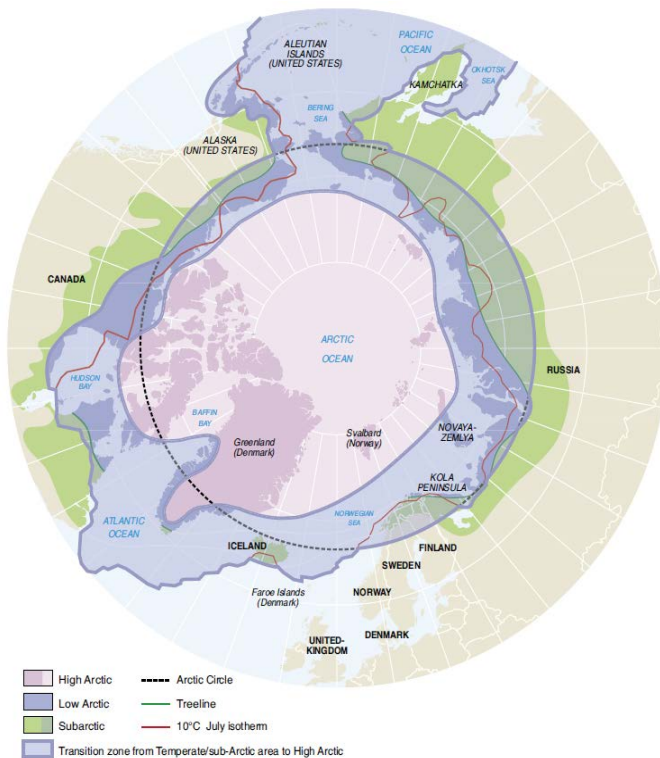


Figure 1.3. Boundaries of the Arctic. Picture adapted from http://www.grida.no/graphicslib/detail/definitions-of-the-arctic_12ba#. Sources: AMAP, 1998. AMAP Assessment Report: Arctic Pollution Issues. AMAP, 1997. Arctic Pollution Issues: A State of the Arctic Environment Report. CAFF, 2001. Arctic Flora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

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

Arctic tundra and climate change

The arctic tundra occur in the northern most regions (Fig. 1.3) where cold temperatures prohibit tree growth, spanning a total area of ca. 7,567,000 km² (appr. 5% of Earth's land surface), spread over Russia, Norway, Iceland, Greenland, Canada and the U.S.A. (Callaghan *et al.*, 2005). The climate of the Arctic is largely driven by the relatively low solar angles relative to the Earth surface. Additionally, most arctic tundra surface is located near the Arctic Ocean and, the energetic balances between land and atmosphere are also greatly influenced by sea ice cover dynamics. Climatically, the arctic tundra is often defined as the area where the average temperature for the warmest month is below 10 °C (Köppen, 1931), however, mean annual air a temperature varies greatly according to location, even at the same latitude. The growing season is short, varying between 3.5 to 1.5 months from the southern to the northern boundaries. The cool summers and prolonged and cold winters produce a continuous permafrost soil layer, and a snow cover that lasts for two thirds of the year (Sturm *et al.*, 2005).

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

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

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

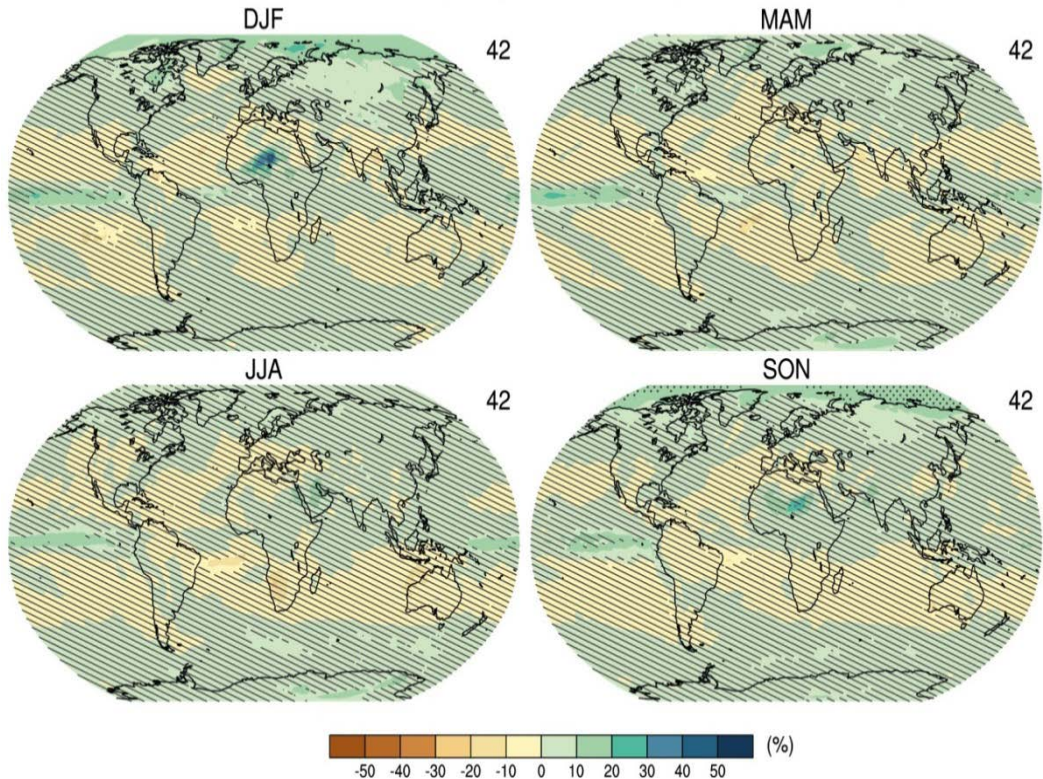


Figure 1.4. CMIP5 multi-model ensemble mean of projected changes (%) in precipitation for 2016–2035 relative to 1986–2005 under RCP4.5 for the four seasons. The number of CMIP5 models used is indicated in the upper right corner. Hatching indicates areas where projected changes are smaller than one standard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

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

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

Arctic fungi and climate changes

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

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

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

Fungi functional diversity

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

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

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

Belowground ECM fungal mycelium morphology can be divided in two parts, the ectomycorrhizae, a morphological structure composed of fungal hyphae and plant roots, and the extramatrical mycelium (EMM), i.e. the mycelium external to the ectomycorrhizae that grows into the surrounding soil with the crucial functions of foraging the litter and/or mineral layers for nutrients and of seeking new roots for colonization (Martin *et al.*, 2001; Anderson & Cairney, 2007). The EMM may form an

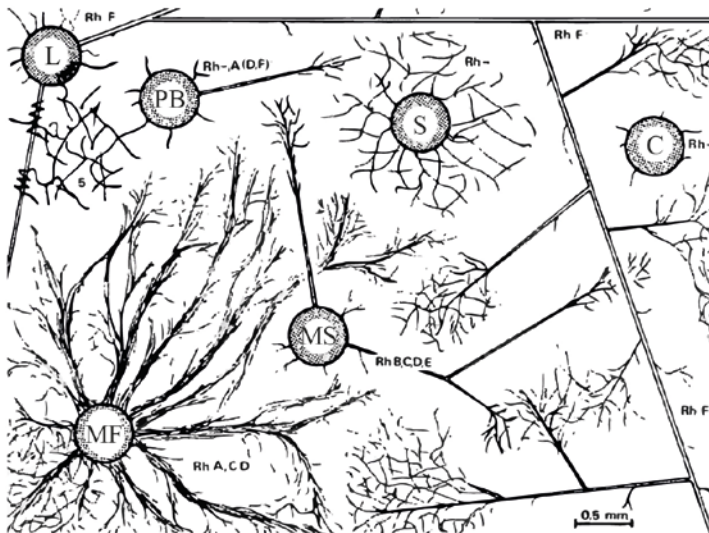


Figure 1.5. Schematic drawings of different extramatrical mycelia exploration types. Legend: L, long-distance; PB, pick-a-back; S, short-distance; C, contact; MS, medium-distance smooth; MF, medium-distance fringe; Rh, rhizomorphs (with classification according to Agerer 1987-2004); Rh-, lack of rhizomorphs.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

intricate hyphal network interconnecting plant roots that pave the way for inter-plant C and nutrient movements (Selosse *et al.*, 2006). EMM of different taxa are known to have distinct anatomical and physiological features that are attributable to various foraging strategies (Colpaert *et al.*, 1992; Agerer, 2001; Hobbie & Agerer, 2010). The main characteristics to classify EMM are the mycelium exploration types (ET), presence/absence of rhizomorphs (vessel-like structures) and hyphae

hydrophobicity (Fig. 1.5) (Agerer, 2001; Hobbie & Agerer, 2010; Peay *et al.*, 2011; Lilleskov *et al.*, 2011; Cairney, 2012). Several studies linked the EMM characteristics with the type of N pools they explore in the soil, and with their roles in soil-plant interaction, taking into account energetic cost-benefit for both fungi and plant host (e.g., Agerer, 2001; Lilleskov *et al.*, 2002; Hobbie & Agerer, 2010; Lilleskov *et al.*, 2011; Cairney, 2012). Additionally, species with abundant EMM generally showed stronger

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

Aims, thesis outline and methodological overview

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

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

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

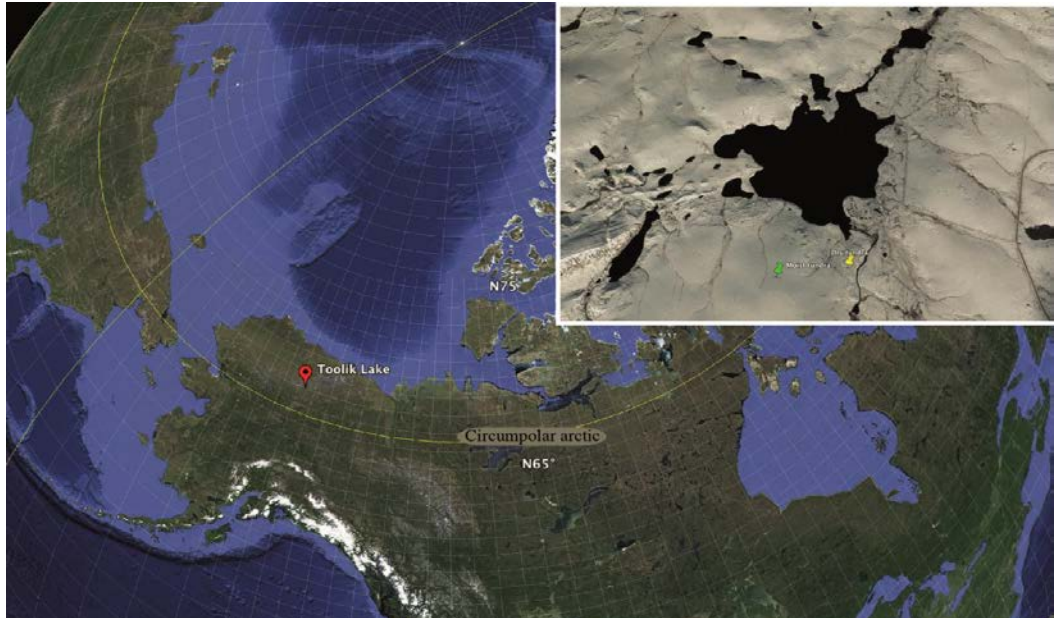
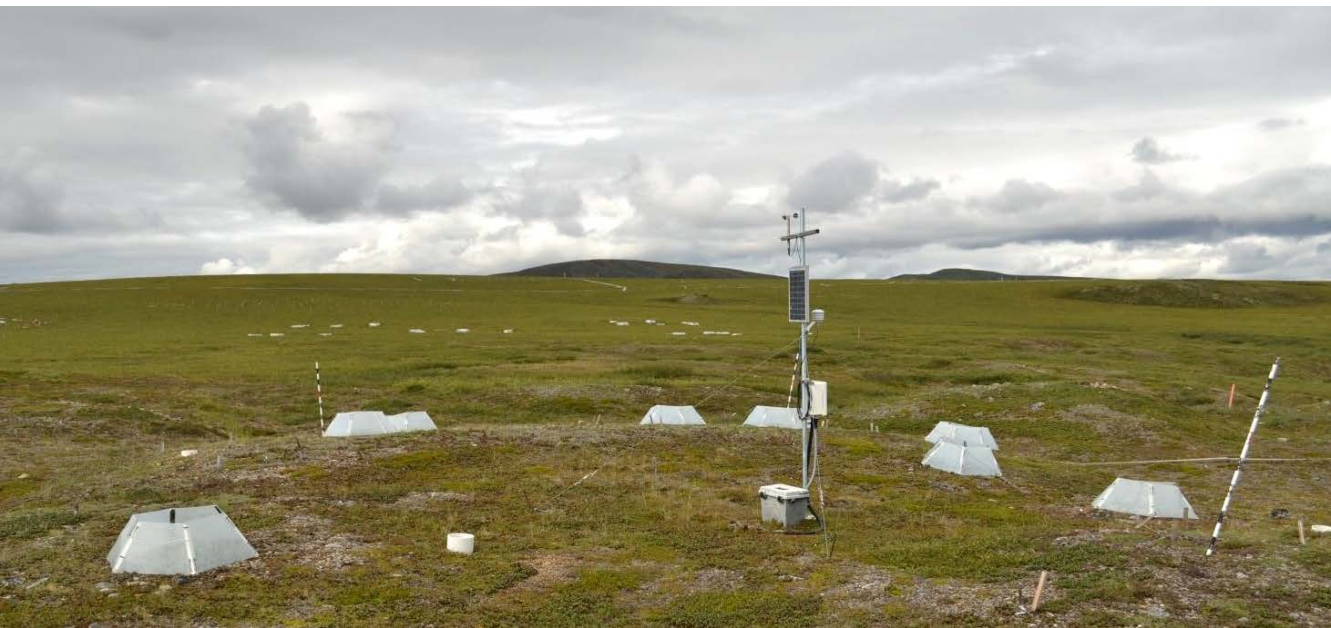


Figure 1.6. Toolik Lake location with close up (inset) with sampling localities, dry tundra with yellow pin and moist tundra with green pin. Source: Google Earth accessed 16 September 2015.

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



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