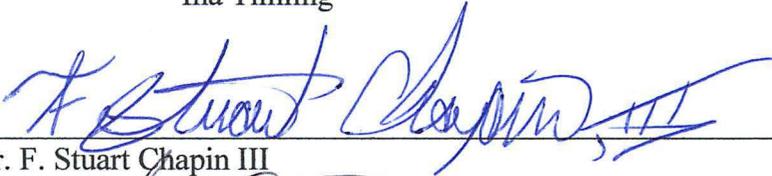


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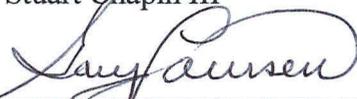
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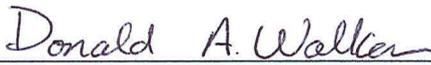
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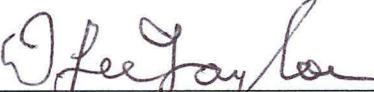
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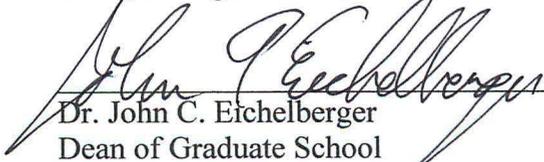


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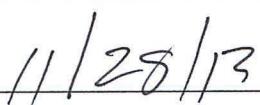
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PEEKING THROUGH A FROSTY WINDOW: MOLECULAR INSIGHTS INTO THE
COMMUNITIES OF ARCTIC SOIL FUNGI

A
DISSERTATION

Presented to the Faculty
of the University of Alaska Fairbanks

in Partial Fulfillment of the Requirements
for the Degree of

DOCTOR OF PHILOSOPHY

By

Ina Timling, M.S.

Fairbanks, Alaska

December 2013

ABSTRACT

Fungi are thought to be one of the most diverse groups of organisms in the Arctic. They drive mineral and energy cycles and influence the occurrence of other organisms as mutualists (mycorrhizae, endophytes, lichens), decomposers and pathogens. Nevertheless, information on fungal biodiversity and distribution patterns in relation to environments across the Arctic is still sparse. Molecular methods were used to examine the diversity and community structures of ectomycorrhizal fungi (EMF) associated with two principal arctic host plants, *Salix arctica* and *Dryas integrifolia*, as well as total soil fungal communities of adjacent disturbed and undisturbed areas of patterned-ground features across the five bioclimatic subzones (A-E) of the North American Arctic. Key findings include the following: (1) More diverse fungal communities had been observed than previously known. These communities encompass nearly all fungal phyla and included all fungal guilds. However, a few species-rich fungal families dominated these fungal communities. (2) Surprisingly, species richness did not decline with latitude. (3) The most abundant fungal taxa were widely distributed in and beyond the Arctic. Yet root (EMF) and soil fungal communities showed niche preferences in regard to bioclimatic subzones. Furthermore, disturbed and undisturbed patterned ground features harbored different soil fungal communities with the exception of the coldest subzone A. In contrast, EMF community composition was not affected by host plant identity. (4) Fungal communities in the warmest subzone E were distinct from the other arctic subzones and the majority of taxa matched fungi from the boreal forest. (5) Key drivers

of fungal community and guild composition along the bioclimatic gradient included regional climate, pH as well as vegetation composition and productivity across the subzones. At the local scale of patterned-ground features, fungal communities were correlated with vegetation composition and microclimate. With a warming climate, I would expect an enhanced colonization of patterned-ground features by vascular plants that would then affect fungal community structure not only at the species level, but also at the level of fungal guilds. In particular I would expect increases in fungi that are symbiotic with plants and a northward shift of both plant and fungal taxa.

DEDICATION

To my father and uncle Jochen who gave me wings and
to my mother and sister who gave me roots

TABLE OF CONTENTS

	Page
SIGNATURE PAGE.....	i
TITLE PAGE	iii
ABSTRACT.....	v
DEDICATION.....	vii
TABLE OF CONTENTS	ix
LIST OF FIGURES	xiv
LIST OF TABLES	xv
LIST OF APPENDICES	xvi
LIST OF SUPPORTING INFORMATION.....	xvii
ACKNOWLEDGEMENT	xix
CHAPTER 1 General introduction	1
<i>1.1. References</i>	<i>7</i>
CHAPTER 2 Peeking through a frosty window: molecular insights into the ecology of Arctic soil fungi.	13
<i>2.1. Abstract.....</i>	<i>13</i>

2.2.	<i>Introduction</i>	14
2.3.	<i>Arctic soil - an extreme environment</i>	16
2.4.	<i>Arctic soil fungal diversity</i>	18
2.5.	<i>Fungal distribution patterns in Arctic soils</i>	20
2.6.	<i>Responses of Arctic soil fungi to climate change</i>	26
2.7.	<i>Adaptations of soil fungi to Arctic environments</i>	30
2.8.	<i>Future challenges in Arctic soil mycology</i>	34
2.9.	<i>Acknowledgements</i>	36
2.10.	<i>References</i>	37
CHAPTER 3 Distribution and drivers of ectomycorrhizal fungal communities		
across the North American Arctic..... 57		
3.1.	<i>Abstract</i>	57
3.2.	<i>Introduction</i>	59
3.3.	<i>Material and methods</i>	63
3.3.1.	<i>Study area</i>	65
3.3.2.	<i>Sampling and processing</i>	66
3.3.3.	<i>Molecular Analysis</i>	68
3.3.4.	<i>RFLP screening</i>	68
3.3.5.	<i>Cloning and Sequencing</i>	69

3.3.6.	<i>Bioinformatics & Statistical Analysis</i>	70
3.3.7.	<i>OTU Clustering and Alignment</i>	71
3.3.8.	<i>Nomenclature:</i>	72
3.3.9.	<i>Ordination Analysis</i>	73
3.3.10.	<i>Diversity Analyses</i>	75
3.4.	<i>Results</i>	75
3.4.1.	<i>EMF diversity in the Arctic</i>	75
3.4.2.	<i>Patterns of EMF communities</i>	77
3.4.3.	<i>Drivers of EMF community structure</i>	79
3.5.	<i>Discussion</i>	81
3.5.1.	<i>EMF diversity in the Arctic</i>	81
3.5.2.	<i>Large Scale EMF community patterns in the Arctic</i>	83
3.5.3.	<i>Factors shaping EMF communities</i>	86
3.6.	<i>Acknowledgements</i>	90
3.7.	<i>References</i>	92
3.8.	<i>Figures</i>	107
3.9.	<i>Tables</i>	112
3.10.	<i>Appendices</i>	115
3.11.	<i>Supporting information</i>	117

CHAPTER 4	Rich and cold: Diversity, distribution and drivers of fungi in patterned-ground ecosystems of the North American Arctic	119
4.1.	<i>Abstract</i>	119
4.2.	<i>Introduction</i>	121
4.3.	<i>Material and Methods</i>	124
4.3.1.	<i>Study areas and patterned ground features</i>	124
4.3.2.	<i>Sampling and processing</i>	125
4.3.3.	<i>Molecular Analysis</i>	126
4.3.4.	<i>Bioinformatics</i>	126
4.3.4.1.	<i>Sequence Clean up</i>	126
4.3.4.2.	<i>Diversity Analysis</i>	128
4.3.4.3.	<i>Ordination Analysis</i>	129
4.3.4.4.	<i>Distribution of OTUs</i>	130
4.4.	<i>Results</i>	130
4.4.1.	<i>Fungal diversity in the Arctic</i>	130
4.4.2.	<i>Species richness along the latitudinal gradient</i>	132
4.4.3.	<i>Community structure across the bioclimatic subzones</i>	133
4.4.4.	<i>Fungal community structure within patterned-ground complexes</i>	135
4.4.5.	<i>Drivers of fungal community structure</i>	136
4.5.	<i>Discussion</i>	137
4.5.1.	<i>Fungal diversity in arctic soils (General description)</i>	137
4.5.2.	<i>Large-scale fungal community patterns in the Arctic</i>	139

4.5.2.1.	Wide distribution of arctic fungi.....	139
4.5.2.2.	Fungal species richness along latitudinal gradient.....	141
4.5.2.3.	Community structures and environmental drivers.....	142
4.6.	<i>Conclusions</i>	147
4.7.	<i>Acknowledgements</i>	148
4.8.	<i>References</i>	150
4.9.	<i>Figures</i>	160
4.10.	<i>Tables</i>	165
4.11.	<i>Supporting information</i>	166
CHAPTER 5	Conclusions	169
5.1.	<i>Fungal diversity</i>	170
5.2.	<i>Fungal distribution</i>	171
5.3.	<i>Key drivers of fungal communities in the Arctic</i>	173
5.4.	<i>Future work</i>	174
5.5.	<i>A fungal ‘outlook’</i>	175
5.6.	<i>References</i>	179

LIST OF FIGURES

	page
Figure 2.1 Daily mean soil temperatures at 10 cm depth	53
Figure 2.2 Soil profile at Isachsen on Ellef Ringnes Island.....	54
Figure 2.3 Patterned ground (frost boils) at Howe Island.....	55
Figure 2.4 Soil fungal OTU (species) richness (Mau Tao).....	56
Figure 3.1 Sampling map showing bioclimatic subzones.....	107
Figure 3.2 Species richness.....	108
Figure 3.3 Distributions of four of the ten most abundant OTUs.....	109
Figure 3.4 Linear regression of observed (Mao Tau)	110
Figure 3.5 NMDS ordination of EMF communities.....	111
Figure 4.1 Map of (a) sampling sites along the North American Arctic Transect.....	160
Figure 4.2 Distribution of the 50 most abundant Operational Taxonomic Units	161
Figure 4.3 Linear regression of (a) observed (Mao tau) and estimated (Chao1).....	162
Figure 4.4 NMDS biplots of all zonal mesic sites across the NAAT	163
Figure 4.5 Relative abundance of fungal families	164

LIST OF TABLES

	page
Table 3.1 Description and sampling scheme of study sites	112
Table 3.2 The ten most abundant OTUs	113
Table 3.3 Correlation coefficients for variables in the NMDS	114
Table 4.1 Locations and characteristics of the zonal mesic sampling sites	165

LIST OF APPENDICES

	page
Appendix 3.1 Rank Abundance Curve of OTUs	115
Appendix 3.2 Two way cluster analysis of all OTUs	116

LIST OF SUPPORTING INFORMATION

	page
Table S3.1 Presence of OTUs associated with <i>D. integrifolia</i> and <i>S. arctica</i>	117
Figure S3.2.1-37 Maximum likelihood phylograms and their alignment files.....	117
Table S4.1 Locations and characteristics of the dry and wet sampling sites	166
Table S4.2 Distribution of clones/OTUs assigned to various taxonomic levels.....	166
Table S4.3 NCBI BLAST-matches and distribution of 1834 OTUs	166
Table S4.4 Geographic distribution of NCBI matches	167
Table S4.5 Indicator species of zonal mesic patterned-ground ecosystems	167
Table S4.6 Indicator species for zonal mesic PGF and bPGF along the NAAT.	167
Table S4.7 Correlations coefficients (r^2) for variables in the NMDS ordination	167
Table S4.8 Spearman Rank Correlation coefficients of Thawing Degree Days.....	167

ACKNOWLEDGEMENT

I would like to thank Bill Gould, Grizelle Gonzalez, Lee Turner and Andrew Borner, who introduced me in an unforgettable way to the Arctic tundra of Alaska; my tireless advisors Lee Taylor and Skip Walker, who taught me so much; my committee members Christa Mulder, Gary Laursen, Terry Chapin for being always constructive; Martha (Tako) Raynolds for being a steady support and for sharing the office; Edie Barbour and Hilmar Maier for all their help with graphic designs; the 'Biocomplexity team', especially Gary Michelson, Chien-Lu Ping, Alexia Kelley, Vladimir Romanovsky, Corinne Vonlanthen, Anja Kade and Martha Raynolds for sharing all their valuable environmental data and for being fun at expeditions; Anne Sudkamp for answering all my questions about English writing; my mother, friends, Paul, Annemarie and Tane for always being there for me.

I would like to acknowledge the generous support of the US National Science Foundation (ARC-0425517, ARC-0632332), and fellowships by EPSCoR, the UAF-Graduate School, the Institute of Arctic Biology and Global Change Student grant. Finally I would like to thank VECO Polar Resources and Aklak Air for logistical support, and Parks Canada and the Innuvialuit community for supporting our study on their lands.

CHAPTER 1 General introduction

After it had rained, my father would take us to the pine forests surrounding the village to hunt for mushrooms. Entering the forests meant entering a different world: one in which plants, animals, light, smell, sound and the ground were so different that it seemed magical. This world did not reveal itself easily. Looking for mushrooms (Suillus sp., Xerocomus sp.) that would grow only here created a sense of adventure, and finding a mushroom would be a moment of fulfilled anticipation. We had noticed that the mushrooms would always grow in the vicinity of tree trunks and, therefore, we could predict their occurrence. While this predictability helped us fill our baskets quickly, it was also most curious to us. Similarly, to find the most desired mushroom (Boletus edulis), we had to go to the edge of the forest where the oaks were growing. Years later, I would learn that the curious, close occurrence of the mushrooms and trees was the result of a mutualistic symbiosis (ectomycorrhizae) between fungi and trees in which they exchange resources. As a result, they allow and support each other's existence. This phenomenon turned out not to be limited to the forests around our village, but to be present across the planet.

(Ina Timling)

Soil fungi are key components in all terrestrial ecosystems; they drive mineral and energy cycling, and influence the occurrence of other organisms, especially plants due to their roles as mutualists (mycorrhizae, endophytes, lichens), decomposers and pathogens. During the last two decades scientists have begun to apprehend the complexity of these communities — largely because of new molecular tools for detecting fungi in soils in an unprecedented way (Buee et al. 2009, Geml et al. 2012, Taylor et al. 2013). Worldwide, fungal biodiversity is estimated to comprise six million species, of which less than 2% are currently described (Taylor et al. 2013)

Fungi in the Arctic have sparked curiosity for nearly 150 years (reviewed in Dahlberg et al. 2013). However the vast expanse of the Arctic, combined with difficulty of access and the ephemeral and irregular nature of fungal fruiting, has limited understanding of their identity, distribution and ecology. Historically, studies of fungal biodiversity in soils were restricted to collections of sporocarps (mushrooms) of macrofungi, microscopy of roots or isolation using fungal cultures. It is now known that these approaches detect only a very small fraction of total fungal biodiversity.

The Arctic is divided into five bioclimatic subzones (A-E) that are defined by summer air temperature and plant growth forms (CAVM Team 2003). Subzone A, which is the coldest and most northern subzone, is dominated by cushion forbs, mosses and lichens. Subzone E, which is furthest south, is the warmest and is characterized by low shrubs, tussock sedges and mosses. Vascular plant species richness and cover as well as plant productivity decrease from subzone E to A (Walker et al. 2005).

In the harsh environment of the Arctic, soil fungi are thought to represent one of the most diverse groups of organisms (Dahlberg et al. 2013). Indeed, a recent molecular study of fungal communities in the Arctic indicates the presence of all major fungal phyla (Wallenstein et al. 2007). However, most of the previous molecular studies focused on ectomycorrhizal fungi (EMF) that form mutualistic symbioses with important shrubs, sedges and forbs (Bjorbaekmo et al. 2010, Blaalid et al. 2012, Deslippe et al. 2011, Fujimura et al. 2008, Fujiyoshi et al. 2011, Geml et al. 2012, Yao et al. 2013). These studies report high fungal diversity with a preponderance of certain fungal families. Two prior molecular studies in the European Arctic suggested that some fungal species have wide distributions (Bjorbaekmo et al. 2010, Geml et al. 2012). Furthermore, Bjorbaekmo et al. (2010) documented no species richness decline of EMF associated with *Dryas octopetala* across two of the five bioclimatic subzones. Nevertheless the majority of the previous molecular studies were limited in geographic scope and comparisons among these studies are difficult due to variation in sampling schemes and molecular methods applied. Therefore, general patterns of fungal diversity and distribution across the Arctic remain unknown.

This gap in knowledge inspired me use molecular methods to address the following questions in my dissertation: What fungi occur across the entire bioclimatic gradient of the Arctic? Do fungi follow the classic latitudinal diversity gradient observed for plants? Do fungal communities vary across the bioclimatic subzones of the Arctic, as observed for plants? Furthermore, I was interested in whether different host plants (shrubs) would

harbor different fungal communities, as had been observed in temperate and Mediterranean climates (Ishida et al. 2007, Morris et al. 2009) (Ch.3).

To address these questions, I examined root-associated EMF (Ch.3) and total fungal communities in soils (Ch.4) along the North American Arctic Transect (NAAT). The NAAT represents a 1,800 km long bioclimatic-latitude gradient across the five Arctic bioclimatic subzones. The transect was established in 2002 as part of the Biocomplexity of Patterned Ground (BPG) project for the study of the interaction of cryoturbation, vegetation, soils, and biogeochemistry in the self-organization of patterned-ground ecosystems (Walker et al. 2008). To date it is the most intensively studied Arctic-scale transect that spans all bioclimatic subzones.

The Arctic is a cold biome underlain by permafrost. Permafrost processes result in patterned-ground features that are abundant throughout the Arctic. These features include non-sorted circles and polygons, which are mostly bare ground interspersed by vegetated areas, with spatially heterogeneous plant communities and soils (Raynolds et al. 2008, Walker et al. 2011). The pairing of the disturbed patches associated with these features with the adjacent undisturbed zonal vegetation provided an opportunity to examine how soil disturbance is related to soil fungal communities across the bioclimatic gradient (Ch.4).

Ongoing climatic changes in the Arctic include a pan-arctic shrub expansion (Tape 2006), an increase in tundra productivity (Bhatt et al. 2010) and warming and thaw of permafrost (Anisimov et al. 2007). Palaeobotanical studies and contemporary warming

experiments indicate that not only do plant communities respond to the effects of warming, but fungal communities do as well (Clemmensen & Michelsen 2006, Deslippe et al. 2011, Elmendorf et al. 2012, Walker et al. 2006). Because fungi are integral components of terrestrial ecosystems, it is imperative to not only gain an understanding of their diversity and distribution, but to also understand key drivers of community composition in order to assess the potential effects of climate change in the Arctic.

Environmental and bioclimatic gradients are powerful tools to study patterns of organisms in relation to various environments. For example, bioclimatic gradients across the Arctic, such as the NAAT, can be used as proxies for climate change. The extensive environmental data set generated by the BPG project along the NAAT, enabled me to correlate fungal communities with environmental factors and plant communities in order to determine the key drivers of fungal communities in the Arctic. I determined key drivers for root associated EMF (Ch.3) and for soils of patterned ground features (Ch.4) in the Arctic.

The dissertation is composed of a general introduction, a review chapter, two data chapters and general conclusions.

In Chapter 2, I review recent molecular studies on the diversity, distribution patterns and ecologies of fungi in the Arctic. I describe the extreme environment of arctic soils and the

adaptation of fungi to this environment. Further, I describe the fungal responses to climate change and future challenges in Arctic mycology.

In Chapter 3, I describe the diversity and distribution patterns of ectomycorrhizal fungi (EMF) associated with two abundant dwarf shrubs, *Dryas integrifolia* and *Salix arctica*, across the bioclimatic gradient in the North American Arctic. I use molecular methods to identify EMF from individual root tips. I assess whether or not species richness of EMF communities declines with latitude. Furthermore I address the effect of host plant identity on the associated EMF communities. To determine the drivers of EMF communities, I correlate the fungal communities with an extensive dataset of environmental factors collected along the NAAT (Walker et al. 2011).

In Chapter 4, I use high-throughput sequencing to describe the total fungal communities in soils of patterned-ground features (PGF) along the NAAT. I assess the effect of bioclimatic subzones and the disturbance associated with PGF on the distribution and community structure of arctic soil fungi. Again, I use the extensive dataset of environmental factors collected along the NAAT to identify the key drivers of soil fungal communities along the bioclimatic gradient.

In the final chapter, I summarize findings of the two data chapters and discuss their implications for our understanding of fungi in the Arctic and future perspectives.

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CHAPTER 2

Peeking through a frosty window: molecular insights into the ecology of Arctic soil fungi.¹

2.1. Abstract

Fungi are ubiquitous in Arctic soils, where they function as symbionts and decomposers and may affect the carbon balance of terrestrial ecosystems subjected to climate change, and yet little is known about soil fungi at high latitudes. Here we review data from recent molecular studies to determine broad patterns in Arctic soil fungal ecology. The data indicate comparatively high fungal diversity in Arctic soils, with currently no evidence for lower species richness at higher latitudes. The dominant fungi, and particularly ectomycorrhizal-forming fungi, appear to be cosmopolitan species. Arctic soil fungi are capable of growth at sub-zero temperatures, melanized forms are frequent, host specificity is low and there is evidence that community composition alters under experimental warming. Future challenges are to determine the drivers of fungal diversity, whether or not diversity alters at higher latitudes and how apparently cosmopolitan fungi are able to survive the extreme environments encountered in Arctic habitats.

¹ published as: Timling I, Taylor DL, 2012. Peeking through a frosty window: molecular insights into the ecology of Arctic soil fungi. *Fungal Ecology* **5**, 419-429

2.2. Introduction

Fungi are ubiquitous in the cold soils of the Arctic (Laursen & Miller 1977; Robinson *et al.* 1996, 1998; Bergero *et al.* 1999; Alias & Suhaila 2008; Newsham *et al.* 2009). They are also found in Arctic sediments, glaciers and permafrost, and constitute a major fraction of the living biomass of Arctic soils (Laursen 1975). Fungal communities in these soils include representatives of all major fungal phyla (Wallenstein *et al.* 2007), which function as decomposers, plant symbionts, parasites, pathogens and lichens. Fungi in Arctic soils perform the same key ecosystem roles - e.g., decomposition and symbiotic interactions with living plants - as those in less extreme environments. However, they survive, reproduce, and carry out a wide range of biogeochemical transformations in soils that are extremely cold, often dry, and mostly snow covered. Nevertheless, our current knowledge of the identities and activities of these fungi is limited.

A decade ago, a widely-cited review, *the molecular revolution in ectomycorrhizal ecology: peeking into the black-box*, dealt with the changing views of diversity and community structure that were emerging from molecular analyses of ectomycorrhizal fungi (EMF) on roots (Horton & Bruns 2001). Five years earlier, a review by Gardes & Dahlberg (1996) surveyed the available information on Arctic and alpine mycorrhizas, and concluded that '*distribution patterns of species diversity are unknown for ericoid and arbuscular mycorrhizal fungi and limited for ectomycorrhizal species*'. Both reviews

suggested that molecular methods held great promise for revealing the identities of soil fungi, as well as the relationships between particular species and environmental gradients. Over a decade later, our view of fungal diversity in the Arctic based upon data from molecular studies is still rather opaque, but tantalizing glimpses of patterns and processes in the ecology of Arctic soil fungi have appeared.

Fungal activity in Arctic soils is important to the future of the biosphere. Recent studies have reported that the North American Arctic contains considerably higher stocks of organic carbon in soils and permafrost than was previously anticipated, with an estimated total of 98 gigatonnes of organic carbon being present in the region (Ping *et al.* 2008a). Considerable microbial metabolic activity occurs in Arctic soils under snow packs, even at temperatures below freezing (Fahnestock *et al.* 1998; Sturm *et al.* 2005). Winter respiration is hence critical to global carbon cycles and to predicting feedbacks to atmospheric CO₂ levels and global warming. As Arctic soils warm and permafrost thaws, the decomposition of organic carbon in Arctic soils by saprotrophic fungi has the potential to release substantial amounts of CO₂ to the atmosphere and hence influence the Earth's climate.

In this review, we summarize molecular work describing the diversity and community structure of fungi – and typically EMF - in Arctic soils that has emerged since the publication of Gardes & Dahlberg (1996). Our focus is on the active layer, the zone of

soil that annually thaws and which is located above the permafrost, rather than on permanently frozen soil (see Wagner 2008). We take a species-oriented perspective, and hence do not consider data from ‘black box’ studies that measure net microbial processes (see Schimel & Chapin 2006). Lastly, we focus on belowground studies in the Arctic, rather than studies on aboveground sporocarps (Kobayasi & Kenkyujo 1967; Miller *et al.* 1973, 1982; Laursen *et al.* 1987, 2001). Major topics that we consider are the characteristics of the soils that fungi inhabit in the Arctic, the diversity and distribution patterns of fungi found in Arctic soils, the responses of fungal communities to past and simulated climate change, and the adaptations that allow fungi to survive in Arctic soils. Lastly, we consider future challenges in the study of Arctic soil fungal ecology.

2.3. Arctic soil - an extreme environment

The Arctic climate is characterized by short, cool summers and a prolonged cold season. Sub-zero temperatures in winter and the lack of warmth in summer lead to continuous permafrost. Soils at 10 cm depth on Banks island in the High Arctic can stay frozen for up to 74% of the year, which is twice as long as in a North American temperate grassland (Fig 2.1). Even Low Arctic soils at Toolik Lake in Alaska can remain frozen for 69% of the year (Fig 2.1). These soils also experience wide temperature fluctuations, with soil temperatures at 10 cm depth ranging between -27°C and 14 °C in the High Arctic, between -7°C and 11 °C in the Low Arctic and between -2°C and 22 °C in a temperate grassland (Fig 2.1). Precipitation decreases from the Low to the High Arctic (Serreze & Barry 2005), resulting in a decrease in mean snow depth (Raynolds *et al.* 2008; Walker *et*

al. 2008). The non-uniform distribution of snow across the landscape can cause large temperature differences in surface soils (Coulson *et al.* 1995), with higher soil temperatures under deeper snow packs. For example, Buckeridge & Grogan (2008) found the temperature of soil under 1 m of snow pack to be -11 °C, compared with -18 °C under a 0.3 m of pack. Soils under snow packs can also be subjected to substantial temperature changes in autumn and winter, caused by occasional warm winds. On Banks Island in the High Arctic, a rapid rise in air temperature over several days from -45 °C to -5 °C led to an increase in soil temperature from -23 °C to -16°C under 15 cm of snow cover and from -31 °C to -13 °C under 5 cm of cover (Geophysical Institute Permafrost Laboratory 2011).

During the short growing season, which can last from 6 weeks in the High Arctic to 4 months in the Low Arctic, the active layer typically thaws to a depth of 30-60 cm (Fig 2.2a; Tarnocai 2009). During thaw, the underlying permafrost can prevent drainage of soils and can lead to temporally anoxic conditions. In dry soils, freezing can lead to desiccation and increased salinity, especially in the High Arctic, where salt crusts can form on the soil surface due to high rates of evaporation (Tarnocai 2004). However, Arctic soils are not only shaped by permafrost, but also by cryogenic processes such as repeated freeze-thaw cycles, cryoturbation, frost heaving and thermal cracking, which lead to the formation of needle ice and ice lenses (Fig 2.2b). These processes result in the mechanical movement of soil and the creation of microrelief, including patterned ground (Fig 2.3a), causing considerable small-scale variation in soil moisture, vegetation

structure and microclimate (Ping *et al.* 2008b; Tarnocai 2009). As a result, Arctic soils are extremely heterogeneous at small scales. Soil pH values in the upper horizons can vary between 4 and 9 (Goryachkin *et al.* 2004), which greatly affects plant communities and nutrient availability (Walker *et al.* 2005). Nutrient contents (N, P, K) are generally low, while carbon contents in the active layer and permafrost are high and can vary substantially (Tarnocai 2009). Generally, soil organic carbon and nitrogen contents decrease from the Low to the High Arctic (Michaelson *et al.* 2008), as do plant biomass and plant cover (Raynolds *et al.* 2008). Furthermore, cryogenic processes, in particular cryoturbation, contribute to the patchy distribution of soil nutrients and carbon in Arctic soils, which can cause large differences in the structure and activity of soil microbiota (Torsvik & Øvreås 2008).

Therefore, fungi that inhabit Arctic soils must adapt to prolonged sub-zero temperatures, rapidly fluctuating temperatures, a short growing season, limited inputs of simple carbon compounds, desiccation, high salinity, varying pH, low nutrients, physical perturbation and temporal anoxia (Coulson *et al.* 1995; Tibbett & Cairney 2007; Daanen *et al.* 2008; Tarnocai 2009).

2.4. Arctic soil fungal diversity

Molecular analyses of root tips and soil clones show that the most frequent and species-rich EMF genera found in the Arctic are *Thelephora/Tomentella*, *Inocybe* and *Cortinarius* (Fig 2.3b), followed by *Hebeloma*, *Russula*, *Lactarius*, *Entoloma*, *Sebacina*, *Clavulina*

and *Leccinum* (Bjorbaekmo *et al.* 2010; Fujiyoshi *et al.* 2010; Deslippe *et al.* 2011; Geml *et al.* 2012). While molecular methods corroborate the findings of previous sporocarp collections (Gardes & Dahlberg 1996), they also reveal the frequent occurrence of fungal genera that either lack or produce only cryptic sporocarps, such as *Clavulina*, *Sebacina* and *Thelephora/Tomentella*. Other frequently recorded fungi include *Cenococcum geophilum* and dark septate endophytes (DSE), such as *Phialocephala fortinii* and *Cadophora finlandica* (Clemmensen & Michelsen 2006; Hryniewicz *et al.* 2009; Newsham *et al.* 2009; Bjorbaekmo *et al.* 2010; Fujiyoshi *et al.* 2010; Walker *et al.* 2011).

Previous molecular studies on Arctic fungi have mainly focused on EMF obtained from root tips and soil clones. They usually report a surprisingly high richness (Fujimura *et al.* 2008; Bjorbaekmo *et al.* 2010; Geml *et al.* 2012), which exceeds previous estimates based on surveys of aboveground ectomycorrhizal sporocarps. For example, Bjorbaekmo *et al.* (2010) found 137 operational taxonomic units (OTUs) on the roots of *Dryas octopetala* along a latitudinal gradient from Southern Norway to Svalbard. This observation is corroborated by our findings from a study in North America along a gradient from the Low to the High Arctic, in which we recorded 154 OTUs with *Dryas integrifolia* and 179 OTUs with *Salix arctica* (Timling *et al.* unpublished data). Geml *et al.* (2012) similarly recorded 73 ectomycorrhizal basidiomycete OTUs in soils on Svalbard, while Fujimura *et al.* (2008) found 25-35 fungal terminal restriction fragment polymorphism (T-RFLP) types per site on Ellesmere Island in the High Arctic, values

similar to those seen in T-RFLP studies from lower latitudes. On ericaceous plants, 224 OTUs have been recorded in the roots of three co-occurring species in the Low Arctic (Walker *et al.* 2011).

Plants and animals display strong trends of decreasing species richness at higher latitudes in the Arctic (Walker *et al.* 2005), reflecting the harsh environmental conditions close to the poles. The limited evidence to date, however, does not indicate a similar trend for prokaryotes (Neufeld & Mohn 2005; Fierer & Jackson 2006; Chu *et al.* 2010) or soil fungi, suggesting that microbial biogeographical patterns differ from those of macro-organisms. Only two molecular studies have hitherto investigated fungal diversity along latitudinal gradients through the Arctic. Bjorbaekmo *et al.* (2010) found no significant change in EMF species richness with increasing latitude in Norway. The same pattern has emerged from our work, in which we sampled thousands of soil clones from North American sites spanning the Low to High Arctic, and similarly found no association between fungal species richness and latitude (Fig 2.4). However, neither of these studies achieved saturated sampling, and hence, we still do not have a clear picture of whether or not soil fungal diversity is altered at higher latitudes in the Arctic.

2.5. Fungal distribution patterns in Arctic soils

Prior to the advent of molecular methods, sporocarp surveys demonstrated that many fungi (mainly EMF) found in the Arctic had circumpolar distributions, and that they also occurred in boreal and temperate habitats (Gardes & Dahlberg 1996). Nevertheless, the

question remained as to whether or not the fungi found in these different biomes were conspecific. To address this question, Geml *et al.* (2012) recently collected 600 soil cores on Svalbard in the High Arctic, extracted total DNA, constructed internal transcribed spacer (ITS) region clone libraries and sequenced *c.* 3100 clones. Focusing on ectomycorrhizal basidiomycetes, they found that at least 73% of the phlotypes had been recorded outside of Svalbard. The same picture has emerged from studies of ectomycorrhizal root tips of *Salix arctica* and *Dryas integrifolia* throughout the North American Arctic, in which 73% of the observed ITS-OTUs also occur in regions outside of the Arctic (Timling *et al.* unpublished data). These studies indicate that long-distance dispersal is likely to play a key role in the phylogeography of EMF in the Arctic (Geml *et al.* 2012), as it does for Arctic lichens (Geml *et al.* 2010). They suggest that Arctic soil fungi may not be selected for cold tolerance, but perhaps for efficient dispersal, as has been observed for plants (Brochmann & Brysting 2008). Potential characteristics that may enhance fungal dispersal include small spore sizes and resistance to ultraviolet (UV) radiation, freezing and desiccation, possibly conferred by the synthesis of melanin (Robinson 2001). This view is corroborated by the frequent occurrence of DSE, other dematiaceous ascomycetes and darkly pigmented EMF, particularly *Cenococcum geophilum* and species of *Tomentella*, in soil fungal communities at high latitudes (Newsham *et al.* 2009).

As in other regions, soil chemistry is an important driver of fungal community composition in the Arctic (Wallenstein *et al.* 2007; Fujimura *et al.* 2008; Fujiyoshi *et al.* 2010; Deslippe *et al.* 2011). Bedrock and associated geochemistry, such as pH and nutrient availability, strongly affect EMF communities associated with *Salix arctica* on Ellesmere Island in the High Arctic (Fujimura *et al.* 2008; Fujimura & Egger 2012), with genotype richness (based on T-RFLP analyses) being positively associated with decreasing pH, as well as higher levels of nitrogen and phosphorus and lower C:N ratio (Fujimura *et al.* 2008). Soil pH, a key driver of soil bacterial community composition (Fierer & Jackson 2006; Chu *et al.* 2010), and substratum C:N ratio are apparently significant factors in determining the structure of soil fungal communities in the Arctic and other regions (Fujimura & Egger 2012; Dennis *et al.* 2012). In addition, relief and topographic position affect the microclimate and soils of the Arctic, where upland sites are often xeric and experience harsher temperature fluctuations than do mesic lowland sites. Fujimura *et al.* (2008) accordingly showed that EMF communities associated with *Salix arctica* at a lowland site had a higher species richness than those at an upland site.

Arctic vegetation types, such as tussock and shrub tundra, have been shown to be major drivers of microbial community composition at the phylum and subphylum level. Fungal communities differ greatly between tussock and shrub tundra, with ascomycetes being more frequent in the former plant community, which is dominated by non-mycorrhizal sedges and mosses, and basidiomycetes and zygomycetes being more frequent in the

latter, which is dominated by ectomycorrhizal deciduous dwarf shrubs. Furthermore, plant community types can affect substratum quality through differences in litter input and root turnover, and by altering the physical environment in the soil, such as temperature (Wallenstein *et al.* 2007). For example, shrubs tend to trap more snow due to their greater height, which leads to greater insulation of the soil during the cold season (Sturm *et al.* 2005). Studies outside the Arctic have also shown that different soil horizons harbor distinct fungal communities (e.g., Lindahl *et al.* 2007; Taylor *et al.* 2010). These patterns were confirmed for the Low Arctic, where fungal communities in mineral soils under shrub tundra differed significantly at the order level from those in organic soils (Wallenstein *et al.* 2007).

Communities of fungal symbionts across the globe are strongly affected by plant functional type and to varying degrees by host plant species identity (e.g., Ishida *et al.* 2007; Shefferson *et al.* 2007; Dumbrell *et al.* 2009). However, several studies suggest that host-plant identity within a mycorrhizal guild (i.e. ecto-, arbuscular or ericoid mycorrhizas) does not contribute to niches of EMF and ericoid fungi in the Arctic. Investigations of fungal communities of co-existing ericaceous plant species (*Cassiope tetragona*, *Empetrum nigrum* and *Vaccinium vitis-idea*) in Arctic tundra revealed diverse communities dominated by the *Rhizoscyphus ericae* complex (Ascomycota) and Sebaciniales (Basidiomycota) that were not restricted to specific hosts (Walker *et al.* 2011). Similar observations have been made for EMF on *Dryas integrifolia* and *Salix*

arctica throughout the North American Arctic (Timling *et al.* unpublished data) and for *Dryas octopetala* and *Salix reticulata* at a sub-Arctic alpine site (Ryberg *et al.* 2009).

Whether this lack of host specificity of mycorrhizal fungi is consistent across the Arctic remains to be resolved, but it may prove to be a feature unique to cold regions.

Retreating glaciers provide ideal systems in which to study the importance of fungi in primary succession (Fujimura & Egger 2012; Jumpponen *et al.* 2012). Successional variation in EMF communities associated with *Salix polaris* in soils of glacier forefronts on Svalbard has been studied by Fujiyoshi *et al.* (2010). The density of EMF was low in recently deglaciated soils and the establishment of dwarf shrubs in early successional stages depended on the availability of fungal propagules in the soil. In later stages of succession, established shrubs provided fungal inoculum and facilitated further plant establishment. Overall, EMF species richness increased with time since exposure and the dominant fungi changed from a community dominated by ascomycetes to basidiomycetes (Fujiyoshi *et al.* 2010). The ascomycete *Geopora* sp., which is known to colonize extreme soil environments, was the dominant species in the transient stage, while the ascomycete *Cenococcum*, known to occur in soils with higher organic matter contents, was the dominant species in the late stage of the chronosequence. Changes in EMF communities of the transient and late stage were correlated with changes in pH, and an increase in soil nutrients, especially N (Fujiyoshi *et al.* 2010). These observed patterns

from the High Arctic parallel studies of glacier forefronts from alpine habitats at lower latitudes (e.g., Trowbridge & Jumpponen 2004; Zumsteg *et al.* 2012).

Historically, it was assumed that Arctic soil microbial communities are inactive during the prolonged cold season, when soils are covered with snow and ice. However, it has recently been shown that microbial processes continue during the cold season in the Low and High Arctic (e.g., Schimel & Mikan 2005; Elberling 2007). Outside the Arctic, dramatic seasonal shifts of fungal communities have been documented in alpine tundra, boreal forest and temperate grassland (Schadt *et al.* 2003; Taylor *et al.* 2010; Dumbrell *et al.* 2011). There is also some evidence for seasonal changes in soil fungal community composition in the Low Arctic, with a significant increase in morphotypes related to *Cortinarius saturninus* and *Clavulina* spp. associated with an Arctic-alpine willow during the summer (Clemmensen & Michelsen 2006). Seasonal shifts in fungal community structure at the order level have also been observed in Arctic tussock and shrub soils sampled at the end of the growing season and just after the spring thaw (Wallenstein *et al.* 2007). However, it is unclear as to whether or not Arctic soil fungal communities show the same dramatic changes in dominant species from spring to summer as those observed at lower latitudes, because the ribosomal small sub-unit gene studied by Wallenstein *et al.* (2007) only distinguishes fungi at the family level.

Research in alpine systems in Colorado (Schadt *et al.* 2003; Lipson & Schmidt 2004) as well as in cold boreal systems (Wallander *et al.* 2001) have shown that fungal biomass in soil peaks in late winter, just before snowmelt. Studies at alpine sites and in boreal forest (Schadt *et al.* 2003; Taylor *et al.* 2010) have similarly demonstrated strong seasonal changes in fungal community composition, suggesting differential growth and/or mortality across species. In addition, increases in fungal biomass over winter occur in some cold soils (Lipson & Schmidt 2004), suggesting that sub-zero temperatures are not necessarily stressful to the entire fungal community. In fact, microbial (including fungal) biomass drops sharply during spring thaw in both alpine and Arctic systems, coinciding with the release of a flush of nutrients, possibly derived from microbial biomass (Schmidt *et al.* 1999; Sturm *et al.* 2005).

2.6. Responses of Arctic soil fungi to climate change

The influence of climate change on soil fungi is just beginning to be evaluated in the Arctic. Evidence from both palaeobotanical studies and from contemporary warming experiments indicates that Arctic soil fungal communities have responded to, and are likely to respond to, climate warming. Analyses of DNA preserved in ancient permafrost from Northeastern Siberia has revealed that fungal communities changed in concert with plant communities after the last ice age (Lydolph *et al.* 2005). During the Pleistocene (400,000 - 20,000 yrs ago), Beringia was a tundra steppe dominated by grasses, herbs and willow-like shrubs (Brubaker *et al.* 1995). The fungal communities were composed of basidiomycetes, ascomycetes and zygomycetes, and included darkly pigmented fungi,

cold-adapted yeasts, plant parasitic fungi and lichen mycobionts, reflecting the plant communities and the cool climate. After the Last Glacial Maximum, dramatic changes in the communities started to occur. As the environment altered, the tundra became dominated by shrubs and trees, which expanded into the previous tundra steppe (Brubaker *et al.* 1995), and fungal communities changed from yeast-like and parasitic fungi to communities with root-associated macro-fungi such as *Suillus*, *Cortinarius* and *Entoloma* (Lydolph *et al.* 2005). Furthermore, there are indications that fungal communities have become more diverse since the Holocene (10,000 yrs ago), as have plant communities (Lydolph *et al.* 2005).

In addition to this evidence from palaeobotanical studies, contemporary experiments, typically using open-topped chambers to simulate climate warming, indicate that Arctic soil fungal communities are likely to alter in future decades as the region warms and plant communities alter. Long-term experiments have shown significant changes in the abundance of plant functional types, with a dramatic increase of EMF deciduous shrubs across the Arctic after only 2 – 6 yrs of warming (Walker *et al.* 2006), and an increase in the abundance of *Betula nana* after 6 - 15 yrs of N and P fertilization (Shaver *et al.* 2001). A recent meta-analysis of the responses of tundra vegetation to experimental warming across the Arctic has shown that increases in shrub abundance and height were most pronounced in the Low Arctic, without any signs of saturation after nearly two decades, suggesting that the responses of tundra vegetation to warming might continue into the

future (Elmendorf *et al.* 2012). When comparing the responses of aboveground plant productivity after 2 - 9 yr of warming across different biomes (alpine and Arctic tundra, grassland and forest), Arctic tundra had the greatest increase in aboveground plant productivity (Rustad *et al.* 2001), which in turn is likely to affect fungal communities. Shrub expansion in Arctic tundra (Tape *et al.* 2006; Elmendorf *et al.* 2012), and, in particular, a shift from tussock- to shrub-dominated tundra, is likely to alter soil fungal communities in favor of basidiomycetes and zygomycetes (see *fungal distribution patterns in Arctic soils*, above).

Long-term experiments in the Arctic have studied the effects of climate warming and increased availability of soil nutrients on fungal communities associated with *Salix* spp. and *Betula nana* (Clemmensen & Michelsen 2006; Fujimura *et al.* 2008; Deslippe *et al.* 2011). EMF colonization rates of root tips (which were found to vary between 68% and >80%) are apparently unaffected by warming and fertilization treatments (Clemmensen & Michelsen 2006; Deslippe *et al.* 2011). While warming greatly increased shrub biomass and carbon flow belowground in Arctic tundra (Clemmensen & Michelsen 2006; Fujimura *et al.* 2008), the effects on fungal communities varied with the length of the treatment. After up to a decade of warming, root associated fungal communities showed little change in composition (Clemmensen *et al.* 2006; Fujimura *et al.* 2008). However, after 18 yrs of warming, significant increases in EMF species diversity occurred, with changes in fungal community composition and structure associated with *Betula nana*, one

of the most responsive shrubs to climate change in the Low Arctic. There was a 15-fold increase in clones affiliated with the Cortinariaceae in the warming treatment, and EMF communities changed towards species with high biomass and proteolytic capacity (*Cortinarius* spp.), while fungi with high affinities for labile N (*Rhizocyphus ericae*, *Russula* and *Lactarius* spp.) declined in abundance (Deslippe *et al.* 2011). Since *Cortinarius* spp. form rhizomorphs, have hydrophobic hyphae and belong to the medium distance fringe exploration types, it has been suggested that these changes in EMF communities may increase the connectivity between individual shrubs through mycorrhizal networks (Deslippe *et al.* 2011). These authors further suggested that increased N acquisition by the shrubs and nutrient redistribution through the formation of mycorrhizal networks may facilitate shrub expansion in the Arctic. Fertilization of Arctic tundra also increased fungal biomass on roots and in soils (Clemmensen *et al.* 2006), and caused an increase in saprotrophic fungi, while EMF diversity was reduced after two decades, an effect that was enhanced when fertilization and warming were combined (Deslippe *et al.* 2011). Nevertheless, fertilization apparently also changed EMF community composition, with an increase in more nitrophilic species, such as *Laccaria bicolor* and *Tomentella stuposa* (Deslippe *et al.* 2011). Similar observations have been made in boreal forests, where nearly three decades of N deposition lead to a dramatic decline in EMF species richness, with a shift towards fungi adapted to high N availability (Lilleskov *et al.* 2002). In a long-term study in a sub-Arctic heath, changes in microbial communities (based on PLFA analyses) were only observed after 15 yrs of N, P and K

fertilization, with fertilization increasing, and warming decreasing, the biomass of fungi in soil (Rinnan *et al.* 2007).

Although there are some plant functional types, such as evergreen shrubs, that are resistant to simulated climate change in the High Arctic (Hudson & Henry 2010; Haugwitz & Michelsen 2011), in general, plant community responses to warming and fertilization (Shaver *et al.* 2000; Walker *et al.* 2006; Elmendorf *et al.* 2012) in the region are faster than those of soil fungal (and typically EMF) communities (Clemmensen *et al.* 2006; Fujimura *et al.* 2008; Deslippe *et al.* 2011). These studies indicate that soil fungal communities in the Arctic respond relatively slowly to the selective pressures of climate change, with warming causing pronounced changes in fungal community composition after one or two decades.

2.7. Adaptations of soil fungi to Arctic environments

Temperatures below freezing point exert a variety of stresses on microbes, suggesting that a range of adaptations exist for fungi to survive in Arctic soils. It is important to distinguish active growth at low temperatures from survival in a dormant state. Given the hardiness of many fungal spores (Miller *et al.* 1992; Bruns *et al.* 2009; Peay *et al.* 2009), survival is less of a challenge than growth at temperatures below freezing point. Actively growing fungal cultures are often killed by exposure to sub-zero temperatures under laboratory conditions, although filamentous fungi can usually survive single bouts of

freezing (France *et al.* 1979). Nevertheless, it is clear that some cold-region fungi are capable of growth at very low temperature, with a study showing that the filamentous ascomycete, *Geomyces pannorum*, which is frequent in soil clone libraries from Interior Alaska (Taylor *et al.* 2010), grows at -35 °C (Panikov & Sizova 2007). This observation is corroborated by recent findings of significant microbial activity and growth at temperatures below the freezing point (McMahon *et al.* 2009; Drotz *et al.* 2010), and the survival of EMF after exposure to multiple freeze-thaw events (Ma *et al.* 2011).

Freezing imposes physical stresses on fungal cells. For example, frost heave is likely to shear fungal hyphae. However, mycelia can often be seen in frost-heaved soils (Timling, personal observation), and fungi thus presumably have mechanisms to cope with hyphal breakage, such as the sealing of severed hyphae at the septal pore and re-establishment of connections through anastomosis. In soils subjected to cryoturbation, we might expect ectomycorrhizal species with long-distance exploration types, which form extensive rhizomorphs (Agerer 2001), to be at a disadvantage. Indeed, Ryberg *et al.* (2010) reported a greater proportion of contact and short-distance exploration types in their coldest alpine tundra study site. However, *Cortinarius* spp., all of which have extensive rhizomorphic mycelium, are diverse and abundant at all Arctic sites studied to date (Deslippe *et al.* 2011; Geml *et al.* 2012). It remains to be determined whether particular phenotypes, such as contact exploration types, are better able to withstand the stresses imposed by cryoturbation (Ludley & Robinson 2008).

To survive in Arctic soils, fungi must prevent or withstand freezing at the cellular level. The formation of ice crystals within cells often leads to death through rupture of the cell membrane. Potent anti-freeze proteins (AFPs) have been recorded in several high latitude fungi, including basidiomycete snow moulds such as *Typhula* and *Sclerotia* spp. (Hoshino *et al.* 2003; Hoshino *et al.* 2009; see also Tojo & Newsham 2012). Interestingly, however, these proteins are located outside rather than inside the cell, leading to the suggestion that they help prevent freezing of the soil solution on hyphal surfaces at temperatures below freezing point. This might significantly improve opportunities for resource acquisition. Nevertheless, not all psychrophilic fungi have detectable anti-freeze activity, and so are capable of withstanding intracellular freezing (Hoshino *et al.* 2009). This capability is likely to be critical to many Arctic soil fungi, as AFPs only provide a modest depression in freezing point temperature, though they can also influence the shape and growth of ice crystals (Hoshino *et al.* 2003). The buildup of compatible solutes is likely to be the key to survival and growth of fungal cells at sub-zero temperatures. Several studies have demonstrated that fungal cells accumulate more trehalose, mannitol and sucrose when subjected to temperatures between 10 °C and < 0 °C (Tibbett *et al.* 2002; Tibbett & Cairney 2007; Hoshino *et al.* 2009), which increases tolerance to freezing and desiccation (Tibbett *et al.* 2002). While influencing ice formation, the buildup of osmoticum is also critical to cell hydration, which is important in dry soils subjected to desiccation. However, it has been suggested that the

accumulation of osmoticum also increases the susceptibility of cells to osmotic rupture when dry soils are flooded with nearly pure water derived from snowmelt (Jefferies *et al.* 2010). This may account for the sharp decline in fungal biomass during spring thaw in alpine and Arctic ecosystems (see *fungal distribution patterns in Arctic soils*, above).

At low temperatures, not only do simple chemical reactions slow, but enzyme-mediated reactions also face a number of challenges. As temperature falls, the changing strengths of different types of molecular interactions can cause proteins to denature (Franks *et al.* 1990), and, even for enzymes that remain properly folded, may slow or halt the release of reaction products (Feller *et al.* 1997; Gerday *et al.* 1997). Many microbes exhibit optimization of turnover rate relative to substrate binding, i.e. K_{cat}/K_m , and increased thermolability, such as lower denaturing temperatures (Gerday *et al.* 1997). There is also evidence that different extracellular enzymes with lower thermal maxima are expressed when fungal cells are chilled (Tibbett *et al.* 1998, 1999), and that membrane composition is altered at low temperature (Kerekes & Nagy 1980; Hammonds & Smith 1986; Weinstein *et al.* 2000). However, such adaptations carry with them tradeoffs at higher temperatures. For example, membranes and enzymes that maintain fluidity and function at $<10\text{ }^{\circ}\text{C}$ do not function well at $>20\text{ }^{\circ}\text{C}$ (Hoshino *et al.* 2009). These tradeoffs make the widespread distribution of dominant Arctic fungi in habitats at lower latitudes particularly puzzling.

2.8. Future challenges in Arctic soil mycology

While a number of studies of Arctic soil fungi have focused on diversity issues, we currently lack answers to the basic questions of whether or not fungal diversity alters at higher latitudes, and whether the Arctic hosts any endemic fungal species. We anticipate that these issues will be resolved by bringing together widespread sampling with high throughput sequencing methods, which should provide complete censuses of Arctic soil fungi. However, a number of biological and bioinformatics issues still plague the estimation of OTU richness, even with exhaustive sampling (Kunin *et al.* 2010; Nilsson *et al.* 2010). A question of perhaps greater ecological importance is how soil fungal community composition changes with latitude. There is evidence that fungal community composition in cold regions is correlated with several climate variables and a complex of geological soil factors (Timling *et al.*, unpublished data; Dennis *et al.* 2012), but studies to date have not yet uncoupled latitude and climate from geographical distance at high latitudes. Careful consideration is needed to tease apart the influence of confounded factors such as climate, latitude and geographical distance on fungal community composition.

If further studies support the view that predominant soil fungi in the High Arctic are also widespread at lower latitudes (Geml *et al.* 2012), then we will be confronted with the puzzle of how these species have evolved the adaptations necessary for survival under such extreme conditions. We can imagine at least three possible explanations. Firstly,

perhaps such adaptations evolve extremely rapidly, so that the current neutral species-level diagnostics (e.g., 97% identity across the ITS region) fail to discriminate distinct populations or recently evolved species. Secondly, perhaps genetic variation in the adaptive genes is large, and a combination of gene-flow and strong selection allow polar populations to maintain the necessary genetic architecture. Thirdly, perhaps some abundant high latitude fungi recolonize sites on an annual basis, and thus do not need to survive winter extremes *in situ* (Robinson 2001). These intriguing possibilities call for detailed population genetic studies of dominant High Arctic soil fungi.

Another priority in future research should be an increased emphasis on fungal physiology and function *in situ* throughout the cold season. It is critical to work on organisms that are numerically dominant or otherwise keystone players in the environment, rather than simply a narrow subset of species that can be easily isolated and manipulated in culture. For example, RNA-based and stable isotope probing methods (Leigh *et al.* 2007; Deslippe & Simard 2011) offer promise for revealing the identities and activities of fungi that actively grow under snowpack.

Glimpses from molecular studies to date suggest several potentially unique attributes of Arctic soil fungal communities in comparison with biomes at lower latitudes, including a high frequency of melanized fungi, frequent growth at sub-zero temperatures, efficient long-distance dispersal, and low host specificity. However, variation in sampling

regimes, molecular methods and OTU designation across studies currently limits our ability to make rigorous comparisons across biomes. High priorities going forward should be to use standardized methods in cross-latitude and cross-biome studies, to elucidate further characteristics of fungal communities inhabiting Arctic soils.

2.9. Acknowledgements

The authors thank Jessie Cherry, Dan Bahauddin, Mark Seeley and Bill Cable for providing soil climate data. Clare Robinson, Jozsef Geml, Donald Walker, Dan Darrow, two anonymous reviewers and particularly Kevin Newsham provided helpful comments on the manuscript. This material is based on work supported by the National Science Foundation through award ARC-0632332. Any opinions, findings and conclusions or recommendations expressed in this material are those of the authors and do not necessarily reflect those of the National Science Foundation.

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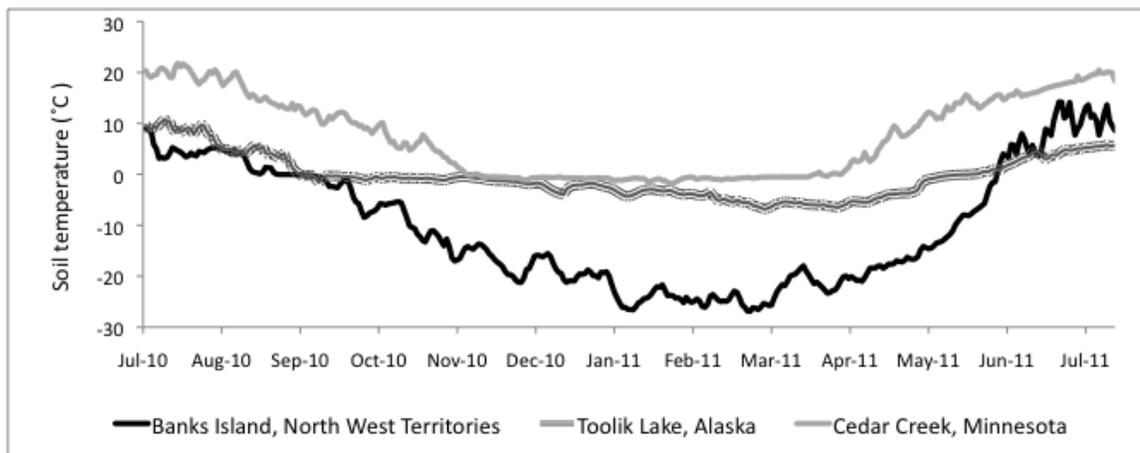


Figure 2.1 Daily mean soil temperatures at 10 cm depth between July 2010 and 2011 on Banks Island in the North West Territories in the High Arctic, at Toolik Lake in Alaska in the Low Arctic and in temperate grassland at Cedar Creek, Minnesota. Data from the Geophysical Institute Permafrost Laboratory and the Institute of Arctic Biology at the University of Alaska, Fairbanks, and from the Cedar Creek Ecosystem Science Reserve.

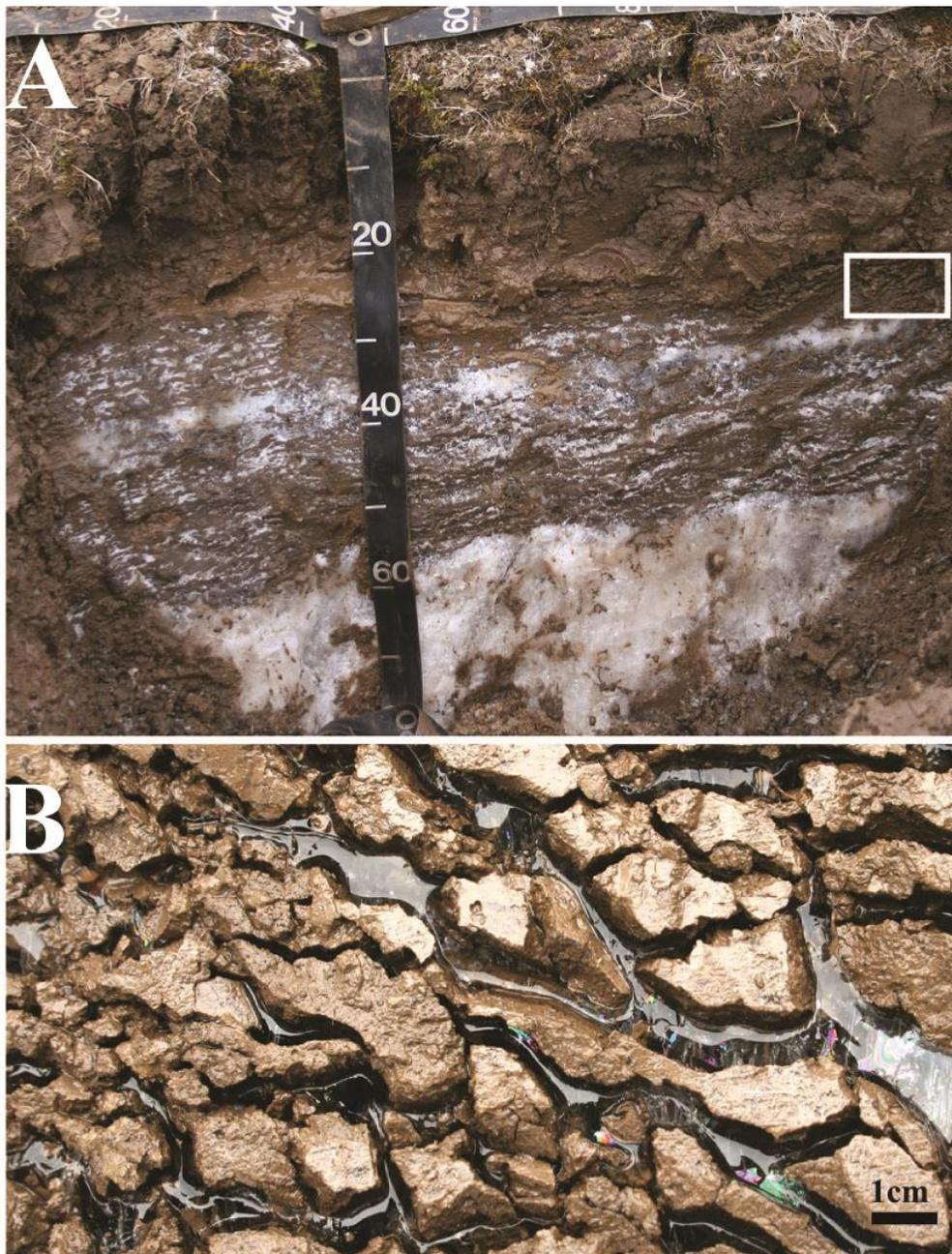


Figure 2.2 Soil profile at Isachsen on Ellef Ringnes Island (A) Soil profile at Isachsen on Ellef Ringnes Island in the High Arctic with permafrost at 25 cm depth, (B) ice lenses in permafrost from the rectangle marked in (A).

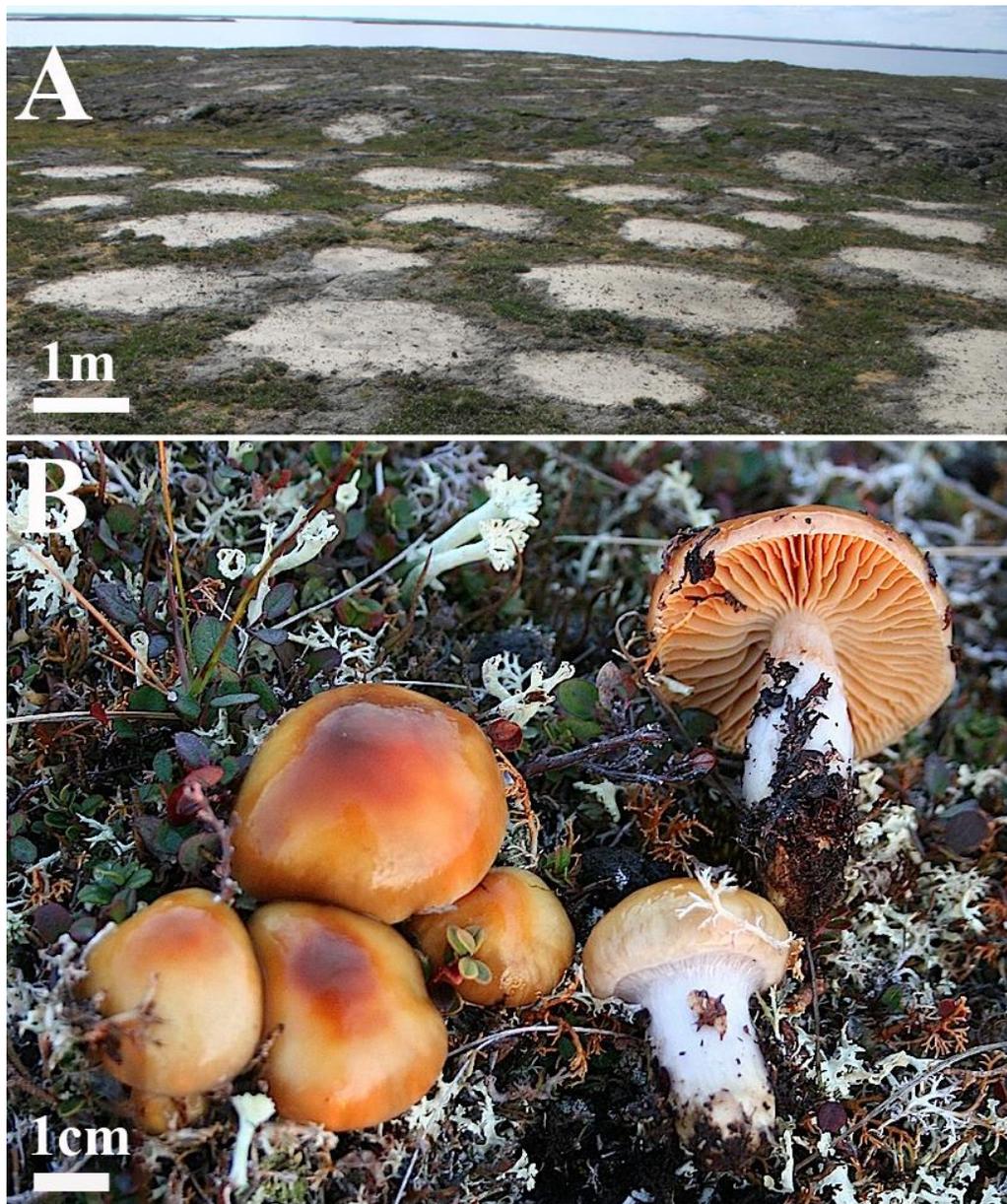


Figure 2.3 Patterned ground (frost boils) at Howe Island (A) Patterned ground (frost boils) at Howe Island, Alaska, (B) *Cortinarius favrei*, a common Arctic basidiomycete, at Toolik Lake, Alaska.

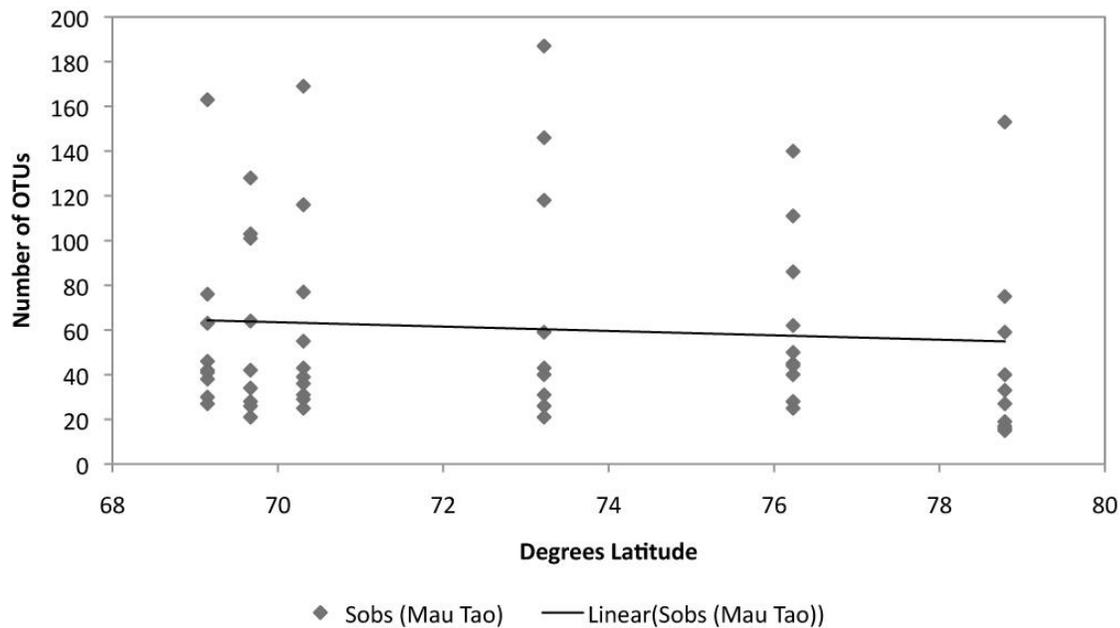


Figure 2.4 Soil fungal OTU (species) richness (Mau Tao) Soil fungal OTU (species) richness (Mau Tao) along a latitudinal gradient through the North American Arctic (Timling *et al.* unpublished data). The presence of potential chimeras was reduced by excluding singletons from the data set. Linear regression showed no influence of latitude on OTU richness ($P = 0.54$).

CHAPTER 3

Distribution and drivers of ectomycorrhizal fungal communities across the North American Arctic.¹

3.1. Abstract

Ectomycorrhizal fungi (EMF) form symbioses with a few plant species that comprise a large fraction of the arctic vegetation. Despite their importance, the identity, abundance and distribution of EMF in the Arctic, as well as the key drivers controlling their community composition, are poorly understood. In this study, we investigated the diversity and structure of EMF communities across a bioclimatic gradient spanning much of the North American Arctic. We collected roots from two principal Arctic ectomycorrhizal host plants, *Salix arctica* and *Dryas integrifolia*, typically growing intermingled, at 23 locations stratified across the five bioclimatic subzones of the Arctic. DNA was extracted from ectomycorrhizal root tips and the ITS region was sequenced and phylogenetically analyzed. A total of 242 fungal Operational Taxonomic Units (OTUs) were documented, with 203 OTUs belonging to the Basidiomycota and 39 to the Ascomycota, exceeding the number of previously morphologically described EMF in the Arctic. EMF communities were dominated by a few common and species-rich families

¹ published as: Timling I., Dahlberg A., Walker D.A., Gardes M., Charcosset J.Y., Welker J.M., Taylor D.L. 2012. Distribution and drivers of ectomycorrhizal fungal communities across the North American Arctic. *Ecosphere* 3 (11): 111.

such as *Thelephoraceae*, *Inocybaceae*, *Sebacinaceae*, *Cortinariaceae*, and *Pyronemataceae*. Both host plants showed similar species richness, with 176 OTUs on *Salix arctica* and 154 OTUs on *Dryas integrifolia*. Host plant identity did not affect EMF community composition. The ten most abundant OTUs had a wide geographic distribution throughout the Arctic, and were also found in boreal, temperate and Mediterranean regions, where they were associated with a variety of hosts. Species richness did not decline with increasing latitude. However, EMF community structure changed gradually across the bioclimatic gradient with the greatest similarity between neighboring bioclimatic subzones and locations. EMF community structure was correlated with environmental factors at a regional scale, corresponding to a complex of glaciation history, geology, soil properties, plant productivity and climate. This is the first large-scale study of EMF communities across all five bioclimatic subzones of the North American Arctic, accompanied by an extensive set of environmental factors analyzed to date. While our study provides baseline data to assess shifts of plant and fungi distribution in response to climate change, it also suggests that with ongoing climate warming, EMF community composition may be affected by northward shifts of some taxa.

3.2. Introduction

Ectomycorrhizal fungi (EMF) are critical to growth and survival of their host plants because they provide water and limiting nutrients in exchange for photosynthetic carbon (Smith and Read 2002). They are thought to be particularly important in harsh environments such as the Arctic tundra, a permafrost-underlain cold-dominated biome with low nutrient availability north of the treeline. EMF have been studied up to 79° N and were found to co-occur with their host plants (Bledsoe et al. 1990, Kohn and Stasovski 1990). Although in the Arctic EMF associate with only 6% of the vascular plant species, including shrubs, a few sedges and forbs (e.g., *Dryas*, *Salix*, *Betula* and *Polygonum*), these plants are important. For example, they are dominant species in plant community types that cover up to 69% of the ice-free Arctic (Walker et al. 2005).

The Arctic is divided into five bioclimatic subzones (A-E), with A being the coldest and E the warmest (Walker et al. 2005). With increasing latitude, organisms face harsher conditions due to decreasing air and soil temperatures (Billings 1992). Vascular plant species richness, including that of EMF host plants, decreases. Simultaneously, plant communities change from a zone of low shrubs in the Low Arctic (subzone E) to a zone of cushion forbs without shrubs in the High Arctic (subzone A) (Walker 2000). This change in plant community composition across the bioclimatic gradient is driven primarily by climate and soil pH (Walker et al. 2011).

Recent climatic changes in the Arctic have led to a pan-arctic shrub expansion (Tape et al. 2006) and an increase in tundra productivity and greening of the Arctic (Bhatt et al.

2010). With continuing warming, further large changes in arctic plant communities are expected (Callaghan et al. 2004). Paleobotanical studies show that plant and fungal communities underwent major changes during past glacial and interglacial cycles, with an increase in shrubs and trees and their root associated EMF (Lydolph et al. 2005, de Vernal and Hillaire-Marcel 2008). Experimental warming of Low Arctic tundra has shown not only a shift of plant communities toward increased shrub dominance (Clemmensen and Michelsen 2006, Walker et al. 2006, Elmendorf et al. 2012), but also changes in the community structure of their associated EMF (Deslippe et al. 2011). EMF are expected to play an important role in these ongoing climate-driven changes in plant communities, particularly shrub expansion, since Arctic shrub species are all ectomycorrhizal.

To understand the factors underlying shrub expansion in the Arctic, we need to include studies of their EMF. In particular, we need to know their identity, their distribution and what factors shape these communities in the Arctic. However, despite the ubiquity and significance of EMF for Arctic tundra and the fact that they have been reported for more than a century (Hesselman 1900, Bledsoe et al. 1990, Kohn and Stasovski 1990, Gardes and Dahlberg 1996, Newsham et al. 2009), our knowledge of the identity, distribution and ecology of arctic EMF is only fragmentary (reviewed in Timling and Taylor 2012).

Thus far, arctic EMF studies have relied primarily on sporocarp (mushroom) collections of macrofungi. Additionally, studies have been limited in geographic and

temporal scope because the Arctic is not easily accessible and occurrence of sporocarps is ephemeral and irregular. Lists of macrofungi from Greenland, Svalbard, the Russian Arctic and Iceland report about 2600 morphologically described macrofungi, with at least 150 ectomycorrhizal species (Elvebakk & Prestrud 1996, Karatygin et al. 1999, Hallgrímsson & Eyjólfsson 2004, Borgen et al. 2006). Such lists have not yet been compiled for North America. Patterns from morphological sporocarp-descriptions of macrofungi indicate that arctic fungi are widely distributed in arctic and alpine habitats on all continents. Some widely distributed EMF genera that have a preponderance in arctic and alpine conditions include *Inocybe*, *Cortinarius*, *Hebeloma*, *Russula*, *Thelephora*, *Tomentella*, *Cenococcum*, and *Laccaria* (Gardes and Dahlberg 1996, Mühlmann and Peintner 2008, Ryberg et al. 2009, Deslippe et al. 2011, Fujiyoshi et al. 2011). However, reliance on morphologically recognized species may underestimate fungal diversity (Taylor et al. 2006). Hence, the reported species richness for arctic EMF provides only a conservative estimation.

Outside the Arctic, many fungi have distribution patterns corresponding with geography (e.g., Taylor et al. 2006, Geml et al. 2008b, O'Donnell et al. 2011). Distinctive phylogeographic patterns have been observed for fungi in boreal, temperate and tropical regions (Taylor et al. 2006, Geml et al. 2008a). In the Arctic however, studies of lichen-mycobionts and EMF have not revealed discrete distributions at the continental or smaller scales, but instead highlight the importance of wide-ranging dispersal (Buschbom 2007, Geml et al. 2010a, Geml et al. 2012b). Thus we might expect that species of EMF in the

Arctic are widely distributed. A wide distribution of EMF would be facilitated by little or no host preference. Indeed low host preference has been shown for EMF communities associated with several alpine and arctic dwarf shrubs (Kernaghan and Harper 2001, Ryberg et al. 2009, Ryberg et al. 2010, Fujimura and Egger 2012). However, most of these studies were conducted on local (~5 km) scales, and hence require confirmation at a much broader geographic scale. In contrast to the relatively low diversity of EMF host plants in the Arctic, studies exploring EMF diversity in the Arctic using molecular methods (Clemmensen and Michelsen 2006, Wallenstein et al. 2007, Fujimura et al. 2008, Bjorbaekmo et al. 2010, Deslippe et al. 2011, Fujiyoshi et al. 2011, Geml et al. 2012b) have revealed species-rich EMF communities; this parallels findings from temperate and boreal biomes (Tedersoo et al. 2006, Buee et al. 2009, Taylor et al. 2010). The largest-scale study to date, exploring EMF diversity of *Dryas octopetala* across two of the five bioclimatic subzones in the European Arctic, observed no decline in species richness with increasing latitude (Bjorbaekmo et al. 2010). Whether this pattern applies across the five bioclimatic subzones and to other dwarf shrubs and regions of the Arctic has yet to be determined.

The objectives of our study were (1) to characterize patterns of EMF community composition along a bioclimatic gradient across all five subzones of the North American Arctic and (2) to identify key drivers controlling their community composition. We sampled two widespread and important shrubs of the arctic tundra, *Salix arctica* Pall and

Dryas integrifolia Vahl (Hultén 1968) in the North American Arctic and carried out detailed molecular phylogenetic analyses of EMF colonizing their root tips.

To identify patterns of EMF community composition along the bioclimatic gradient in the Arctic we hypothesized that:

- (1) EMF richness decreases with latitude, parallel to species richness of macro-organisms.
- (2) EMF communities of *S. arctica* and *D. integrifolia* change gradually across bioclimatic subzones, as observed in plant communities.
- (3) Dominant EMF associates of *S. arctica* and *D. integrifolia* have wide distributions across Arctic and Alpine habitats. However, these dominants will be restricted to cold-dominated regions due to cold adaptation.
- (4) Climate and abiotic soil factors are the most important drivers of EMF community composition, while host plant identity of *S. arctica* and *D. integrifolia* exerts little influence.

In our study, we have sampled for the first time all five bioclimatic subzones of the Arctic and analyzed key drivers of EMF community structure based on a wide range of environmental factors along the gradient.

3.3. Material and methods

This paper reports the combined work of two research initiatives conducting descriptive surveys in the North American Arctic. Because the collaboration was established after sampling and molecular analysis had been completed, we provide information regarding

these steps separately, where they differ. The first dataset comprises sampling sites throughout the Canadian Arctic Archipelago (CAA) (Eriksen et al. 2006), and the second dataset consists of sampling sites along the North American Arctic Transect (NAAT) (Walker et al. 2008).

In both datasets, we sampled sites in five bioclimatic subzones of the Arctic (A-E) as portrayed on the Circumpolar Arctic Vegetation Map (Walker et al. 2005) (Fig. 3.1). A ‘bioclimatic subzone’ is defined based on a combination of summer air temperature and dominant plant growth forms. Subzone A is the coldest, with a Mean July Temperature (MJT) of 0-3°C. The dominant plant growth forms are cushion forbs, mosses and lichens. This coldest subzone has a very limited extent of about 2% of the nonglacial Arctic. Subzone B has a MJT of 3-5°C and is characterized by prostrate dwarf shrubs. Subzone C has a MJT of 5-7°C, and is dominated by hemi-prostrate dwarf shrubs, sedges and mosses. Subzone C represents the subzone with the largest extent in the circumpolar Arctic tundra. Subzone D has a MJT of 7-9°C, with erect dwarf shrubs, sedges and mosses. Subzone E is the most southern subzone and has a MJT of 9-12°C, with low shrubs, tussock sedges and mosses. Vascular plant diversity and plant cover increases from subzone A to E (Walker et al. 2005).

To analyze these data, we structured our observations into two datasets. The first consisted of all data collected from both the CAA and the NAAT. We refer to this as the ARCTIC dataset. This dataset represents a large geographical scale, but is described with fewer environmental factors than the second dataset, which consists of data collected

only along the NAAT. While this dataset contains fewer sites in each bioclimatic subzone, it is accompanied by a more extensive collection of vegetation and environmental data for each study site.

Due to the different strengths of these two datasets, we used the ARCTIC dataset to assess the overall EMF diversity associated with *S. arctica* and *D. integrifolia*, the species richness along a latitudinal gradient, the geographical distribution patterns of the observed fungal taxa, and the effect of host plant and environment on the ECM fungal communities. We used the NAAT dataset to investigate changes in EMF community structures between sampling locations and bioclimatic subzones. Finally, we used the NAAT dataset to identify key environmental factors affecting fungal communities along this bioclimatic gradient.

3.3.1. Study area

NAAT.-We sampled ectomycorrhizal root tips associated with *S. arctica* and *D. integrifolia* at eight sites along the 1800 km North American Arctic Transect (NAAT), covering bioclimatic subzones A-E (Table 3.1). All sites represent mesic zonal sites that were extensively studied by the 'Biocomplexity of Patterned Ground Project' (Walker et al. 2008). Detailed descriptions of the study sites are provided in Kade et al. (2005), Vonlanthen et al. (2008), and Reynolds et al. (2008). Samples along the NAAT were collected at the end of July in 2005, 2006 and 2009. In 2006, we obtained samples of *D. integrifolia* and *S. arctica* from Thule (Greenland). This location was a mesic site from subzone C, and can be described as prostrate dwarf-shrub forb tundra.

3.3.2. Sampling and processing

CAA.-The material for this study was collected July 1st to September 1st 1999 as part of the Tundra North West 1999 expedition in the Canadian Arctic, spanning 14 sites (Eriksen et al. 2006). These sites were selected to represent longitudinal and latitudinal gradients, to encompass vegetation spanning the Low to High Arctic and four bioclimatic subzones (B-E). Sites included mesic and dry conditions (Table 3.1). Further information can be found in Eriksen et al. (2006).

CAA. -At five sites, two intensively studied 20 x 20 m plots were established, with one in mesic conditions and one in dry conditions. These plots were not more than 500 meters apart. Each plot was surveyed for vegetation and soil characteristics within the Biodiversity Program of Tundra North West 1999 (Bölter 2006, Bölter et al. 2006, Eriksen et al. 2006), and eight plants of both *S. arctica* and *D. integrifolia*, including their full root systems, were randomly collected from each plot, (Table 3.1). In order to obtain additional mycorrhizal samples from less intensively studied sites and to cover a larger area and, hence, a larger potential proportion of local species richness, 2-10 plants of *S. arctica* were randomly collected along 500 m transects at each of the fourteen sites and 3-10 plants of *D. integrifolia* were collected at three sites along the same transect. At the five intensively studied sites, transects were an outward extension beyond the plot area. In total, 164 *S. arctica* and 89 *D. integrifolia* root systems were obtained. Roots were processed in a similar fashion as samples from the NAAT.

Single or multiple representative root tips of all distinguished morphotypes from each plant were sorted and placed individually in 1.5 ml tubes. From each plant, at least five mycorrhizal tips were collected, regardless of whether one or several morphotypes were detected. All discernible mycorrhizal morphotypes were sampled from each plant. Immediately after detaching, each mycorrhizal root tip was submerged in 300 μ L of 2X CTAB lysis and kept frozen at -20° C until DNA extraction was performed as described by Gardes and Bruns (1996).

NAAT. -At each site, we randomly chose three to eight plants from each shrub species in the immediate vicinity of the 10 m x10 m grid established for the 'Biocomplexity of Patterned Ground Project', representing mesic zonal conditions (Kade et al. 2005, Reynolds et al. 2008, Vonlanthen et al. 2008, Walker et al. 2008). Entire plants were excavated along with soil surrounding the root system, for a total of 34 *S. arctica* and 39 *D. integrifolia*. (Table 3.1). The plants were stored in a cooler and transported to the laboratory within ten days, where the root systems were immediately washed and rinsed with de-ionized water. All root tips were harvested and morphotyped following Agerer (1987-2002). Each morphotype was kept separate for each plant throughout analysis (i.e. no attempt was made to define shared morphotypes across samples because this has shown to be an error-prone practice (Sakakibara et al. 2002). Two root tips from each morphotype were placed individually in cryovials and frozen at -80° C, then lyophilized prior to DNA extraction.

3.3.3. Molecular Analysis

CAA: -DNA Extraction and PCR.-Cell disruption was performed by three freeze/thaw cycles in liquid nitrogen/65°C. EMF root tips were crushed with a motorized mini-pestle (Kontes, Vineland, NJ, USA) before incubation at 65°C for 1h. After chloroform extraction and DNA precipitation of 1856 samples, the ITS region was amplified using the primer set ITS1-F and ITS4 as above using a PTC-200 thermal cycler (MJ Research Inc., Waltham, MA, USA) following the protocol by Gardes and Bruns (1993).

NAAT:- DNA Extraction and PCR.-The lyophilized roots were ground with 3.2mm stainless steel beads (BioSpec. Products, Inc.) using a DNA Mixer Mill (Retsch®, Haan, Germany). Ground root tips were immersed in buffer and RNase for 12 hours before DNA extraction to increase yield. DNA was extracted from 776 single root tips, using the E.Z.N.A. SP Fungal DNA Kit (Omega Bio-tek, Doraville, GA). Extracted DNA was used to amplify the entire ITS region using fungal specific primer ITS1-F (Gardes and Bruns 1993) and generic primer ITS4 (White et al. 1990) with a PTC-225 Thermal Cycler (MJ Research, Waltham, MA, USA). The polymerase chain reaction (PCR) program was 2 min at 96°C, then 34 cycles of 30 s at 94°C, 40 s at 54°C, 1 min at 72°C followed by 10 min at 72°C.

3.3.4. RFLP screening

PCR products (700-800 bp) were digested with the restriction enzyme *HaeIII* (BioLabs. Inc., New England, USA) following manufacturers instructions and visualized on the 2% Nusieve GTG plus 1% agarose gels. Restriction fragment length polymorphism (RFLP)

patterns were identified using AlphaImager 3400 (Alpha Innotech, San Leandro, CA, USA). Only bright bands that added up to the correct total fragment size for the ITS region were included in the designation of particular RFLP patterns, while weak bands were excluded. RFLP profiles were grouped with the program GERM 1.01 (Dickie et al. 2003). To minimize the underestimation of EMF-diversity, we tabulated RFLP types for each plant species separately and matched RFLP types only within a site. We applied the following settings: maximum forward and backward error 10bp, maximum sum error 100bp, lower measurement limit 100bp, and the joint matching and ranking method (Dickie et al. 2003). For the CAA mycorrhizae, 71% of the sequenced samples were digested with four restriction enzymes (*Cfo*I, *Hinf*I, *Hae*III, and *Mbo*I) independently, 24% with two enzymes (*Cfo*I, *Hinf*I), and 4% with three enzymes (*Cfo*I, *Hinf*I, *Hae*III). Identical RFLP-patterns were then used to molecularly characterize each mycorrhiza.

We recorded the number and abundance of each RFLP type for each plant at each site. Multiple samples from different sites for each RFLP type were chosen for sequencing. More sequences were obtained for frequent RFLP-types, up to a maximum of 22 sequences from 10 sites for one RFLP-type. Altogether, this allowed us to later combine RFLP types from different sites and plant species having similar or identical sequences into a single operational taxonomic unit (OTU).

3.3.5. Cloning and Sequencing

RFLP types indicating colonization of a root tip by a single fungal taxon were purified using QIAquick PCR Purification Kit (Qiagen Sciences, Valencia, CA, USA). Purified

DNAs were cycle sequenced with Applied Biosystems (ABI) Big Dye v.3.1 kit using ITS1-F and ITS4. Cycle sequence products were purified using SephadexTM – G50 (GE Healthcare- Bio Sciences – AB, Uppsala, SWEDEN) before they were sequenced on a capillary DNA sequencer 3130x/ Genetic Analyzer (Applied Biosystems, USA) at the University of Alaska (Fairbanks, USA) or Genome Express (Meylan, France).

NAAT samples that contained multiple taxa were cloned as follows. PCR products were purified and concentrated with Zymo DNA Clean and Concentrator-5 columns (Zymo Research, Irvine, CA, USA). DNA concentration was determined with a NanoDrop ND-1000 Spectrophotometer (NanoDrop Technologies, Rockland, DE, USA). For cloning, we used the TOPO TA Cloning Kit for Sequencing (Invitrogen, Carlsbad, CA, USA) following the manufacturer's instructions. From each sample/plate, five clones were randomly chosen and PCR amplified with M13F and M13R primers. PCR products were digested with *Hae*III. From these five clones, one representative for each unique clone RFLP type was sequenced as above using the primers M13F and M13R. All sequences were deposited in GenBank under the accession numbers JX630331 - JX630750 and JX630751 - JX630968.

3.3.6. Bioinformatics & Statistical Analysis

Sequence quality filtering. -Sequences were cleaned according to Taylor and Houston (2011). In brief, vector sequences were removed and the two reads for each sequence were assembled using Aligner v.1.3.4 (Codoncode, Dedham, MA, USA). Consensus sequences were exported in FASTA format. Base calls with a phred score <20 were

masked with the letter 'N' using a Perl script. Ambiguous ends of sequences were trimmed using the program TRIMSEQ (EMBOSS) at <http://cbi.labri.fr/utis/Pise/trimseq.html>, with a window size set to 20 and a threshold ambiguity set to 5%. Trimmed sequences were submitted to the program Bioinformatics ToolBox (DNA 2.0) at <http://www.dna20.com/index.php?pageID=151> in order to identify and eliminate sequences with >2% Ns.

3.3.7. OTU Clustering and Alignment

In order to assess phylogenetic diversity based on the ITS1 - 5.8S - ITS2 region, we clustered the sequences into OTUs using CAP3 (Huang and Madan 1999). OTUs were defined as sequences sharing 97% sequence identity in the overlapping regions. We set 'overlap percent identity' to 97, 'maximum overhang percent length' to 60, and 'clipping range' to 6. All other settings were left at defaults. For each OTU, the number and the sampling location of clones and matching RFLP patterns were recorded. For further community analysis, we recorded the presence/absence of an OTU in each plant replicate at a sampling site. For example, if eight *Salix* plants were sampled from a particular site, the range of possible abundances for an OTU was 0 to 8. We included all sequences that fell within an OTU for phylogenetic analyses.

In order to identify the OTUs, we compared the ITS sequences to a curated database derived from GenBank sequences hosted on the Fungal Metagenomics website (<http://biotech.inbre.alaska.edu/fungal-metagenomics/>), using the FASTA matching algorithm (Pearson 1999). From these search results, named sequences with the highest

similarity were included in sequence alignments and phylogenetic analyses. Sequences were considered intergeneric chimeras when they had >90% sequence similarity to different genera in ITS1 and ITS2 and intrageneric chimeras when ITS1 and ITS2 had >97% sequence similarity to different species.

Because the ITS regions of different genera cannot be aligned, we grouped the sequences based on their BLAST search result into genera/families. OTU sequences of the various genera were aligned in Clustalw (Kuo-Bin-Li 2003). Initially we built a maximum parsimony tree in PAUP* 4b10 (Swofford 2003) to identify major clades within genera that were too divergent to be aligned with confidence. Sequences from the resulting major clades were aligned separately in Clustalw, as above. The alignments were further adjusted by eye in the alignment editor Se-Al (Rambaut 1996). PAUP* 4b10 (Swofford 2003) and Modeltest 3.7 (Posada and Crandall 1998) were used to determine the best-fit evolutionary model, with the Hierarchical Likelihood Ratio Test (hLRT). Trees were constructed using the maximum likelihood method in Garli 0.951 (Zwickl 2006). Statistical branch support was estimated using 100 maximum likelihood bootstrap replicates. A consensus tree was computed based on 50% majority rule.

3.3.8. Nomenclature:

OTUs were named on the basis of the phylogenetic results. OTUs were considered to be identified to species level when the closest match in the 90% bootstrap supported clade was a single taxon with >97 % sequence similarity (i.e. all clade members represent the same species, or include one fully identified species and other unidentified taxa). We

applied ‘affinity’ (aff.) when the closest matches were fully identified (genus + species) and showed 93-97% sequence similarity and there was no species incongruence among the members of the clade regarding the species name. We used ‘species’ (sp.) when the closest matches were only described at the genus level, as well as when the closest matches with >95% similarity were fully identified, but had different species names (incongruence in the terminal clade). A sequence similarity below 93% was used to assign taxa at the family level, and below 83% at the order level (Deslippe et al. 2011).

3.3.9. Ordination Analysis

We used nonmetric multidimensional scaling (NMDS) to investigate relationships between the distributions of ectomycorrhizal fungi and environmental factors. Analyses were executed in PC-ORD 5 (McCune and Grace 2002). We used the abundance-based version of the Sorensen index (Bray-Curtis) as a distance measure. Prior to ordination, we conducted outlier tests for OTUs. If the deviation exceeded 2 SD, an OTU was considered an outlier and was excluded from the analysis. We determined the dimensionality of the ordination and chose the lowest dimensionality that captured most of the variation. Both the ARCTIC and NAAT datasets were best described by 3-dimensional solutions that had instabilities below 0.00001. To avoid solutions involving local minima in stress values, each analysis was run 50 times using a random seed, 50 runs of real data, and 500 iterations with Monte Carlo randomizations to test for significance.

The environmental factors included in the analysis consisted of measurements taken in the field, as well as remotely sensed data from satellites. Categorical factors included ‘host plant’ and ‘location’. Numerical environmental factors are listed in Table 3.3. Before NMDS of the ARCTIC dataset, we square-root transformed the main matrix to down-weight the importance of abundant OTUs (McCune et al. 2002). In order to investigate whether ectomycorrhizal fungal communities differed according to location (23 locations), bioclimatic subzone (A-E), host plant (*S. arctica*, *D. integrifolia*), or soil moisture (mesic, dry), we used Non-Parametric Multiple Response Permutation Procedures (MRPP) in PC-ORD 5. MRPP determines whether defined groups vary statistically from those derived by random assembly. It measures within-group homogeneity, A (analogous to ‘effect size’), which can reach a maximum of 1, when all communities within a group are identical and have no overlap with other groups. In contrast, if within-group variation occurs by random chance, then $A = 0$. If $A > 0$, there is among-group variation that is not completely explained by within-group variation, and we can calculate the probability that group differences are due to chance (McCune et al. 2002). We report the chance-corrected effect size. Because we used MRPP in conjunction with NMDS ordination, we chose the same distance measure Sorensen (Bray Curtis), as recommended by McCune et al. (2002).

To illustrate the changes of EMF community composition across the bioclimatic subzones, we applied a two-way cluster analysis in PC-ORD 5. The two-way cluster dendrograms present OTUs of selected genera that occurred more than once in the ARCTIC across the five bioclimatic subzones.

3.3.10. Diversity Analyses

Due to the different number of samples taken at various sampling sites, we randomly subsampled three plants to estimate measures of species richness. Species richness (Mao Tau, Chao2) of the fungal communities were calculated in EstimateS 8.0 (Colwell 2006). Rarefaction curves were also computed for each bioclimatic subzone using EstimateS 8.0. The number of OTUs was determined by randomly subsampling the observed OTUs 50 times. To examine the relationship between the observed and estimated OTU richness (Mao Tau, Chao2) along the latitudinal gradient, we performed a linear regression analysis in R (R-Development Team 2008).

3.4. Results

3.4.1. EMF diversity in the Arctic

To identify EMF diversity in the Arctic, we sampled 326 plants (198 *S. arctica* and 128 *D. integrifolia*) across the North American Arctic. Following morphotyping of root tips, extraction of DNA and RFLP analysis we obtained 644 fungal, non-chimeric sequences. Clustering these sequences across the entire ITS region resulted in 242 OTUs with 203 OTUs belonging to the Basidiomycota and 39 to the Ascomycota. When considering the abundance of OTUs (based on the presence/absence of each OTU across all plants), we found that the 203 Basidiomycete OTUs occurred 751 times, whereas the 39 Ascomycete OTUs occurred 91 times (Table S3.1, Supporting information). The rank abundance curve shows a log-normal distribution, with a few abundant OTUs and many rare species.

In fact, 111 OTUs (46%) were singletons (Appendix 3.1). We identified all OTUs in 37 separate maximum likelihood phylograms (Fig. S3.2.1-37, Supporting information).

Overall biodiversity was dominated by only a few fungal families and orders, which included *Thelephoraceae*, *Inocybaceae*, *Sebacinaceae*, *Cortinariaceae* and *Pyronemataceae* (Fig.3.2). Twenty nine OTUs (12%) were identified to the species level, 194 OTUs (80%) to the genus level and 19 OTUs (8%) could be identified to family or order. Fifty eight OTUs (24% of all OTUs) showed < 95% similarity to any publicly available sequences on GenBank (as of December 2010). Of these, 44 OTUs were Basidiomycota and 14 were Ascomycota. The genus *Inocybe* had the most (21 OTUs) sequences without matching reference sequences in GenBank.

The ten most abundant OTUs (number of OTUs in parentheses) belonged to the genera *Cortinarius* (5), *Hebeloma* (1), *Tomentella* (1), *Sebacina* (1), *Inocybe* (1) and *Laccaria* (1). Their phylogenetic relationships to the closest matches in and outside the Arctic are shown in Supporting information 3.2 (Fig. 1, 7, 9, 12, 13, 14, 21, 23). They were found in 7-16 of the 23 sampling sites. The geographic distributions of four of the ten most abundant OTUs are shown in Figure 3.3. BLAST-searches in GenBank revealed that all of the most abundant taxa had close matches outside of the Arctic, and they also occurred at other Arctic locations. Additionally, these OTUs were found in a variety of habitats from the Arctic to the Mediterranean and associated with a range of host plants, including angio- and gymnosperm tree species, orchids, a sedge and a perennial forb (Table 3.2). Overall, 73% of all OTUs (176) had matches in GenBank with >97%

similarity in the ITS region that originated in other regions of the Arctic and beyond the Arctic.

The six OTUs found on *S. arctica* in subzone A included *Tomentella* sp. 27, *T. stuposa* 1, *T. aff. terrestris* (OTU 22, 25, 188), *Sebacina* sp. 23 (OTU 21), *Cortinarius* sp. 15 (OTU 39) and *Laccaria* sp. (OTU 20). These OTUs occurred from one subzone to all five subzones and also on *D. integrifolia*. Closest matches ($\geq 97\%$ similarity) in GenBank showed that most of them occurred outside the Arctic on a variety of host plants. In fact, OTUs 39 and 20 were among the ten most abundant OTUs throughout our study area, and OTU 39 in particular had a wide geographic distribution outside the Arctic and occurred on a wide array of host plants (Table 3.2). These results indicate that neither ectomycorrhizal fungi in High or Low Arctic are limited to the Arctic, to arctic/alpine (tundra) habitats, nor restricted to a particular host (*Salix*, *Dryas*).

3.4.2. Patterns of EMF communities

Species richness along the latitudinal gradient. -Linear regression analysis of observed and estimated species richness (Mau Tao, Chao2) showed a non-significant decrease in EMF species richness (OTUs) associated with *S. arctica* and *D. integrifolia* with increasing latitude (Fig. 3.4).

Community structure across the bioclimatic subzones. -NMDS ordination of the NAAT dataset revealed changes in EMF community structure across the bioclimatic subzones (Fig. 5.5A). Additionally, two way cluster analysis supports the observation that neighboring subzones are most similar. Only OTU 39 (*Cortinarius* sp. 15) occurred

across all bioclimatic subzones while many OTUs (belonging to the genera *Tomentella*, *Thelephora*, *Cortinarius*, *Inocybe*, *Entoloma*, *Russula*, *Lactarius*, *Clavulina*, *Sebacina*, and *Cenococcum*) were just observed in subzone C. However, the indicator species test did not show any species that were uniquely associated with particular bioclimatic subzones. This result might be due to low sampling intensity in our study and therefore lack of statistical power.

Despite the lack of indicator species, MRPP analysis showed a significant effect of ‘bioclimatic subzone’ on EMF community structure along the NAAT ($A = 0.037$, $P < 0.001$) and across the ARCTIC dataset ($A = 0.007$, $P < 0.001$). To refine these differences in community structures, we considered the distribution of OTUs from the most abundant genera, across the subzones B-E (excluding subzone A, because it was represented by only three plants of *S. arctica*). Using MRPP, we found that OTUs belonging to *Tomentella* were significantly different among all four subzones ($A = 0.032$, $P < 0.001$, Appendix 3.2A). *Sebacina* had no differences in the Low Arctic subzones (D-E), while differences occurred between the High Arctic subzones (B-C) and between the High and Low Arctic subzones ($A = 0.026$, $P < 0.001$) (Appendix 3.2B). *Inocybe* showed mixed results, with no difference between the High Arctic subzones (B-C), and also no differences between the Low Arctic subzone E with the High Arctic subzones B and C. Nevertheless, *Inocybe* differed among subzones in the Low Arctic ($A = 0.015$, $P < 0.001$, Appendix 3.2C). In contrast, OTUs of *Cortinarius* did not differ among the subzones ($A = 0.001$, $P < 0.556$, Appendix 3.2D). In summary, across the bioclimatic subzones, we

find opposing distribution patterns between *Tomentella* and *Cortinarius* (different community structures among all subzones for *Tomentella* and none for *Cortinarius*) and between *Sebacina* and *Inocybe* (different community structures between the High and Low Arctic subzones for *Sebacina* and none for *Inocybe*).

3.4.3. Drivers of EMF community structure

Host plant identity. -Overall 176 OTUs were associated with *Salix arctica* and 154 OTUs with *Dryas integrifolia*. A third of the observed EMF species were found only once (singletons). From all OTUs, only 81 OTUs were recorded more than 3 times and 61 OTUs more than 4 times. There were 87 OTUs shared between the two host plants, which included the following fungal families (number of OTUs in parentheses): *Thelephoraceae* (28), *Inocybaceae* (22), *Cortinariaceae* (12), *Sebacinaceae* (10), *Pyronemataceae* (4), *Tuberaceae* (3), *Russulaceae* (2), *Clavulinaceae* (2), *Tricholomataceae* (1), *Sordariaceae* (1), *Entolomataceae* (1), *mitosporic Dothideomycetes* (1). MRPP showed that plant host identity had only a marginal effect on fungal communities in both the ARCTIC ($A = 0.003975$, $P < 0.001$) and the NAAT dataset ($A = 0.0049$, $P = 0.06$). Low host preference is also demonstrated in the ordination space, where EMF communities associated with the two host plants along the NAAT always clustered by sampling site, rather than by host plant (Fig. 3.5A).

Environmental drivers. -Three-dimensional NMDS ordinations were used to describe variation in EMF community structure across the Arctic. The ARCTIC dataset consisted of 239 OTUs spanning 38 sites (23 locations*2 host plants), after three outliers were

removed. Five sites were only represented by one host plant species. The three axes accounted for 65.7% of the variation in community composition. Axis 1 was correlated with temperature-related factors such as subzone and Summer Warmth Index (SWI), which is the sum of the mean monthly temperatures above 0°C (Walker et al. 2011). Axes 2 and 3 correlated with SWI, geographic location ('longitude'), and geology-related factors ('landscape age', 'pH') (Fig. 3.5B).

The NAAT dataset consisted of 128 OTUs from 12 sites (6 locations * 2 host plants), after removing seven outliers. The environmental matrix included 35 numerical (Table 3.3) and two categorical factors. A three dimensional NMDS ordination was used to describe variation in fungal community structure along the NAAT (Fig. 3.5A). The three axes accounted for 70.1% of the variation in the dataset. Axis 1 correlated to soil moisture, while axis 2 corresponded with vegetation factors, including an increase in vascular plant species richness and plant productivity. Other contributors to axis 2 included soil carbon content, and summer and winter precipitation to the south along the NAAT. Axis 3 was best interpreted as a complex of factors relating to geographic location, soil chemistry and air and soil temperature (Table 3.3). MRPP analysis of dry and mesic sites across five locations (MI, BI2, EL, DI, BFS) showed that EMF communities of *S. arctica* and *D. integrifolia* were weakly, but significantly ($A = 0.005$, $P = 0.001$) affected by these two categories of soil moisture. The NMDS ordinations of both datasets (ARCTIC, NAAT) showed that EMF communities along the bioclimatic

gradient in the Arctic shift along the bioclimatic gradient, mainly in concert with a complex set of temperature and soil factors associated with each geographic location.

3.5. Discussion

3.5.1. EMF diversity in the Arctic

We found diverse EMF communities associated with *S. arctica* and *D. integrifolia* in the North American Arctic. The observed 242 OTUs on two hosts exceed the number of previously reported sporocarps and morphologically described mycorrhizas (approximately 150) in the Arctic across all regions and host species. With 176 OTUs on *S. arctica* and 154 OTUs on *D. integrifolia*, we found a species (OTU) richness similar to that previously observed on *D. octopetala* (137 OTUs) along a latitudinal gradient from Alpine Norway to Svalbard (subzones B and C) (Bjorbaekmo *et al.* 2010). As in most microbial studies, our rarefaction curves did not approach an asymptote and 46% of the OTUs were singletons. This result indicates that we did not exhaust EMF species richness and that the actual diversity is certainly greater than observed in our study.

The major genera found in our study (*Thelephora*, *Tomentella*, *Sebacina*, *Inocybe*, *Cortinarius*, *Russula*, *Hebeloma*, *Laccaria*, *Clavulina*) are characteristic of arctic and alpine environments (Mühlmann and Peintner 2008, Ryberg *et al.* 2009, Bjorbaekmo *et al.* 2010, Deslippe *et al.* 2011, Geml *et al.* 2012b). While the *Thelephoraceae* have a worldwide distribution (Kõljalg 2000), they seem especially species-rich in the Arctic, as they comprised nearly a third of all observed species in our study. Moreover, the

proportional increase of melanized fungi (e.g., *Thelephoraceae*) from warm to cold subzones in our study corresponds with the increase of thelephoralean fungi from central Norway to Svalbard (Bjorbaekmo et al. 2010), demonstrating the fitness of this fungal group to the arctic environment.

In contrast to other studies in the Arctic (Hryniewicz et al. 2009, Bjorbaekmo et al. 2010, Fujiyoshi et al. 2011), *Cenococcum geophilum* and species in the genera *Russula* and *Lactarius* were less abundant with latitude along the bioclimatic gradient. We found *Cenococcum geophilum* only in subzone C; it is often associated with high carbon content in the soil, which in fact was highest in subzone C in our study. The genera *Russula* and *Lactarius* are abundant and species-rich EMF in the Low Arctic and the Boreal Forest (Geml et al. 2009, Geml et al. 2010b, Taylor et al. 2010, Deslippe et al. 2011), while they were less abundant and species-rich in our higher latitude study. This suggests, that these genera may prefer EMF host plants of the Low Arctic and Boreal and/or may be less adapted to the climatic conditions in the colder subzones of the Arctic.

Notably, members of the Russulaceae, Thelephoraceae, and *Cenococcum* are ubiquitous and dominant components of ECM communities in temperate forests throughout the world (e.g., Gardes and Bruns 1996, Jonsson et al. 1999, Tedersoo et al. 2006). Species of *Inocybe* and *Cortinarius* do occur in temperate regions, but do not appear to be ubiquitous dominants in warm-temperate regions, while they become increasingly common in the boreal forests of Europe and North America (Jonsson et al. 1999). These patterns suggest that, at the genus level, *Tomentella-Thelephora* are climate

generalists, while *Inocybe* and *Cortinarius* may be more specialized to cold climates and the Russulaceae to warmer climates, though extending to boreal regions.

3.5.2. Large Scale EMF community patterns in the Arctic

Wide distribution of arctic EMF. The majority (73%) of OTUs in our study matched GenBank sequences recovered from other regions both within and beyond the Arctic. Thus, our study supports patterns from sporocarp records that have claimed a wide distribution of arctic fungi across arctic and alpine habitats spanning all continents (Ronikier and Ronikier 2010). It also agrees with molecular studies showing a pan-arctic distribution of *Lichenomphalia* (Geml et al. 2012a) and another study, which showed that 73.3% of ectomycorrhizal phylotypes observed on the isolated Arctic archipelago of Svalbard also occurred outside of Svalbard (Geml et al. 2012b).

Analysis of the ten most abundant OTUs showed that they were not restricted to arctic and alpine regions. Instead they also occurred in boreal, temperate and Mediterranean regions, in a wide variety of habitats and with a wide range of host-plants (Table 3.2). This suggests either that these fungal species have very wide ecological amplitudes and niches or that there has been recent population differentiation, which is not reflected among our ITS groupings at 97% similarity.

The distribution patterns we observed suggest the contribution of both terrestrial and transoceanic dispersal over long distances across multiple scales to the assembly of arctic communities. While individual long-distance dispersal events (especially transoceanic) are considered rare, it has occasionally been hypothesized for hypo- and epigeous fungi at

lower latitudes (e.g., Halling et al. 2008, Hosaka et al. 2008). Sufficient dispersal over large distances to homogenize populations has been demonstrated to occur in some lichens (Buschbom 2007, Geml et al. 2010a) and plants occurring in the Arctic (Tremblay and Schoen 1999, Alsos et al. 2007). Vectors for such dispersal of plants over wide areas of the Arctic include wind, snow, birds, driftwood, sea ice and mammals (reviewed in Alsos et al. 2007). All of these vectors could likely have been used by fungi as well, though there is limited direct evidence concerning fungal dispersal at high latitudes (see discussion in Robinson 2001). While dispersal over long distances must have occurred in the ubiquitously distributed dominant OTUs found in our study, our data do not permit us to pinpoint the direction nor the timing of dispersal. Nevertheless, the wide distribution of EMF taxa observed in the North American Arctic and in Svalbard (Geml et al. 2012b) seem to contrast with EMF distribution patterns outside the Arctic, which often show strong phylogeographic patterns with particular taxa restricted to continents or sub-continental regions (e.g., Taylor et al. 2006, Geml et al. 2012b).

EMF species richness along latitudinal gradient. -Although a latitudinal gradient in diversity is one of the most fundamental and striking patterns observed for many macro-organisms (Hillebrand 2004), we found no evidence of a decline with latitude in EMF species richness associated with *S. arctica* and *D.integrifolia*. This contrasts with the generally observed latitudinal species decline for vascular plants and animals in the Arctic, but our findings agree with the only other published EMF study along a latitudinal gradient, which was carried out in the European Arctic (Bjorbaekmo et al. 2010). Our

study also agrees with findings for bacterial communities along latitudinal gradients in and outside the Arctic, which showed no decline in species richness (Neufeld and Mohn 2005, Fierer and Jackson 2006, Chu et al. 2010). Together, these studies suggest that species richness of prokaryotic and eukaryotic microbes may be influenced by similar factors, which appear to be distinct from those affecting macro-organisms. One confounding factor, however, is that our analysis, as well as that of Bjorbaekmo et al. (2010) are based on the number of EMF species recorded per plant rather than the number of species per habitat or unit area. Populations of *S. arctica* and *D. integrifolia* decrease in size and are increasingly fragmented with latitude. Hence, given the well-established relationships between species richness and area, it may be that EMF species richness of these two plants declines with latitude when aggregated at the level of habitat or unit area.

EMF community structure across bioclimatic subzones. -Despite the wide distribution of the most abundant OTUs in our study, EMF community structures varied amongst bioclimatic subzones. This agrees with findings from plant communities along the same gradient (Walker et al. 2011). As observed for plant communities, EMF communities from adjacent bioclimatic subzones showed the greatest similarity. Furthermore, despite the wide distribution of the most abundant OTUs, we found a nearly complete species turnover from the coldest to the warmest subzone (Appendix 3.2A-D). This result

indicates that environmental conditions, particularly temperature, contribute to shaping these communities.

3.5.3. Factors shaping EMF communities

Host plant identity. -The two host-plants, *S. arctica* and *D. integrifolia*, which grow intermingled in throughout most the Arctic (subzone B to D), shared more than a third of the observed EMF and did not harbor distinct fungal communities. The high species richness associated with *S. arctica* and *D. integrifolia* demonstrates that both hosts are broadly receptive to fungal symbionts. Our findings are in accordance with Ryberg et al. (2009, 2010), who observed species-rich EMF communities on *S. reticulata*, *S. herbacea*, *S. polaris* and *D. octopetala* in a subarctic alpine tundra, with no host preference. Also, EMF communities were less host-specific in alpine tundra than in a subalpine forest (Kernaghan and Harper 2001). The apparent lack of host specificity seems to be more pronounced in the arctic and alpine tundra than in boreal and temperate forests and Mediterranean woodlands, where host plants belonging to the same genera (Morris et al. 2008) or order (Ishida et al. 2007) can have an important influence on EMF fungal communities (Kernaghan et al. 2003, Ishida et al. 2007, Morris et al. 2008, Tedersoo et al. 2008, Taylor et al. 2010). The lack of a host effect on the EMF community suggests that environmental factors might be stronger drivers of this community than in some other systems.

Environmental drivers. -EMF communities were clearly correlated with environmental factors across regional scales and our ordinations show a strong distinction

between the EMF communities from Alaska versus Canada. This distinction is likely primarily due to the glaciation history of the area and its geology. The Canadian sites were glaciated during the last Glacial Maximum and are only 10,000 -16,000 years old. In contrast, the sites in Alaska, which are estimated to be 500,000 – 900,000 years old, were not glaciated during the Pleistocene and were part of the Beringian refugium (Raynolds and Walker 2009). Further, the sites in Canada and Alaska have a different geology resulting in different parent material and mineral contents of the soils. The sites in Canada are glacial tills and clays derived from sedimentary rocks and marine shale and are correlated with high Mg^{2+} contents. In contrast, the sites in Alaska are loess, which is derived from limestone, and are correlated with higher Ca^{2+} contents (reviewed in Walker et al. 2011).

EMF communities are often affected by complex site and soil factors, such as bedrock or parent material. Effects of bedrock chemistry, manifested in different pH and nutrient availability, have been observed in EMF communities associated with *S. arctica* on Ellesmere Island (Fujimura et al. 2008, Fujimura and Egger 2012). However, in our study, the differences in parent material affected the Mg^{2+} and Ca^{2+} content more than pH. All of our sites were non-acidic; the pH ranged from 6.4 to 7.8, representing non-acidic tundra. Not surprisingly, then, pH explained less of the variation of EMF community structure than in previous studies. Instead, pH becomes a key driver and strong predictor in sites with a wider range of pH values. Studies that observed such strong effect of pH on community structures include plants in the Arctic (Walker et al.

2011) and bacteria and fungi across different biomes (Erland and Taylor 2002, Fierer and Jackson 2006, Toljander et al. 2006, Chu et al. 2010, Taylor et al. 2010)

Temperature appears to be a key driver for EMF community structure along the bioclimatic gradient. This parallels findings for plant communities along the same gradient (Walker et al. 2011), as well as findings for soil fungi and bacteria along an environmental gradient in Antarctica (Yergeau et al. 2007). Experimental support for the effect of temperature on EMF community structure comes also from a long-term warming experiment in the Low Arctic tundra, where ectomycorrhizal communities of *Betula nana* that were dominated by *Russulaceae* (*Russula*, *Lactarius*) shifted to a community dominated by *Cortinarius* after nearly two decades of warming (Deslippe et al. 2011). While in our study *Russula* and *Lactarius* are less abundant in the High Arctic, EMF communities shifted from being *Tomentella* dominated in subzone B to *Cortinarius* dominated in subzone E. To the degree to which the bioclimatic gradient in our study is a suitable analog for climate change, this suggests that long-term climate warming of the Arctic will lead to changes in EMF communities, with an increased abundance of EMF from warmer subzones to colder subzones. The highest rates of temperature increase are observed in coastal areas of the North American Arctic (Bhatt et al. 2010) where we would expect EMF communities to change first.

Another key driver of EMF community composition is nitrogen (NO_3^- , NH_4^+) availability, as shown in temperate and boreal forests (e.g., Toljander et al. 2006, Kjoller et al. 2012) as well as in bacterial and fungal communities in Antarctic soils (Yergeau et

al. 2007). Nitrate and NH_4^+ increased from subzone A to D and were correlated with EMF community structure, extending the patterns seen in previous studies in the Arctic. In contrast to other studies, which have suggested the importance of soil moisture on EMF abundance and community structure (e.g., Erland and Taylor 2002), soil moisture under the mesic to dry conditions in our study was not correlated with EMF community structure.

In conclusion, this is the first study of EMF communities across all five bioclimatic subzones of the North American Arctic. Our study corroborates previous findings of diverse EMF communities associated with arctic dwarf shrubs. EMF communities associated with *S. arctica* and *D. integrifolia* are dominated by a few species-rich fungal families, such as *Thelephoraceae*, *Inocybaceae*, *Sebacinaceae*, *Cortinariaceae*, suggesting that these families are particularly adapted to arctic conditions. The wide distribution of most observed EMF in and beyond the Arctic, supports emerging evidence that widespread dispersal might be a common phenomenon for fungi in the Arctic. In contrast to macro-organisms, EMF species richness does not decrease with increasing latitude and harshness of the arctic climate, at least not at the scale of individual host plants, suggesting that EMF species richness is not governed by temperature. Nevertheless, as seen in plant communities, temperature is a key factor shaping EMF community structure across the bioclimatic subzones of the Arctic. EMF communities were not affected by host identity of *S. arctica* and *D. integrifolia*, but were correlated with environmental factors across a regional scale, encompassed by a complex of

glaciation history, geology, soil properties, plant productivity and climate. Our study provides important baseline data to assess climate change. Using the bioclimatic gradient as an analog for climate change, it indicates that long-term climate warming may affect EMF community structures in the Arctic by causing shifts of some EMF taxa from the Low to the High Arctic

3.6. Acknowledgements

The authors would like to thank Frédéric Teulières and Sophie Manzi for performing molecular typing of TNW99 samples, Hilmar Maier and Shalane Frost for assistance with the maps, Edie Barbour for helping with the graphical design, Anne Sudkamp and Donie Bret-Harte for editing earlier manuscripts, Rebecca Brenton for assisting with morphotyping, Annemarie Timling, Tane Timling and Ronald Daanen for helping in the field, Vladimir Romanovsky for climate data, Alexia Kelley, Gary Michelson and Chien-Lu Ping for soil data, Naoki Takebayashi for assistance with R, Martha Raynolds for conceptual discussions, VECO Polar Resources and Aklak Air for logistical support, and Parks Canada and the Innuvialuit community for supporting our study on their lands. We thank the Swedish Polar Research Secretariat for the opportunity to take part in the Tundra Northwest Expedition of 1999. The authors also acknowledge the generous support of US National Science Foundation grants ARC-0425517 to DAW, ARC-0632332 to DLT, ARC-0531305 to JMW, the Swedish University of Agricultural Sciences financial support to A.D, the CNRS and Université Toulouse 3 Paul Sabatier financial support to M.G, and fellowships by EPSCoR to IT and a Global Change Student

Research grant to IT.

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3.8. Figures

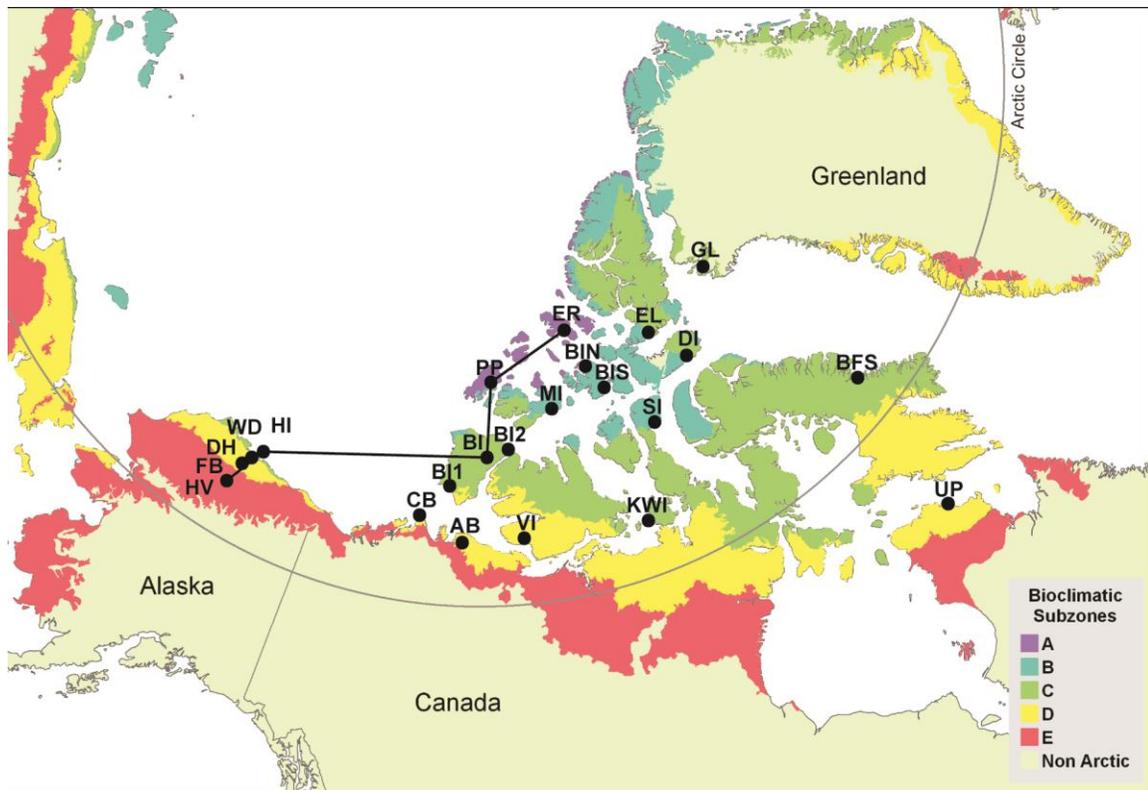


Figure 3.1 Sampling map showing bioclimatic subzones (CAVM Team, 2003). The sites connected by a line represent the North American Arctic Transect (NAAT). The remaining sites represent the Canadian Arctic Archipelago (CAA).

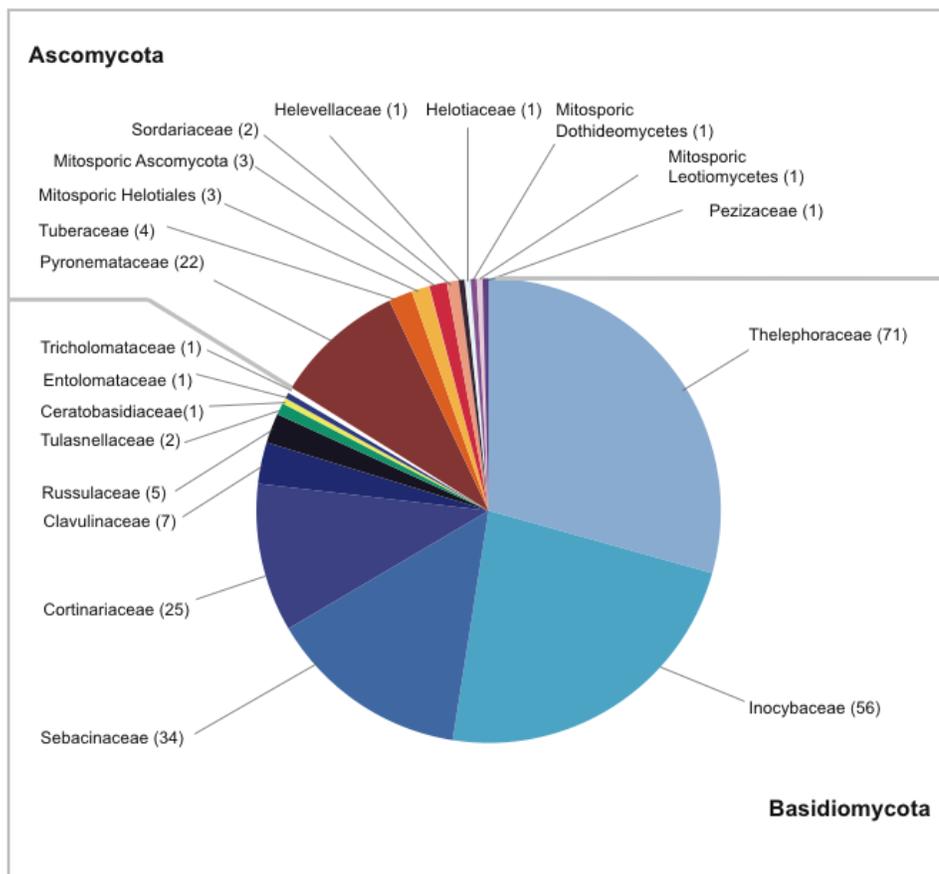


Figure 3.2 Species richness (number of OTUs in parentheses) within detected fungal families associated with *S. arctica* and *D. integrifolia* across the North American Arctic.

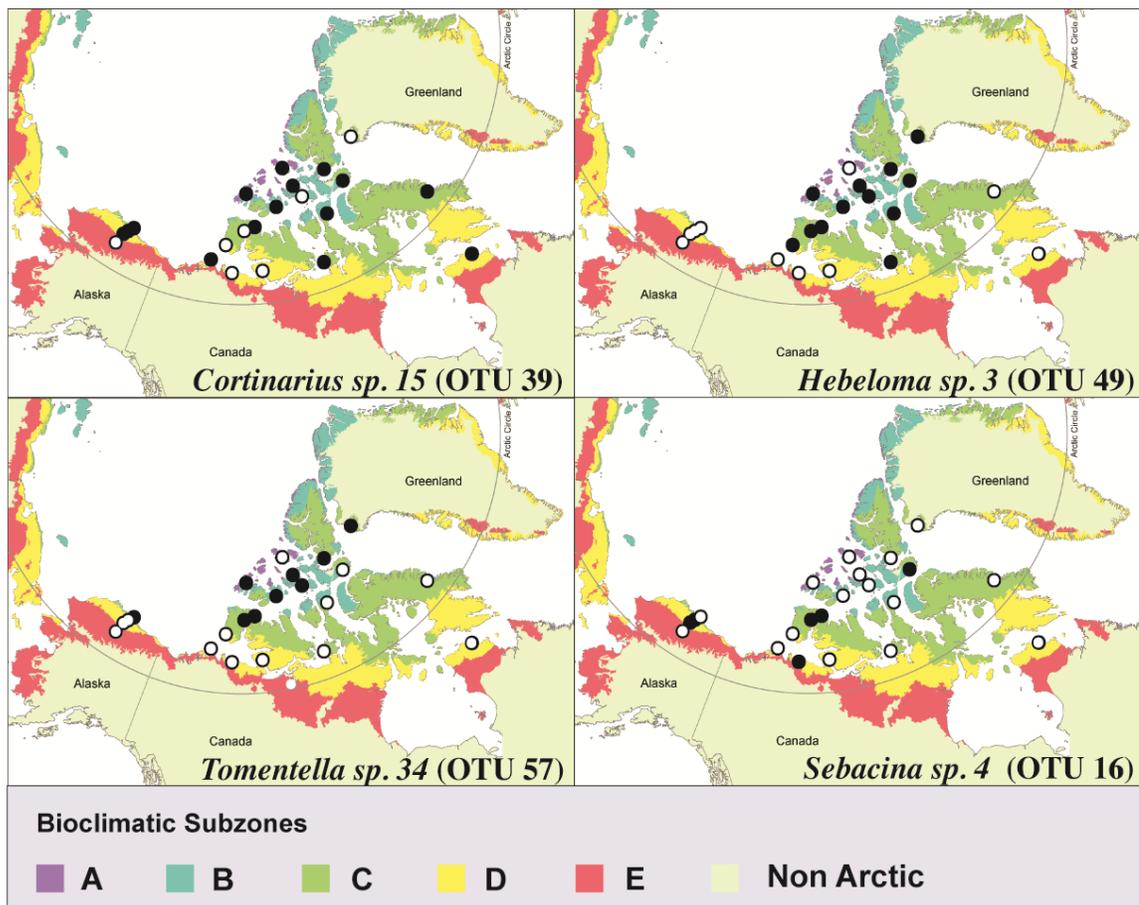


Figure 3.3 Distributions of four of the ten most abundant OTUs associated with *S. arctica* and *D. integrifolia* across the North American Arctic. Black dots indicate presence of an OTU, white dots indicate absence of an OTU in the samples studied.

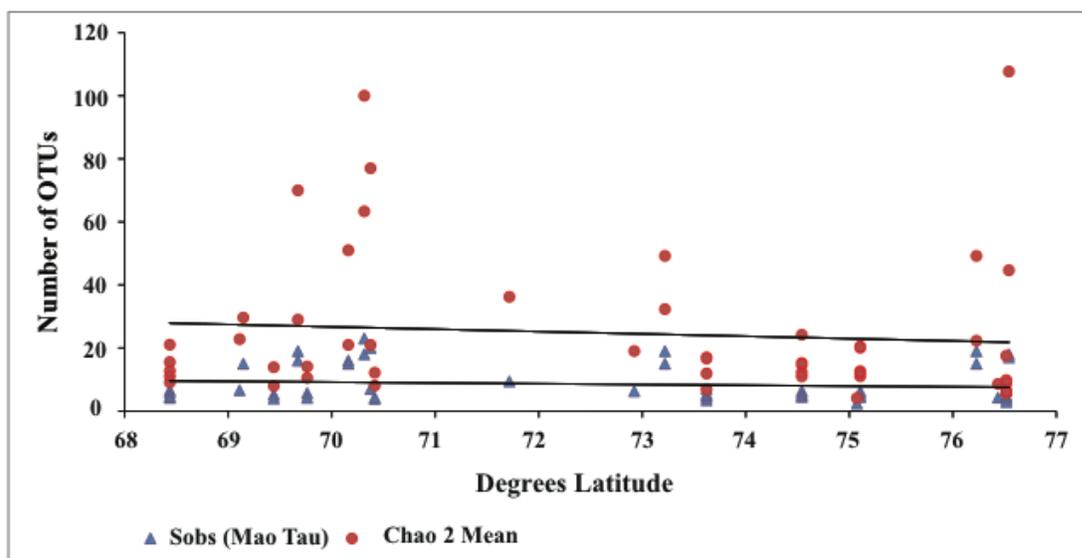


Figure 3.4 Linear regression of observed (Mao Tau) and estimated (Chao2) EMF species (OTU) richness associated with *Salix arctica* and *Dryas integrifolia* along the latitudinal gradient. Species richness was rarified to three root systems. EMF species richness associated with both host plants does not significantly decline with latitude ($P = 0.815$ (MaoTau), $P = 0.967$ (Chao2)). We excluded Ellef Ringnes (ER) and Ungava Peninsula (UP) due to low sampling size (≤ 3 *S. arctica* root systems).

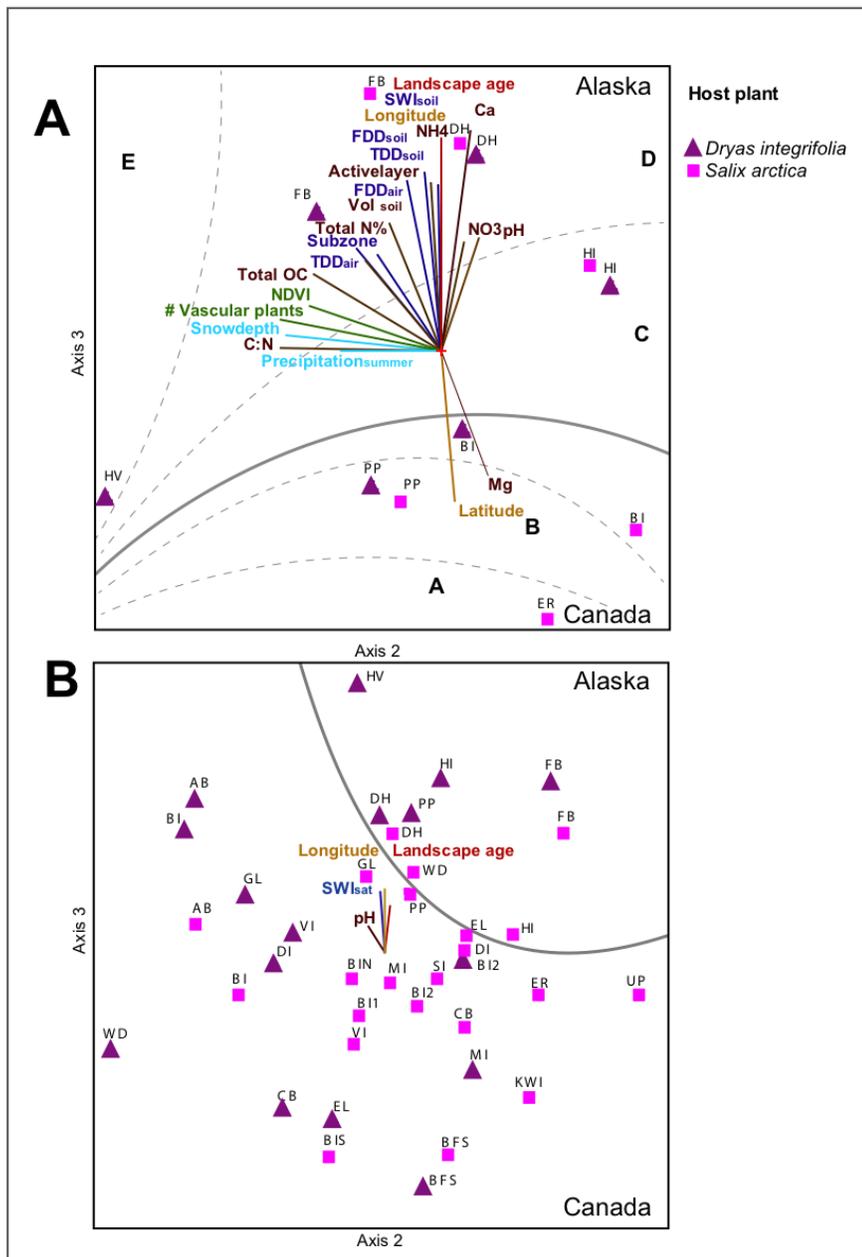


Figure 3.5 NMDS ordination of EMF communities associated with *S. arctica* and *D. integrifolia* for (A) the NAAT and (B) the ARCTIC datasets. The biplot diagram shows variables with $r^2 = 0.350$ (a) and $r^2 = 0.100$ (b). Samples were coded according to location of the study sites and host plant.

3.9. Tables

Table 3.1 Description and sampling scheme of study sites across the North American Arctic, including the Canadian Arctic Archipelago (CAA) and the North American Transect (NAAT)

Location	Data set	Code	Subzone	Latitude	Longitude	Mesic		Dry		Transect	
						SA†	DI§	SA	DI	SA	DI
Ellef Ringnes Island	NAAT	ER	A	78° 47' N	103° 33' W	3
Prince Patrick Island	NAAT	PP	B	76° 13' N	119° 17' W	3	3
Melville Island	CAA	MI	B	75° 06' N	107° 40' W	8	8	6	7	8	...
Bathurst Island South	CAA	BIS	B	75° 04' N	98° 34' W	8	...
Bathurst Island North	CAA	BIN	B	76° 25' N	97° 40' W	8	...
Banks Island	NAAT	BI	C	73° 13' N	119° 33' W	3	3
Thule (Greenland)	NAAT	GL	C	76° 32' N	68° 46' W	3	3
Howe Island	NAAT	HI	C	70° 18' N	147° 59' W	3	3
Westdock	NAAT	WD	C	70° 22' N	148° 32' W	3	3
Banks Island North	CAA	BI2	C	73° 37' N	115° 50' W	8	8	7	5	6	...
Ellesmere Island	CAA	EL	C	76° 28' N	86° 55' W	8	8	7	7	7	...
Devon Island	CAA	DI	C	74° 33' N	82° 48' W	7	7	8	6	7	...
Baffin Island South	CAA	BFS	C	68° 27' N	66° 50' W	8	8	8	8	7	...
Somerset Island	CAA	SI	C	72° 54' N	93° 30' W	6	...
King William Island	CAA	KWI	C	69° 06' N	98° 56' W	5	...
Deadhorse	NAAT	DH	D	70° 09' N	148° 27' W	8	8
Franklin Bluffs	NAAT	FB	D	69° 40' N	148° 43' W	8	8
Ungava Peninsula	CAA	UP	D	62° 22' N	73° 47' W	2	...
Victoria Island	CAA	VI	D	69° 23' N	114° 47' W	4	3
Banks Island South	CAA	BI1	D	71° 43' N	123° 43' W	6	...
Happy Valley	NAAT	HV	E	69° 08' N	148° 50' W	...	8
Albert Bay	CAA	AB	E	69° 46' N	122° 05' W	5	5
Cape Bathurst	CAA	CB	E	70° 29' N	127° 50' W	10	9
Totals CAA		39	39	36	33	89	17
Totals NAAT		34	39
Totals ARCTIC		73	78	36	33	89	17

† *Salix arctica* (SA)

§ *Dryas integrifolia* (DI)

Table 3.2 The ten most abundant OTUs associated with *S. arctica* and *D. integrifolia* across the North American Arctic and their occurrence on different continents, climate zones and association with different host plants as reported in GenBank.

Name	OTU #	Climate zone					Trees	Perennials	Continents			GenBank Accession number
		Arctic/ Alpine	Boreal	Temperate	Mediterranean/ Tropic	Gymnosperm	Angiosperm	Kobresia sp.	Forbs	Orchids		
<i>Cortinarius sp.17</i>	6	x	...	x	x	NA, EU‡	EU326170, AY669673, FJ865564, GQ159898
<i>Inocybe tjallingiorum</i>	7	...	x	EU	FN550899
<i>Sebacina sp.4</i>	16	x	x	...	x	x	NA, EU	AF440643, EU018581, FJ196973, AY243530
<i>Cortinarius sp.10</i>	18	x	x	x	x	NA	EU498732, GQ159875, EU292263
<i>Laccaria sp.</i>	20	x	x	NA, EU	DQ149866, DQ149864, GU234157
<i>Cortinarius sp. 27</i>	27	x	x	x	...	x	x	x	x	...	NA, EU, Asia	FN393101, HM146805, DQ481838, AY748857, FJ378752, FJ626948, EF655689, AJ889970, AY641471, GU234029
<i>Cortinarius sp.3</i>	28	x	x	x	x	x	NA, EU	EU516955, EU292302, FN429009, AY669690, AY916076, EF644125, AY351630, FJ197948, DQ102686
<i>Cortinarius sp.15</i>	39	x	x	x	x	x	x	x	NA, EU, Asia	GU246988, AY083178, AY083180, DQ402509, FN428998, EU668287, AY748856, AF495455, AY640413, FN428989, EU554694, EF434114, FN428994, GU234113
<i>Hebeloma sp.3</i>	49	x	x	x	x	...	x	x	NA, EU, Asia	EU292525, FJ436320, AY312984, AY311519, AB211272, AY312980, EF635824, AJ875352, AY320395, AY948195
<i>Tomentella sp.34</i>	57	x	x	x	x	...	x	x	NA, EU, Asia	HM105527, AF272914, AJ893300, AJ421255, FJ897226, AF184747, DQ974779

¶ NA: North America; ‡ EU: Europe

Table 3.3 Correlation coefficients for variables in the NMDS Ordination along the NAAT and throughout the ARCTIC

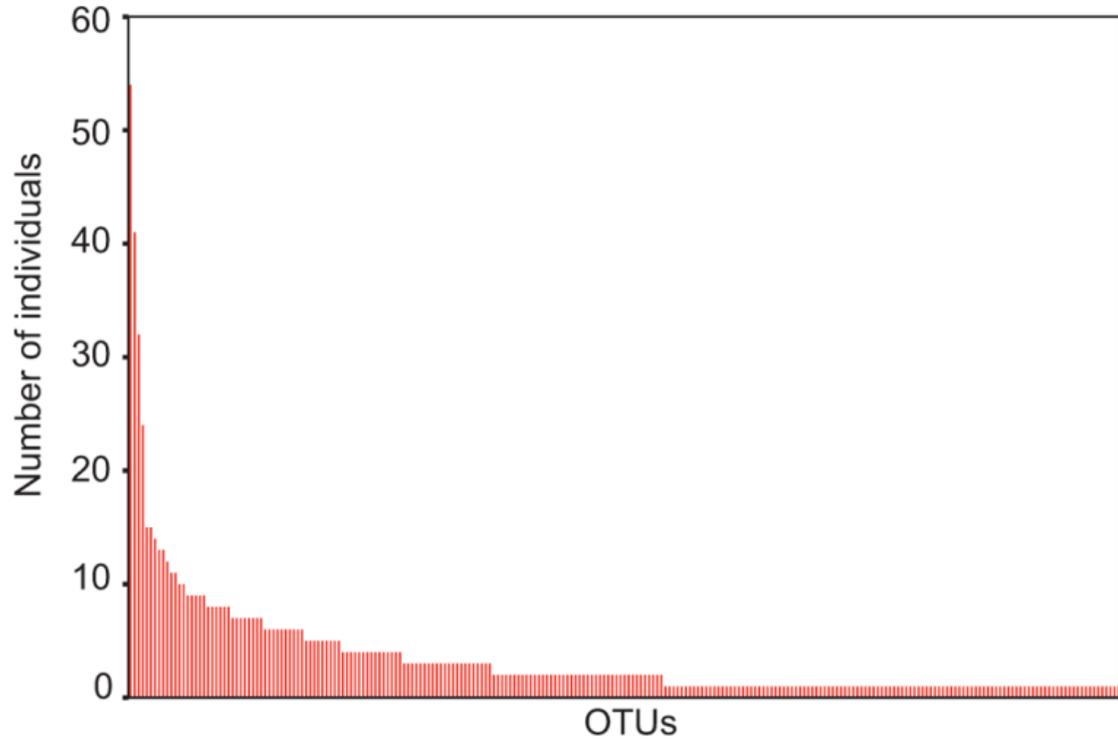
Group	... Environmental factor	NAAT NMDS, N=12			ARCTIC NMDS, N=38		
		1	2	3	1	2	3
Location	Latitude	0.001	0.052	0.575	0.010	0.024	0.000
	Longitude	0.017	0.065	0.680	0.012	0.006	0.256
Vegetation	NDVI (sat) §	0.030	0.458	0.170	0.001	0.014	0.096
	Number of Vascular plants	0.154	0.612	0.120
	Host Plant Cover	0.085	0.001	0.001
Precipitation	Precipitation summer (sat)	0.004	0.379	0.000	0.005	0.003	0.000
	Precipitation annual (sat)	0.001	0.182	0.180	0.018	0.020	0.000
	Snowdepth	0.026	0.617	0.038
Temperature	Subzone	0.029	0.243	0.365	0.087	0.038	0.060
	SWI (sat) †	0.001	0.152	0.219	0.022	0.018	0.227
	SWI air	0.001	0.216	0.249
	SWI soil/interboil	0.011	0.000	0.742
	TDDair ‡	0.003	0.291	0.346
	FDDair ¶	0.005	0.000	0.619
	TDDsoil	0.002	0.013	0.632
	FDDsoil	0.002	0.132	0.650
Soil	Landscape age (1000 years)	0.073	0.000	0.815	0.003	0.019	0.173
	Bulk density	0.104	0.262	0.148
	Gravimetric soil moisture	0.181	0.012	0.269
	Volumetric soil moisture	0.224	0.197	0.490
	Active layer depth	0.019	0.002	0.652
	Sand	0.059	0.122	0.041
	Silt	0.038	0.084	0.140
	Clay	0.025	0.050	0.024
	Mg	0.082	0.178	0.474
	P	0.005	0.218	0.090
	K	0.001	0.168	0.164
	Ca	0.000	0.109	0.838
	Na	0.068	0.181	0.184
	pH	0.126	0.141	0.43	0.008	0.063	0.100
	NH ₄ ⁺	0.134	0.039	0.643
	NO ₃ ⁻	0.053	0.086	0.417
Total N (%)	0.121	0.322	0.390	
Total OC (%)	0.065	0.490	0.294	
C:N	0.033	0.588	0.011	

§ Normalized Difference Vegetation Index (NDVI) is an index of vegetation greenness and is commonly used as indicator for biomass (Walker et al. 2011)

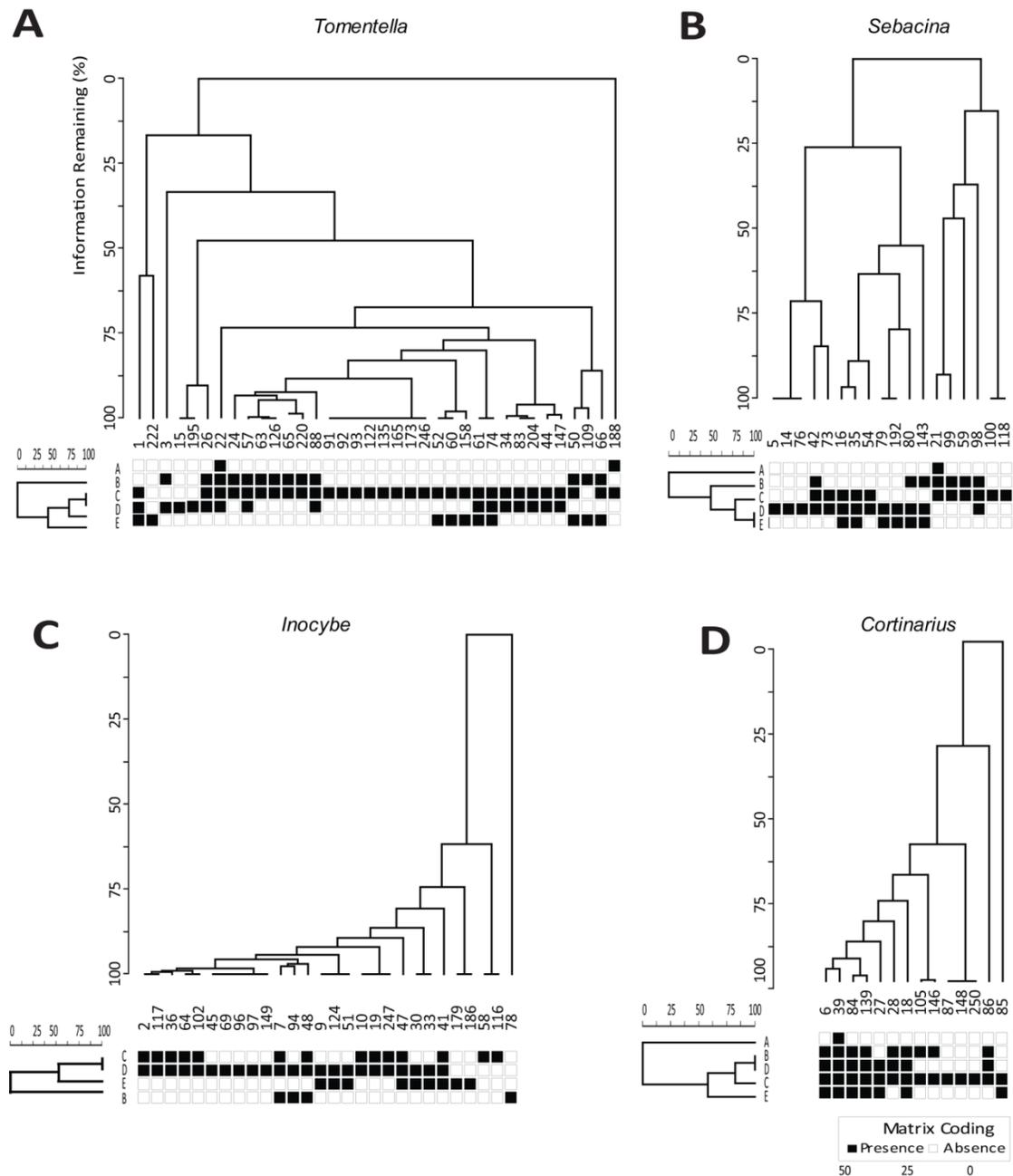
† Summer Warmth Index (SWI) is the sum of mean monthly temperature above 0°C (Walker et al. 2011)

‡ Thawing Degree Days (TDD) is the sum of mean daily temperatures > 0°C over a year (Walker et al. 2011)

¶ Freezing Degree Days (FDD) is the sum of mean daily temperature < 0°C over a year (Walker et al. 2011)

3.10. Appendices

Appendix 3.1 Rank Abundance Curve of OTUs associated with *D. integrifolia* and *S. arctica* in the North American Arctic



Appendix 3.2 Two way cluster analysis of all OTUs occurring more than once belonging to the genus (A) *Tomentella*, (B) *Sebacina*, (C) *Inocybe*, (D) *Cortinarius* across the five bioclimatic subzones of the Arctic. The horizontal dendrogram indicates the bioclimatic subzones of the Arctic (A-E). The vertical dendrogram represents the observed OTUs.

3.11. Supporting information

Table S3.1 Presence of OTUs associated with *D. integrifolia* and *S. arctica* across 23 sampling sites in the North American Arctic. The supplementary table contains presence data (based on presence/absence in a host plant) of all Operational Taxonomic Units (OTUs) identified at $\geq 97\%$ similarity in ITS region across 23 sampling sites in the North American Arctic. The rows represent the OTUs with their identified taxon name. The columns represent the locations in each subzone (A-E) and the hosts (SA: *Salix arctica*, DI: *Dryas integrifolia*). The last column contains the GenBank accession numbers of deposited sequences.

Figure S3.2.1-37 Maximum likelihood phylograms and their alignment files for ectomycorrhizal fungi associated with *Salix arctica* and *Dryas integrifolia* across the North American Arctic.

Maximum-likelihood phylograms (Fig.) of (1) Tomentella1, (2) Tomentella 2, (3) Pseudotomentella, (4) Inocybe 1, (5) Inocybe 2, (6) Inocybe 3, (7) Inocybe 4, (8) Inocybe 5, (9) Sebacina 1, (10) Sebacina 2, (11) Sebacina 3, (12) Cortinarius 1, (13) Cortinarius 2, (14) Cortinarius 3, (15) Cortinarius 4, (16) Clavulina, (17) Russula, (18) Tulasnella, (19) Ceratobasidium, (20) Entoloma, (21) Hebeloma, (22) Lactarius, (23) Laccaria, (24) Pyronemataceae, (25) Tuber, (26) Tuberales, (27) Sordariaceae, (28) Phialocephala, (29) Cenococcum, (30) Angulospora, (31) Hymenoscyphus, (32) Leohumicola, (33) Cadophora, (34) Leptodontidium, (35) Peziza, (36) Balsamia, (37) Tetracladium. taxa

inferred from the ITS rDNA datasets showing the phylogenetic spread of the OTUs observed in our study (highlighted by a grey box), including their presence across the bioclimatic subzones of the Arctic among representatives of congeneric taxa in GenBank. Branches with $\geq 90\%$ bootstrap support are highlighted. Each phylogram is accompanied by the sequence alignment files (nex) used for each phylogram.

CHAPTER 4

Rich and cold: Diversity, distribution and drivers of fungi in patterned-ground ecosystems of the North American Arctic.¹

4.1. Abstract

Fungi are thought to be one of the most diverse groups of organisms in the Arctic. Nevertheless, information on fungal biodiversity and distribution patterns in relation to environments across the Arctic are sparse. We examined the diversity and community structures of soil fungi at six locations across the five bioclimatic subzones (A-E) along the North American Arctic Transect (NAAT) through sequencing of ~9000 fungal ITS-LSU clones. Furthermore, we analyzed the effect of disturbance within patterned-ground features compared to the adjacent undisturbed areas at each location. Our key findings include: (1) We found 1834 Operational Taxonomic Units (OTUs) spanning eight phyla, 24 classes, 75 orders, 120 families, and 214 genera, distributed among all major functional groups of fungi. (2) Surprisingly, species richness did not decline with latitude. Instead, richness of mycorrhizal fungi decreased with latitude, but was offset by a parallel increase in lichen species richness.

¹ Timling I, Walker DA, Nusbaum C, Lennon NJ, Taylor DL. Rich and cold: Diversity, distribution and drivers of fungi in patterned-ground ecosystems of the North American Arctic. Prepared for submission in *Molecular Ecology* (3) The

most abundant OTUs were widely distributed in and beyond the Arctic. Yet fungal communities showed niche preferences in regard to regional climate and vegetation across the bioclimatic subzones and to local disturbance and vegetation of the patterned-ground features. (4) Fungal communities in subzone E were quite distinct from the other arctic subzones and the majority of OTUs matched fungi from the boreal forest. (5) At the local scale, fungal communities on relatively disturbed patterned-ground features (PGF) differed significantly compared to relatively stable areas in between patterned-ground features (bPGF), except in the coldest subzone A, where relatively small scale of the patterned-ground features (PGF) presented no clear differentiation between the features. Indicator species of PGFs included lichens and saprotrophic fungi, and bPGFs were characterized by ectomycorrhizal and pathogenic fungi. (6) Key drivers of fungal communities and guild composition along the NAAT included regional climate, pH and vegetation. At the local scale of patterned-ground features, fungal communities were correlated with vegetation composition and microclimate. With a warming climate, we would expect an enhanced colonization of patterned-ground features by vascular plants that would then affect fungal community structure not only at the species level, but also at the level of functional groups. In particular we would expect increases in fungi that are symbiotic with plants.

4.2. Introduction

Fungi are ubiquitous components of Arctic soils: they drive mineral and energy cycles, and influence the occurrence of other organisms as mutualists (mycorrhizal taxa, endophytes, lichens), decomposers and pathogens. Despite their ubiquity and importance for ecosystem function, we have only begun to uncover fungal biodiversity and distribution patterns in the Arctic (reviewed in Timling & Taylor 2012). Ongoing climate change has been especially rapid in the Arctic and long-term experiments show that climate change affects plant and fungal communities (Clemmensen & Michelsen 2006; Deslippe *et al.* 2011; Rinnan *et al.* 2007; Walker *et al.* 2006). However, in order to assess the potential effects of climate change on Arctic fungi, it is first necessary to assess the biodiversity and distribution of fungal groups at local, and regional scales of the Arctic.

Though cold, the Arctic encompasses great variation in climate and plant communities, which has led to the designation of five bioclimatic subzones (A-E). The subzones are defined by summer air temperature and plant growth forms. Subzone A is the coldest and is dominated by cushion forbs, mosses and lichens, while subzone E is the warmest and is characterized by low shrubs, tussock sedges and mosses. Vascular plant species diversity and cover increase from subzone A to E (Walker *et al.* 2005) (Table 4.1). Across the Arctic patterned-ground features (PGFs), which in this study include non-sorted circles and small non-sorted polygons are abundant (Washburn 1980, Walker *et al.* 2008). They are formed by permafrost processes and result in spatial heterogeneity of plant communities and soils at the sub-meter scale. The PGFs generally are composed

of a central area that is highly disturbed by frost heave and covered variously with bare ground, biological soil crusts, and plants adapted to the disturbance (Walker et al. 2008). Areas between the patterned-ground features (bPGFs) are more continuously vegetated and experience much less frost heave (Walker et al. 2008, Daanen 2012). The diameter of the PGFs range from 10-30 cm in subzone A to 3 meters in subzone C (Fig. 4.1b); they are nearly bare, with interspersed soil crusts, in subzones A-C, but are nearly entirely vegetated in subzone E (Raynolds *et al.* 2008; Walker *et al.* 2011). Environmental conditions within these features differ not only across the bioclimatic subzones, but also on a local scale, including variation in thermal regimes, thaw depth, nutrient content and soil moisture (Michaelson *et al.* 2008), which is reflected in differences in the associated plant communities (Kade *et al.* 2005; Vonlanthen *et al.* 2008; Walker *et al.* 2011).

While all major fungal phyla are present in arctic soils (Wallenstein *et al.* 2007), most molecular studies have focused on ectomycorrhizal fungi (EMF) (Bjorbaekmo *et al.* 2010; Blaaid *et al.* 2012; Fujimura & Egger 2012; Fujiyoshi *et al.* 2011; Geml *et al.* 2012b; Timling *et al.* 2012). Patterns that emerge from studies of EMF associated with abundant dwarf shrubs show species-rich fungal communities that are dominated by several fungal families such as *Thelephoraceae*, *Inocybaceae*, *Sebacinaceae*, *Cortinariaceae*, and *Pyronemataceae*. Several studies report no decline in EMF species richness with latitude (Bjorbaekmo *et al.* 2010; Timling *et al.* 2012). Furthermore, it appears that some arctic fungal species, including EMF (Geml *et al.* 2012b; Timling *et al.* 2012), lichens (Geml *et al.* 2012a) and saprotrophic fungi (Jurgens *et al.* 2009), have

wide distributions throughout and beyond the Arctic. Despite the wide distributions of dominant species, EMF community structure varied gradually along a bioclimatic gradient through the North American Arctic Transect (NAAT) (Timling *et al.* 2012), paralleling associated plant communities (Walker *et al.* 2011). Other than for mycorrhizal taxa, no information exists on the diversity and distribution patterns of total fungal communities in soil across the arctic bioclimatic gradient.

Early work in the Alaskan tundra included sporocarp surveys of patterned-ground features such as high and low centered ice wedge polygon tops, rims and troughs. Especially EMF species were documented on polygons tops and rims, while more decomposer occurred in the low center basins and troughs (Laursen 1975). Prior molecular studies of individual sites in cold-dominated biomes have shown that fungal communities are correlated with regional climate, soil chemistry and plant communities, which are, in turn associated with various landscape features (Fujimura & Egger 2012; Wallenstein *et al.* 2007; Yergeau *et al.* 2007). Similarly, EMF communities associated with *Dryas integrifolia* and *Salix arctica* along the NAAT were strongly correlated with regional environmental factors corresponding to geology and soil properties, glaciation history, climate and plant productivity, while host plant identity did not have a significant effect (Timling *et al.* 2012). Still, key drivers for total fungal communities in patterned-ground ecosystems across the full bioclimatic gradient are unknown for the Arctic.

In the present study, we use molecular methods to examine variation of soil fungi associated with zonal soils of small patterned-ground features along the NAAT (Walker

et al. 2008). A comprehensive environmental dataset of sites along the NAAT was used to investigate correlations between fungal communities and environmental factors (Walker *et al.* 2011).

Specifically, we addressed the following questions:

- (1) What taxa occur in soil fungal communities across the Arctic?
- (2) How do species richness and composition of soil fungal communities vary a) between patterned-ground features and b) across the latitudinal and bioclimatic gradient in the Arctic?
- (3) What are the primary environmental drivers of fungal community composition in the Arctic?

4.3. Material and Methods

4.3.1. Study areas and patterned-ground features

We sampled soils from six locations along the North American Arctic Transect (NAAT), arrayed to represent the five bioclimatic subzones (A through E) of the Arctic (CAVM-Team 2003) (Fig. 4.1a, Table 4.1). The zonal study sites selected represent typical local mesic vegetation that develops under the existing climate on fine-grained soils with no extreme moisture, slope, soil chemistry or disturbance (Razzhivin 1999). The patterned-ground features varied from 20-30 cm small non-sorted circles, mostly between *Eriophorum vaginatum* tussocks, in subzone E to 2-3 meters diameter large well developed non-sorted circles in subzone C and D to small, 20-30 cm, non-sorted

polygons in subzone A and B. Non-sorted circles are mainly a consequence of differential frost heave (Peterson and Krantz 2008), whereas non-sorted polygons are a result of fine scale thermal contraction and desiccation cracking (Washburn 1980). Frost heave in PGFs ranges from 1-9 cm and in bPGFs from 4-20 cm (Walker et al. 2008).

To broaden the environmental context of our study, we also sampled dry sites in subzones A and B and one wet site in subzone B (Table S4.1, Supporting information). All sites were extensively studied by members of the 'Biocomplexity Project' on the same or prior expeditions (Kade *et al.* 2005; Raynolds *et al.* 2008; Vonlanthen *et al.* 2008; Walker *et al.* 2008; Walker *et al.* 2011).

4.3.2. Sampling and processing

Soil samples were collected in July and August in 2005, 2006 and 2007. At each site, we haphazardly collected 20 soil cores (1.8 cm diameter) from each of five PGFs and five adjacent bPGFs. The sampling was in close proximity (within 15 meter radius from the edge of the grids) to 10x10 m grids, where the vegetation, active layer and snow depth were mapped in detail at each location (Raynolds *et al.* 2008). Climate data were obtained from automated climate stations near the grids (<http://permafrost.gi.alaska.edu>).

Each soil core included organic and mineral horizons from the upper 5-10 cm. The 20 soil cores from each feature (PGF, bPGF) were immediately pooled for each feature and stored in 50 ml Falcon tubes in liquid nitrogen and later at -80 °C. A total of 100 pooled soil samples (2000 cores) were collected along the NAAT (60 mesic + 30 dry +10 wet

sites). Prior to DNA extraction, all soils were lyophilized and ground at 4 °C using 0.8 cm steel beads on a Genie Vortex-2 (Scientific Industries).

4.3.3. Molecular Analysis

We followed the approach described in Taylor *et al.* (2013). In brief, we extracted genomic DNA of soil from each pooled sample, quantified extracted DNA and normalized it to 4 ng/μL. Then we amplified the region of the complete internal transcribed spacer (ITS) and partial large subunit (LSU) using a tagging approach as described in Taylor *et al.* (2008), removed small fragments, concentrated DNA and normalized the purified PCR products to 25ng/μL. The pooled fragments were then cloned, and ligation reactions were shipped frozen to the BROAD Institute of MIT and Harvard University, where automated transformation, plating, colony picking, Templiphi reactions and sequencing were performed. Twelve clone-libraries with at least 1536 clones per library were sequenced.

4.3.4. Bioinformatics

4.3.4.1. *Sequence Clean up*

In order to retain only high quality fungal sequences, we followed the approach outlined in Taylor and Houston (Taylor *et al.* 2013; Taylor & Huson 2011). In brief, dirty ends of sequence-reads were trimmed and vector sequences were removed. The two sequences per clone were assembled then assigned to a sample by their tag (Taylor *et al.* 2008) after which orientations were corrected, low quality bases were changed to Ns, and sequences

with excess Ns removed using a set of Perl scripts. The sequences passing these quality control steps were submitted to OTUpipeline (Edgar *et al.* 2011), which uses Uclust and Uchime (Edgar 2011) to cluster sequences into Operational Taxonomic Units (OTUs) and detect chimeric sequences. We clustered sequences at a sequence similarity of 97% across the ITS and partial LSU. Chimeric sequences with a score >1.0 were removed. To identify and eliminate non-fungal sequences, we used MEGABLAST (Altschul *et al.* 1997) to search for matches for each OTU in NCBI and assigned them to taxonomic groups at the kingdom level using MEGAN 4.62.7 (Huson *et al.* 2007). OTU sequences that were not assigned to fungi were aligned, followed by the extraction of the 5.8S region and the LSU, which were then added to a master alignment of known organisms. A phylogenetic tree was then constructed, where sequences were scored by clade. All non-fungal and inconclusive sequences were eliminated from the data set.

We chose a representative sequence for each OTU by using a BLAST search with the consensus sequence as query against the clone sequences contained within the OTU cluster, then chose a real sequence most similar (in most cases identical) to the consensus. In order to identify each OTU, we extracted the ITS1-5.8S-ITS2 region from the chosen representative sequence and submitted them again to BLAST search at NCBI, excluding environmental sequences. In cases where the ITS region was too short (<200 bp), we used the partial LSU region for this step. We used 90% coverage and a range of similarity values as cut-offs for various taxonomic levels. In the ITS1-5.8S-ITS2 region we used similarities of $\geq 97\%$ for the species level, a range from <97 to $>93\%$ for the genus, and a

range from <93% to >83% for family level. In the LSU region we applied $\geq 99\%$ for the species level, $\geq 97.5\%$ for the genus level and $\geq 95.5\%$ for the family level, based on comparisons of identity levels in linked ITS and LSU regions from an array of taxa (Taylor, unpublished data). A representative sequence from each OTU was deposited in GenBank under the Accession numbers KC965108 – KC966374 and KF296719 – KF297285.

4.3.4.2. *Diversity Analysis*

Fungal diversity in the Arctic was assessed across all sampled sites, including wet, mesic and dry sites. In contrast, analyses pertaining to the bioclimatic gradient were restricted to mesic sites, because we were not able to sample dry and wet sites at each location along the gradient. Species richness (Mao Tau, Chao1) of fungal communities was calculated after rarefying clone numbers across the five PGF and bPGF communities of each location to the minimum number of clones (258 clones) in any sample using Estimate S 8.0 (Colwell 2006). Rarefaction curves for each bioclimatic subzone were computed in Estimate S 8.0 by randomly sub-sampling the observed OTU abundances 50 times. To analyze how species richness of the various functional groups changed along the latitudinal gradient, we assigned the OTUs to the functional groups of lichens, mycorrhizal fungi (including ectomycorrhizal, ericoid, arbuscular and dark septate fungi), which were based on identification at the genus level, and a third group comprising saprotrophic, pathogenic and remaining fungi that were identified from family to phylum level (and therefore had undetermined ecologies). Due to unequal sample numbers across

the sites, we calculated the relative OTU richness for each functional group at each site. To determine the relationship of observed and estimated species richness of the rarified OTUs and the relative OTU-richness for each functional group with increasing latitude, we performed a linear regression in R (R-Development Team 2008).

4.3.4.3. *Ordination Analysis*

We used the non-metric multidimensional scaling (NMDS) ordination method in PC-ORD5 to examine the relationship between fungal communities and environmental factors along the bioclimatic gradient (McCune & Mefford 2006). The environmental matrix included two categorical variables (bioclimatic subzone and PGF versus bPGF) and 64 quantitative variables related to geographic location, soil, climate and vegetation (Table S4.7, Supporting information) (Walker et al. 2011). Laboratory processes and the elimination of clone sequences during sequence clean up resulted in different clone numbers per site. Therefore, we normalized the clone number by site totals and excluded OTUs that were found in fewer than two sites prior to analysis. We used the abundance-based Sorensen dissimilarity index (Bray Curtis) as the distance measure. A three-dimensional solution with instabilities below 0.00001 best described the data. Significance of stress values was tested through 500 iterations of Monte Carlo randomization.

To investigate whether fungal communities varied statistically from random assembly among the bioclimatic subzones, and between PGF and bPGF, we applied multiple response permutation procedures (MRPP) in PC-ORD, again using the Bray Curtis index,

as recommended by McCune *et al.* (2002). In order to determine how environment is related to the abundance of the dominant fungal families along the bioclimatic gradient, we calculated Spearman Rank correlations between the two strongest environmental factors identified by NMDS (Thawing Degree Days of the air (TDDair), pH) and the most abundant families. To investigate the effects of plant communities and environment (Kade *et al.* 2005; Vonlanthen *et al.* 2008; Walker *et al.* 2011) on the fungal communities, we performed partial Mantel tests based on 999 permutations in R.

4.3.4.4. *Distribution of OTUs*

To determine the minimum geographic distribution of the 50 most abundant OTUs, we recorded the location of the five top matches from GenBank. In addition, we conducted an indicator species analysis in PC-ORD to investigate whether there were individual OTUs that were specific to PGFs, bPGFs or particular bioclimatic subzones. Shared species analysis was conducted in EstimateS.

4.4. Results

4.4.1. Fungal diversity in the Arctic

We obtained a total of 7834 passing fungal clones, which were grouped into 1834 OTUs. Based on BLAST searches in GenBank, OTUs could be identified at varying levels of taxonomic precision. About 18.9% of the OTUs were assigned at the species level, 19.4% to a genus, 23.3% to family, 28.4% to order and 10% to the phylum level. The observed OTUs spanned eight phyla, 24 classes, 75 orders, 120 families, and 214 genera.

Ascomycota dominated these communities with 4850 clones (1211 OTUs), followed by Basidiomycota with 2706 clones (486 OTUs). The Ascomycota comprised 75 families, with *Verrucariaceae* being the most abundant (803 clones) and the most diverse (97 OTUs), followed by *Helotiaceae* (241 clones, 67 OTUs) and *Herpotrichiellaceae* (133, 45). The Basidiomycota contained 36 families with *Inocybaceae* being the most abundant (817 clones) and most diverse (86 OTUs), followed by *Thelephoraceae* (676, 82) and *Cortinariaceae* (349, 17). The Chytridiomycota included 5 families, with *Olpidiaceae* and *Spizellomycetaceae* being the most abundant, each with two clones representing two OTUs. Zygomycota, Glomeromycota, Blastocladiomycota, and Neocallimastigomycota were represented by one family each, while Cryptomycota were only identified at the phylum level (Table S4.2, Supporting information). Approximately a fifth of the fungi were lichens (18% of all clones and 22.5% of all OTUs). More than half of the OTUs (1003) were singletons (Table S4.2, Supporting information). The rank abundance curve across all sites had a log-normal distribution, with a few abundant and many rare OTUs (data not shown). The rarefaction curves from the mesic sites did not approach an asymptote (data not shown), indicating that we failed to saturate fungal diversity in our sequencing. The 50 most abundant species included fungi of various functional guilds, such as ectomycorrhizae, lichens, saprotrophs, pathogens, and endophytes (Fig. 4.2). BLAST searches in GenBank for matching sequences at the species level showed that the 50 most abundant OTUs are widely distributed not just across the Arctic, but also outside

the Arctic. Matching sequences of these OTUs included location from both hemispheres (Table S4.4, Supporting information).

4.4.2. Species richness along the latitudinal gradient

Linear regression analysis showed no relationship of observed (Mao Tau) and estimated (Chao1) fungal species (OTU) richness with latitude (Fig. 4.3a). This was true both for combined data and separate analyses for bPGF and PGF communities. Observed mean species richness (Mao Tau) along the gradient was 109 ± 18 OTUs (mean \pm SD) in the PGFs and 112 ± 12 OTUs in bPGF, which was not significantly different ($P = 0.8477$). At the site level we found an average of 245 ± 30 OTUs (Mao Tau). Estimated species richness (Chao1) was about threefold higher than the Mao Tau for the PGF (291 ± 43 OTUs) and bPGFs (298 ± 76 OTUs) and twofold for the sites (561 ± 94 OTUs). Rare species, including singletons and doubletons, accounted for $75.4\% \pm 3.4\%$ (mean \pm SD) of the species richness at each site. When considering functional groups of fungi (lichens, mycorrhizal fungi, saprotrophs/pathogens/fungi with undetermined ecologies), we found that the relative OTU richness of lichens at a site increased significantly with latitude. The opposite was found for mycorrhizal fungi, where relative OTU richness at the site declined with latitude (Fig. 4.3b). This pattern was even stronger for lichens in PGFs ($P = 0.0417$, $r^2 = 0.6860$) and for mycorrhizal fungi in bPGFs ($P = 0.0212$, $r^2 = 0.7719$). The group of fungi containing saprotrophs, pathogens and fungi with undetermined ecologies showed no changes in relative OTU richness across sites with latitude (Fig. 4.3b).

4.4.3. Community structure across the bioclimatic subzones.

MRPP showed that overall fungal community structure varied across the bioclimatic subzones ($A = 0.0711$, $P < 0.001$). The effect of subzone was stronger when we separated PGF communities ($A = 0.075$, $P < 0.001$) from bPGF communities ($A = 0.102$, $P < 0.001$). The gradual changes in community structure are also apparent in the NMDS ordination biplot (Fig. 4.4) and were also seen in a cluster-dendrogram (not shown), where neighboring subzones were most similar to each other. Along the bioclimatic gradient, the relative abundance of Ascomycota clones and OTUs in PGF and bPGF communities decrease sharply from subzone E to D and then increased again toward subzone A (Fig. 4.5). The opposite pattern occurred for the Basidiomycota, which increased from subzone E to D and then decreased toward subzone A (Fig. 4.5).

Indicator species analysis showed that subzone A was best characterized by particular lichen species (e.g., *Verrucariaceae* spp), as well as several pathogenic and saprotrophic taxa (e.g., *Phaeosphaeria* sp., *Leptosphaeria* sp.) belonging to the Ascomycota and only a few ectomycorrhizal fungi (e.g., *Sistotrema biggsiae*, *S.* sp.) belonging to the Basidiomycota. From subzones D to B we observed a decreasing abundance of ectomycorrhizal fungi, including families such as *Inocybaceae*, *Thelephoraceae*, *Cortinariaceae*, and *Sebacinaceae* (Fig. 4.5). Several *Tomentella* and *Inocybe* spp. were indicators for subzone D or C, while *Cortinarius minutulus* and *C. rubricosus* were indicator species for subzone B (Table S4.5, Supporting information).

Unlike subzones A-D, in Subzone E, 84 % of the sequences found were unique to this subzone. Several Ascomycota families were much more abundant than in other subzones, including the *Helotiaceae*, *Davidiellaceae*, *Thelebolaceae* and *Hyaloscyphaceae*. Of the Basidiomycota, *Tricholomataceae* was most abundant in Subzone E, while other common families, such as *Thelephoraceae*, *Inocybaceae* and *Cortinariaceae* were less abundant (Fig. 4.5). To shed light on the distribution of the unique sequences, we BLAST searched them against a dataset from our boreal and arctic studies and found that 73 % of the sequences had top matches in the boreal forests and 27% across other arctic study sites. Finally, indicator species for subzone E were dominated by endophytes/DSE, e.g., *Rhizoscyphus* sp., *Phialophora* sp., *Humicola* sp., *Thelebolus* sp. and by saprotrophs, including *Clavaria falcata*, *Mycena* sp. *Cryptococcus terricola* and *Antarctomyces psychrotrophicus*. Only a few lichens (*Umbilicariaceae* sp.) and EMF (*Pseudotomentella tristis*) appeared to preferentially occur in this subzone.

Overall, the findings of the indicator species analysis across all subzones were largely the same as those seen in the 50 most abundant OTUs (Fig. 4.2), where EMF were most abundant and widely distributed in subzones D-B, and less so in subzones A and E. Lichens, saprotrophic and pathogenic fungi are more abundant in the High Arctic (subzones A-C), while the range of endophytic fungi extends from subzone A to E.

Finally, there was no single OTU that occurred in all five subzones. Instead five Ascomycota OTUs, namely *Tetracladium* sp. (OTU3), *Cladosporium cladosporioides* (OTU41), *Gyoeerfyella* sp. (OTU126), *Phialophora* sp. (OTU292), and *Mortierella* sp.

(OTU2465), were found across four of the five subzones. Blast searches in GenBank revealed that these fungi have wide geographic distributions; at least one of these OTUs was found on every continent (data not shown).

4.4.4. Fungal community structure within patterned-ground complexes

Across the bioclimatic gradient, PGFs were dominated by Ascomycota. While Basidiomycota had higher abundances in bPGFs, they were only dominant in subzones D and C (Fig. 4.4). Overall, fungal communities differed between PGF and bPGF when combined across all sites, although the effect size was quite small ($A = 0.0077$, $P < 0.001$). MRPP of PGF and bPGF fungal community structures at each subzone (5 subzones x 2 features (PGF/bPGF)) showed significant differences in subzone E to B ($A = 0.033$ to 0.096 , $P < 0.01$). In contrast, no significant differences occurred between PGF and bPGF fungal communities in subzone A ($A = 0.013$, $P > 0.20$). The separation between PGFs and bPGFs at each location across the gradient can be seen in the NMDS ordination (Fig. 4.4).

Despite the different communities of PGFs and bPGFs, taken as a whole, they shared nearly half of the OTUs (Morisita Horn Index 0.417) across the gradient. Shared species between PGF and bPGF in subzone E are mostly ascomycetous EMF, saprotrophs and endophytes. From subzone D to B shared species were dominated by Basidiomycota and EMF. In subzone A shared species are mostly Ascomycota, including lichens (data not shown). Indicator species for PGFs across the entire gradient included lichens (*Atla* sp.) and saprotrophic fungi (*Tetracladium* sp.) belonging to the Ascomycota. In contrast,

indicator species for the bPGFs included ectomycorrhizal (*Inocybe exilis*, *I. fulvipes*, *Cortinarius helobius*) and pathogenic fungi (*Phoma sp.*, *Venturia polygoni vivipari*) (Table S4.6, Supporting information). These patterns were also seen when considering only the 50 most abundant OTUs (Fig. 4.2).

4.4.5. Drivers of fungal community structure

The three axes of the NMDS accounted for 47.6 % of the variation. Axis 1 was mainly correlated with soil-related factors (e.g., pH, Ca^{2+} , percent silt), as well as summer precipitation, biomass of lichens and cover of bryophytes and erect dwarf shrubs. Axis 2, which is not shown here, was correlated with a different set of soil factors (sand, clay) and with cover of prostrate dwarf shrubs. Axis 3 represents a biogeographic gradient that was correlated mainly with temperature-related factors such as TDD_{air} (Thawing-Degree Days for the air temperature, which is the sum of mean daily temperatures $> 0\text{ }^{\circ}\text{C}$ over a year), SWI (Summer Warmth Index, which is the sum of mean monthly temperatures above $0\text{ }^{\circ}\text{C}$ over a year in thawing-degree months, $^{\circ}\text{C mo}$), FDD_{soil} and FDD_{air} (annual Freezing-Degree Day sum for the soil and air, which is the yearly sum of mean daily temperatures less than $0\text{ }^{\circ}\text{C}$), thaw depth and location (latitude, longitude). Other factors relating to vegetation included NDVI (Normalized Difference Vegetation Index, which is an index of vegetation greenness and is commonly used as indicator for biomass), number of shrub and lichen species as well as annual precipitation and landscape age corresponded with axis 3 (Fig. 4.4, Table S4.7, Supporting information).

Spearman Rank correlations between the strongest environmental factors (TDDair and pH) and the most abundant fungal families revealed that TDDair was significantly positively correlated ($P \leq 0.05$) with the abundance of *Inocybaceae* (0.48), while negative correlations were observed for *Mortierellaceae* (-0.45), *Herpotrichiellaceae* (-0.44) and *Leptosphaeriaceae* (-0.38). Increasing pH was significantly positively correlated with *Thelephoraceae* (0.52), *Inocybaceae* (0.44) and *Verrucariaceae* (0.45), while it was negatively correlated with *Helotiaceae* (-0.58) (Table S4.8, Supporting information). A partial Mantel test showed that fungal communities were significantly correlated with plant communities when we factored out abiotic environment ($r = 0.468$, $P = 0.001$) and, *vice versa*, fungal communities were significantly correlated with abiotic environment when we factored out plant communities ($r = 0.3769$, $P = 0.007$).

4.5. Discussion

4.5.1. Fungal diversity in arctic soils (General description)

In terms of numbers, the 1834 OTUs of our study, which encompass eight of the ten known fungal phyla, equate to about half of the 4350 fungal species currently described from the entire Arctic (Dahlberg et al. 2013). Considering that more than half of our OTUs (1003) were singletons and that the rarefaction curves did not reach an asymptote, we can expect a much higher fungal diversity along the NAAT than has been recorded to date. The dominance of Ascomycota (66% of the OTUs) and Basidiomycota (26.5% of OTUs) reflects currently known fungal patterns from the Arctic (Dahlberg *et al.* 2013)

and paleoecological observations from 16,000-32,000 year old Siberian Permafrost, where Ascomycota comprised 74.2% and Basidiomycota 10.3% of the OTUs (Bellemain *et al.* 2013). This dominance changes in the boreal forest where Ascomycota comprise 55% of the OTUs and Basidiomycota 39.6% (Taylor *et al.* 2013) and becomes the opposite in temperate forests, where Basidiomycota dominate (54% of OTUs), followed by Ascomycota (36.1% of OTUs) (Wubet *et al.* 2012). Nevertheless, similar to the Arctic, Ascomycota dominate soils of semiarid grasslands (Porrás-Alfaro *et al.* 2011) and the Alpine tundra (Lentendu *et al.* 2011; Schadt *et al.* 2003), suggesting that these fungi are well adapted to dry environments with fluctuating temperatures. The dominance of Ascomycota in the Arctic can be attributed to the high diversity of lichen and saprotrophic as well as parasitic microfungi (Dahlberg *et al.* 2013). Basidiomycetes are often associated with the presence of EMF and their hostplants (Orgiazzi *et al.* 2013), or large woody debris (Norden *et al.* 2004). Many melanized fungi occurred across the Arctic, such as *Tomentella*, *Cenococcum*, black yeasts (*Mrakia*, *Exophiala*) and DSE (*Phialophora*, *Phialocephala*). These fungi are often associated with extreme environments in regard to temperature, UV radiation and drought (Porrás-Alfaro *et al.* 2011). While the observed fungi represent all fungal guilds, consisting of mutualists (mycorrhizal taxa, endophytes, lichens), decomposers and pathogens, as in other biomes, there is a preponderance of certain fungal families and genera in the Arctic. The most abundant and diverse families and genera in our study are commonly reported from other regions of the Arctic (Deslippe *et al.* 2011; Geml *et al.* 2012b; Singh *et al.* 2012), the

Alpine (Mühlmann *et al.* 2008; Mühlmann & Peintner 2008) and, to some extent, from Antarctica (Bridge & Spooner 2012; Goncalves *et al.* 2012). Overall, our results support the idea that fungi are one of the most diverse groups of organisms in the Arctic (Dahlberg *et al.* 2013).

4.5.2. Large-scale fungal community patterns in the Arctic

4.5.2.1. *Wide distribution of arctic fungi*

The 50 most abundant fungal taxa were not limited to the Arctic and other cold habitats. Instead, individual OTUs had matches outside the Arctic, encompassing all continents. This wide distribution suggests that fungi in the Arctic might not display continental-scale endemism, which stands in contrast to fungi from lower latitudes, where some fungi do show continental and finer-scale geographic patterns (Geml *et al.* 2008; Taylor *et al.* 2006). Furthermore, the wide distributions suggest that arctic fungal communities are composed largely of generalists. This parallels observations from Antarctica, where most known fungi are considered to be members of cosmopolitan groups while a few taxa appear to be endemic (Bridge & Spooner 2012). Additional support for a wide distribution of fungi found in the Arctic emerges from a variety of studies. A previous study of EMF along the NAAT showed their wide distribution across the Arctic and beyond into boreal, temperate and Mediterranean regions, where they occurred in different habitats with a variety of host-plants, indicating their generalist nature (Timling *et al.* 2012). EMF found on Svalbard had matches at the species level in North America and Eurasia, which led the authors to suggest that EMF communities on Svalbard are a

result of multiple recent transoceanic colonization events by long-distance dispersal (Geml *et al.* 2012b). Lichens from the Arctic extend to boreal and temperate regions and often have a circumpolar and/or bipolar distribution (Printzen 2008). Similarly, certain saprotrophic taxa occur not only in the High Arctic, but also in Antarctica and Europe (Jurgens *et al.* 2009). Pathogenic snow molds are widely distributed in polar regions as well as in temperate climates (Tojo & Newsham 2012). Nevertheless, there are also fungi in the Arctic that are thought to have co-specified with their high latitude hosts. The pathogenic rust *Melampsora epitea* showed distinct morphological and phylogenetic characters when associated with its host *S. arctica* in the High Arctic in contrast to when it was associated with *S. bebbiana*, *S. nigra*, *S. interior* in temperate climates (Smith *et al.* 2004), supporting the idea that the Arctic is not an ‘evolutionary freezer’ (Brochmann & Brysting 2008). These emerging patterns suggest that there might be only a few fungal species that are endemic to the Arctic (Dahlberg *et al.* 2013).

Despite the emerging evidence that fungi in the Arctic have wide geographic distribution patterns in and outside the Arctic, these observations must be interpreted with caution, because we applied only one cutoff for fungal species (97% across the ITS region), which might be not applicable across all fungi and therefore may fail to differentiate certain fungal taxa. We expect that a narrower cut-off and/or use of more sensitive markers would detect further species groups in some taxa and thereby reveal more restricted distributions than are currently documented. To the degree that our ITS types correspond to species, the wide distribution patterns of the dominant fungi from the

Arctic suggests that these fungal taxa must be able to disperse across wide terrestrial and transoceanic distances, and that they are mainly generalists with wide ecological amplitudes, since they appear to be able to cope with wide environmental differences.

4.5.2.2. *Fungal species richness along the latitudinal gradient*

Fungal species richness did not decline with latitude. Our data suggest that the absence of a decline along the bioclimatic gradient is driven mainly by the opposing distribution patterns of two functional groups: lichens and mycorrhizal fungi. While relative lichen species richness increased significantly with latitude, mycorrhizal species richness decreased significantly. The increasing species richness of lichens with latitude coincides with aboveground observations along the NAAT (Kade *et al.* 2005; Vonlanthen *et al.* 2008). Nevertheless, this pattern is not identical to findings from other regions of the Arctic, where species richness of lichens usually declines with latitude (Chernov & Matveyeva 1997; Dahlberg *et al.* 2013). One possible explanation may be related to the decreasing area covered by larger plants (bPGFs) that cause shading and *vice versa* the increasing area with bare ground (PGFs), which is especially high in subzones A-C (Kade *et al.* 2005; Vonlanthen *et al.* 2008). Lichens would likely face less competition and therefore have a greater chance to establish in open areas (Dahlberg *et al.* 2013), which might lead to higher species richness. The species richness decline of mycorrhizal fungi warrants further investigation, using Next Generation Sequencing technologies in order to overcome under-sampling of these diverse communities. Furthermore, the ecology of many fungi is still undetermined.

4.5.2.3. *Community structures and environmental drivers*

Bioclimatic subzones: Fungal communities of neighboring subzones along the bioclimatic gradient of the NAAT were most similar. This corroborates not only our previous findings for root-tip EMF associated with *D. integrifolia* and *S. arctica* (Timling *et al.* 2012), but coincides with changes in zonal plant communities and abiotic environments along the same gradient (Walker *et al.* 2011). Climate, including temperature-related factors and annual precipitation, exerts the strongest effect at the regional scale across subzones. Climate was correlated with changes in plant productivity (NDVI) and variation in plant communities. Especially in warmer subzones, the number of shrub species increases while the number of lichen species decreases, which consequently drives the abundance and diversity of some ectomycorrhizal families (e.g., *Inocybaceae*) and lichen associated fungi. Partial Mantel tests showed that fungal community structures in our study were correlated with both plant communities and abiotic environmental variables. Such interactions were also observed along a maritime Antarctic latitudinal gradient, where fungal communities were correlated with the interaction of latitude-dependent environments and vegetation type (Yergeau *et al.* 2007). In contrast, Dennis *et al.* (2012) did not detect differences in fungal community composition along a latitudinal gradient in maritime and sub-Antarctic and suggests that temperature is not a key driver of these communities at a regional scale. This result may be due to the phylogenetic resolution of the molecular approach applied (T-RFLP of partial SSU), which distinguishes fungal taxa at approximately the ordinal level.

Temperature and precipitation were also among the factors most strongly correlated with EMF species richness along bioclimatic gradients from the tropics to the boreal regions. Interestingly EMF species richness was highest in temperate and boreal forests and showed a negative relation with precipitation and a unimodal relation with temperature (Tedersoo *et al.* 2012). However, EMF composition was mostly affected by the distribution of host plant families (Tedersoo *et al.* 2012). Correlations between fungal and plant community composition have also been observed in boreal (Taylor *et al.* 2010), alpine (Zinger *et al.* 2011), temperate (Wubet *et al.* 2012) and Mediterranean regions (Orgiazzi *et al.* 2013).

While climate and the resulting plant communities seem to be the major drivers in subzones A to D, pH and the resulting plant communities appear to be the key driver at the junction between subzone D and E, where we observed a major transition in fungal communities. The majority of fungal OTUs (84%) in subzone E were unique to this site/subzone. This transition coincides with the change from non-acidic (subzones A to D) to acidic (subzone E) tundra and a change from a sedge/prostrate dwarf-shrub/moss tundra to a tussock-sedge/erect dwarf-shrub/moss tundra; the latter includes an abundance of boreal plant species that are not common in zonal plant communities further north, such as *Betula nana*, *Ledum decumbens*, *Vaccinium vitis-idea*, *Polygonum bistorta*, and *Sphagnum* spp. as well as an increased cover of *Eriophorum vaginatum* (Walker *et al.* 1994). Yurtsev (1994) considers subzone E to be an extension of the boreal region without trees. Indeed, Kade *et al.* (2005) and Vonlanthen *et al.* (2008) found that the

proportion of plant species that are shared with boreal and alpine regions linearly declines from the warm to the cold subzone (D to A), while the proportion of arctic species increases. Though about 50% of the plant species in zonal communities in the coldest subzone (A) are also known from alpine regions, 80% of the plant species in zonal communities in the warmest subzone (E) also occur in alpine and boreal regions (Kade *et al.* 2005; Vonlanthen *et al.* 2008). It is interesting to speculate that the high proportion of fungal OTUs that are shared with boreal regions might facilitate future expansion of treeline into the Arctic. Reithmeier (2011) observed that conifers grew best in soils of *Salix* spp. from above treeline and concluded that the EMF of *Salix* spp. could potentially facilitate establishment of conifers at higher latitudes by acting as ectomycorrhizal nurse plants. Additionally, *B.nana* maintained EMF through tundra fire, potentially facilitating the re-vegetation by shrubs and possibly the expansion of trees into the Arctic (R. Hewitt, personal communication).

Although subzone A lacks woody species, we found EMF in soils. These EMF are most likely from spores, because EMF are obligate mutualists that require hosts for germination and survival (Ishida *et al.* 2008). This indicates that DNA based soil analysis will always include sequences from spores and therefore fungi that are not actively growing at the time of sampling. However, longevity of fungal spores is estimated to vary from one year to potentially several decades (Bruns *et al.* 2009; Ishida *et al.* 2008) and spore germination increases the presence of host plants (Ishida *et al.* 2008). One might

wonder if the present fungi would provide inoculum to facilitate shrub establishment in this subzone.

PGFs and bPGFs: The distinct fungal communities of PGFs versus bPGFs along the bioclimatic gradient parallel structure of plant communities at the same sites (Walker *et al.* 2011). The only exception occurred in subzone A, where fungal community structures did not differ significantly between PGFs and bPGFs, despite hosting different plant communities. This is not surprising because, in comparison to the other subzones, PGFs of the sampled site in subzone A generally experienced less frost heave and strong physical disturbance, and bPGFs develop in soil cracks, which are sparsely vegetated. Most of the patterned-ground at subzone A is due to thermal and possibly desiccation cracking at the fine scale (20-30 cm). The sampling approach used did not clearly differentiate the soils collected from the fine scale microhabitats of cracks *vs.* the non-cracked adjacent area toward the centers of the polygons. These bPGFs host many lichens but lack the woody species that are common in all other subzones (Walker *et al.* 2011). The fundamentally different type of disturbance in subzone A that has less of an effect on the centers (PGFs) and sparse vegetation in bPGFs in subzone A lead to very similar abiotic environments across these features.

The significant differences between fungal communities of PGF and bPGF in subzones B through E are likely a result of the local micro-environments created by different types of physical disturbance, causing patterned ground such as frost heave in subzone C to E and cracking in subzones B to A, and the resulting differences in plant

communities. Temperature related factors (TDD_{Soil} , FDD_{Soil}) and cover and species richness of plant functional types (lichens, bryophytes, graminoids, forbs and shrubs) in particular, were related to fungal communities not only at the regional scale (subzones) but also on the local scale across paired PGF and bPGF. TDD_{Soil} showed the strongest differences between PGF and bPGF in subzone C and D, with PGFs being up to 200-500 thawing degrees warmer than the adjacent bPGFs. Additionally PGFs in subzone D experienced annual frost heave of up to 20 cm, causing distinct disturbances of the soil (Walker *et al.* 2011).

Ascomycota dominate PGFs, where various lichens are significant indicator species. However lichens are not the dominant component of the Ascomycota in PGFs. Instead these Ascomycota include other functional groups, such as mycorrhizal taxa, saprotrophs and pathogens, underlining the functional diversity within these seemingly ‘barren’ features. In contrast, EMF and saprotrophs are the major components of the basidiomycetes and are also indicator species for bPGFs. The dominance of basidiomycetes in bPGFs at subzone C and D, coincides with the highest cover and biomass of deciduous and evergreen ectomycorrhizal shrubs (e.g., *Dryas integrifolia*) (Walker *et al.* 2011). Generally, saprotrophic fungi of both phyla were abundant throughout PGFs and bPGFs. Although organic carbon (%) and C : N ratio and soil moisture were generally higher in bPGFs, PGFs were generally warmer and drier, which could favor decomposition (Michaelson *et al.* 2008). Not surprisingly, pathogenic fungi occurred more often in bPGFs, where plants act as hosts. The high spatial heterogeneity

of fungal communities of PGFs and bPGFs contributes to a high local alpha diversity of these sites along the NAAT.

Overall fungal communities in patterned-ground ecosystems are not only shaped by climate, physical and biological interactions, but also represent an integral part of the interactions that drive patterned-ground ecosystems directly by facilitating plant nutrition and establishment as plant symbionts and decomposers and indirectly by stabilizing the ground in the form of lichens in soil crusts and hyphae binding soil aggregates. Soil crusts facilitate the establishment of plants in these disturbed microhabitats. Eventually, plants will mask the PGFs and change their biophysical properties (Walker *et al.* 2011). These roles are especially apparent in the southern part of the Arctic tundra biome. Future studies should address the functional role of fungi in the evolution of patterned-ground ecosystems.

4.6. Conclusions

This is the first report of soil fungal communities in patterned-ground ecosystems and the first to describe communities across the entire bioclimatic gradient of the North American Arctic. With the inclusion of all fungal groups found in soil in our study, we extend and substantiate previous findings from studies of EMF root-tip communities in the Arctic. Namely, fungal communities in the North American Arctic are diverse, supporting the notion that fungi are the most species-rich eukaryotes in the Arctic. While fungal communities show no species richness decline with latitude, the species richness is maintained by the opposing patterns in the relative species richness of two functional

groups, with lichens increasing and mycorrhizal fungi decreasing with latitude. The wide distribution of the most abundant OTUs supports increasing evidence that many fungi in the Arctic must be excellent dispersers with wide ecological amplitudes. Nevertheless, despite their wide geographic distributions, arctic fungi show niche preferences with regard to climate and vegetation at the regional scale across bioclimatic subzones, and at a local scale across patterned-ground features and their associated abiotic environments and plant communities. Fungal community structures are driven by the same primary abiotic environmental factors as plant communities, which are temperature, disturbance and pH. However, they are also highly correlated with the composition of plant communities with which they interact as symbionts, pathogens and decomposers. The high heterogeneity at the microscale of patterned-ground features contributes to the high alpha diversity of fungi in these sites. If we consider our studies along the NAAT as an analog to climate warming, we expect that a warming climate associated with enhanced colonization of patterned-ground features by vascular plants will initiate a suite of biophysical changes that will affect fungal community composition and structure not only at the species level, but also at the level of functional groups. In particular we would expect an increase in fungi that are symbiotic with plants. These changes, in turn, may alter ecosystem functioning of patterned-ground features in the Arctic.

4.7. Acknowledgements

The authors would like to thank Hilmar Maier and Edie Barbour for helping with graphical designs, Martha Raynolds and Ronald Daanen for conceptual discussions,

VECO Polar Resources and Aklak Air for logistical support, Parks Canada and the Innuvialuit community for supporting our research on their lands. The authors acknowledge the generous support of US National Science Foundation grants ARC-0425517 to DAW, ARC-0632332 to DLT, and fellowships by EPSCoR, the UAF-Graduate School and the Institute of Arctic Biology to IT and a Global Change Student Research grant to IT.

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4.9. Figures

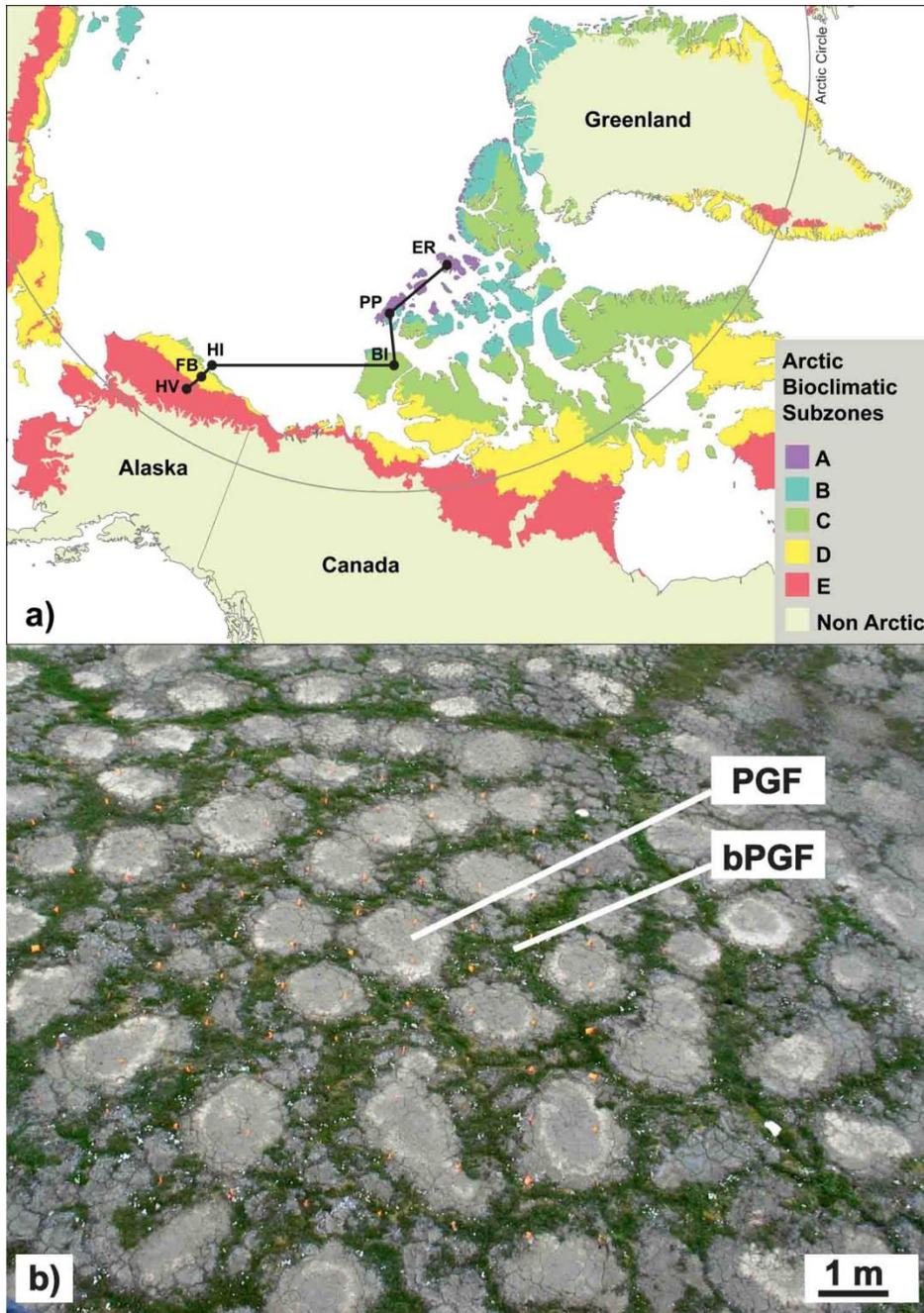


Figure 4.1 Map of (a) sampling sites along the North American Arctic Transect (NAAT), (b) Patterned-ground features (PGF) and areas between Patterned-ground features (bPGF) at Howe Island (subzone C).

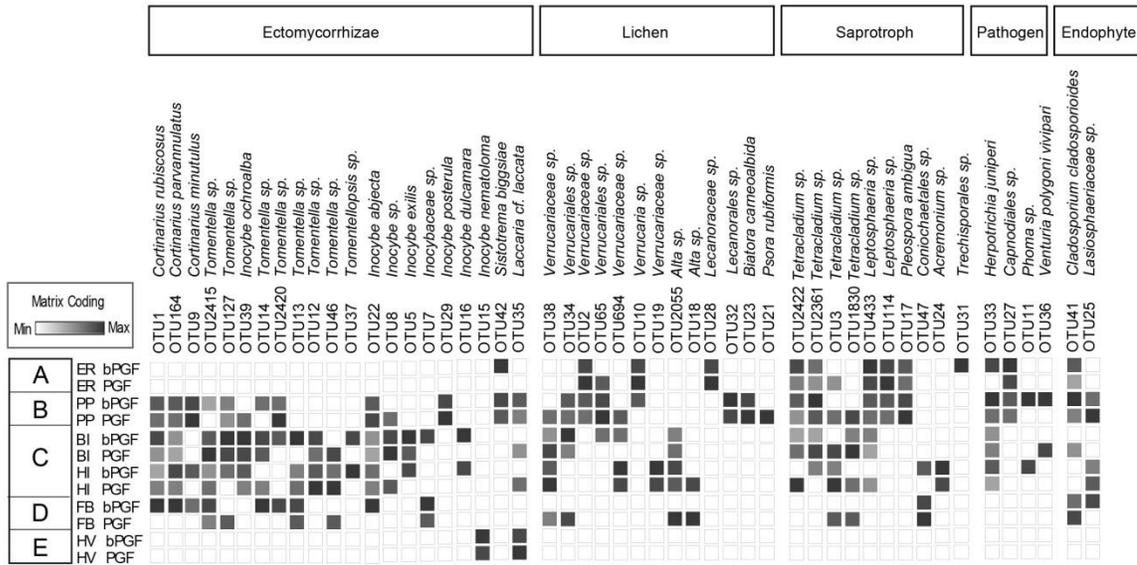


Figure 4.2 Distribution of the 50 most abundant Operational Taxonomic Units (OTUs) along the NAAT

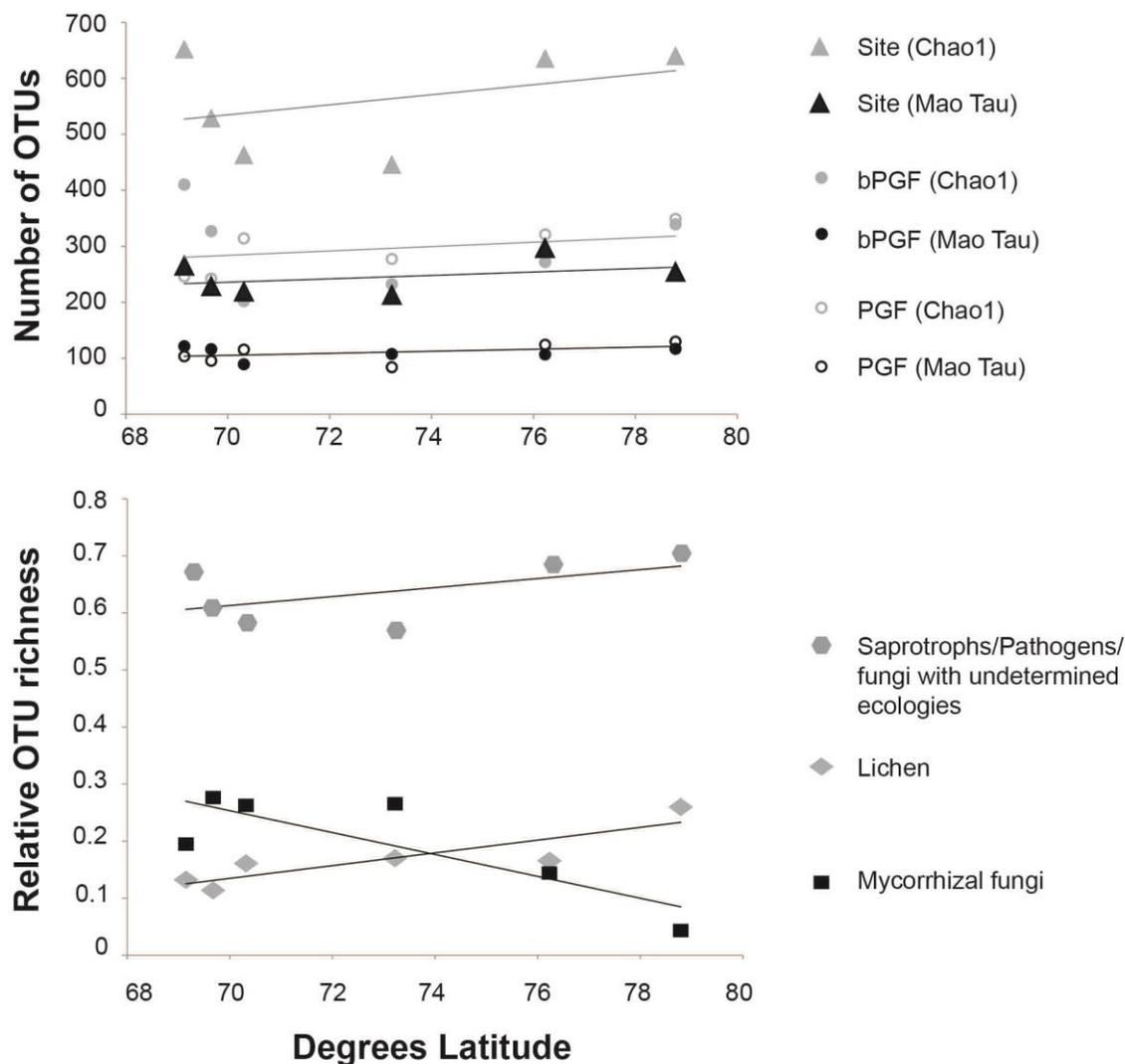


Figure 4.3 Linear regression of (a) observed (Mao tau) and estimated (Chao1) fungal species (OTU) richness of zonal mesic sites and patterned-ground features (PGF, bPGF) along the NAAT. Species richness was based on pooled soil clones from five PGF and bPGF, which were rarified to 258 clones. Observed and estimated fungal species richness of PGF and bPGF, and pooled at the site level shows no significant decline with latitude ($P > 0.12$, $r^2 > 0.16$ (MaoTau), $r^2 > 0.06$ (Chao1)). (b) Relative species (OTU) richness across the sites of the NAAT increased significantly for lichens ($P = 0.02$, $r^2 = 0.75$) and decreased significantly for mycorrhizal fungi with latitude ($P = 0.04$, $r^2 = 0.68$), while there was no relationship between the relative species richness of the group of fungi containing saprotrophs, pathogens and fungi with undetermined ecologies ($P = 0.27$, $r^2 = 0.28$) and latitude.

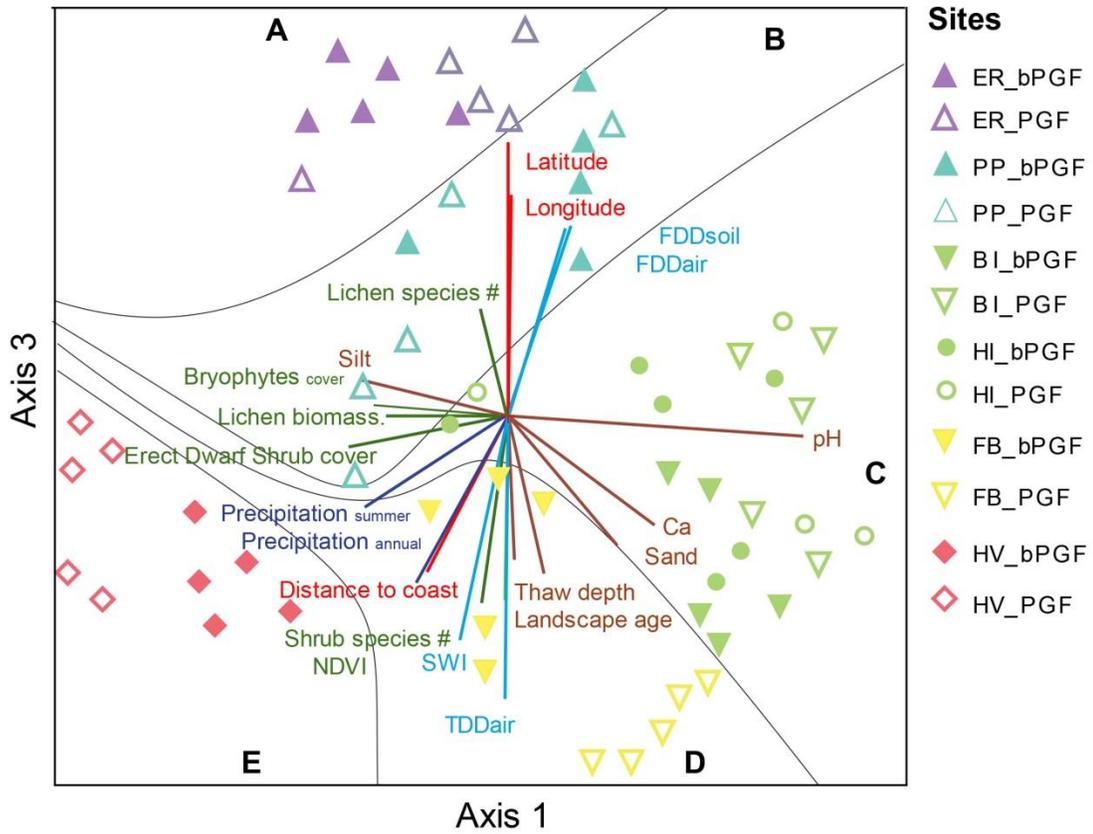


Figure 4.4 NMDS biplots of all zonal mesic sites across the NAAT

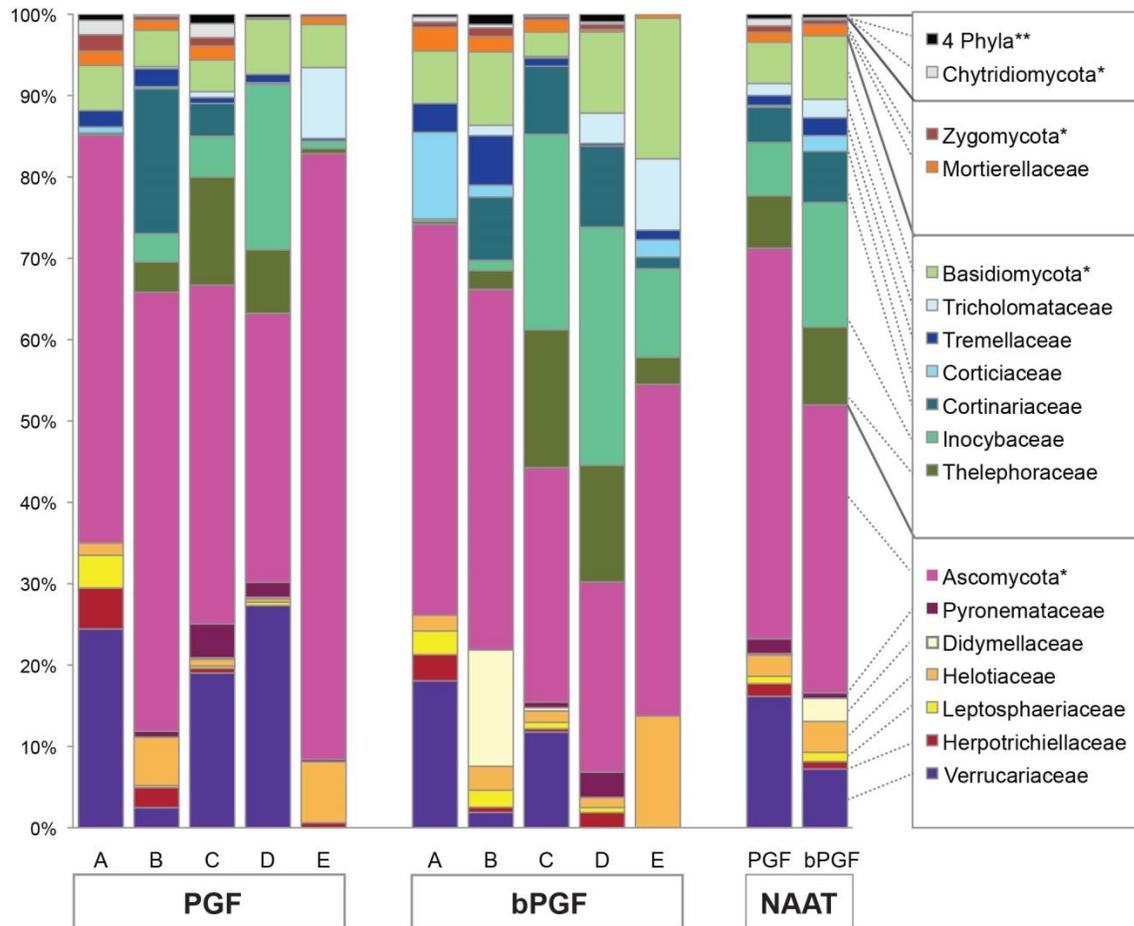


Figure 4.5 Relative abundance of fungal families (based on number of clones) in zonal mesic PGF and bPGF across the NAAT. Only families that represented more than 1% of the clones are displayed.

4.10. Tables

Table 4.1 Locations and characteristics of the zonal mesic sampling sites along the North American Arctic Transect (NAAT)

Location	Site	Latitude/ Longitude	Bioclimatic Subzone	SWI* (°C) †	Soil pH †	Soil C:N †	Vegetation Type ‡	Plant Community‡
Isachsen, Ellef Ringnes Island (ER)	PGF	78° 47.106' N 103° 33.09' W	A	3.6	6.5	13.60	Lichen, forb barren (B1b)	<i>Puccinellia angustata</i> – <i>Papaper radicatum</i>
	bPGF				6.4	13.45	Rush/grass, forb, moss tundra (G1)	<i>Saxifraga-Parmelia omphalodes spp. glacialis</i>
Mould Bay Prince Patrick Island (PP)	PGF	76° 13' 42.8" N 119° 17' 57.9" W	B	6.2	7.4	14.74	Lichen, forb barren (B1b)	<i>Hypogymnia subobscura- Leproloma</i>
	bPGF				7.1	15.23	Graminoid, prostrate dwarf shrubs forb tundra (G2)	<i>Orthotrichum speciosum-Salix arctica</i>
Green Cabin, Banks Island (BI)	PGF	73° 13' 10" N 119° 33' 33" W	C	14.7	8.4	46.12	Bare ground (B1a)	<i>Puccinellia angustata-Potentilla vahliana</i>
	bPGF				7.9	25.14	Prostrate dwarf shrub, herb tundra (P1a)	<i>Dryas integrifolia-Carex rupestri.</i>
Howe Island (HI)	PGF	70° 18' 54" N 147° 59' 37" W	C	14.7	8.6	49.99	Lichen, forb barren (B1b)	<i>Braya purpurascens-Puccinellia angustata</i>
	bPGF				7.8	39.28	Prostrate dwarf shrub, herb tundra (P1a)	<i>Dryas integrifolia-Salix arctica</i>
Franklin Bluffs (FB)	PGF	69° 40' 28" N 148° 43' 16" W	D	27.0	8.0	21.99	Lichen, forb barren; Prostrate dwarf shrub tundra (B1b, P1a)	<i>Junco biglumis-Dryadetum integrifoliae</i>
	bPGF				8.2	38.39	Nontussock sedge, dwarf shrub, moss tundra (G3)	<i>typicum/pedicularetosum Dryado-integrifoliae-Caricetum bigelowii</i>
Happy Valley (HV)	PGF	69° 08' 50" N 148° 50' 49" W	E	30.2	5.2	16.71	Liverwort, moss barren; Prostrate dwarf shrub, herb moss tundra (B1c)	<i>Anthelia juratzkana-Juncus biglumis</i>
	bPGF				5.0	19.43	Tussock sedge, dwarf shrub, moss tundra (G4)	<i>Sphagno-Eriophoretum vaginati</i>

§ Patterned Ground Feature (PGF)

£ between Patterned Ground Feature (bPGF)

* Summer Warmth Index (SWI) is the sum of the monthly means above 0 °C.

† Data are from Walker et al. (2011)

‡ Vegetation types follow physiognomic units of the Circumpolar Arctic Vegetation Map (CAVM). Data are from Reynolds *et al.* (2008).

4.11. Supporting information

Table S4.1 Locations and characteristics of the dry and wet sampling sites at along the North American Arctic Transect (NAAT)

Table S4.2 Distribution of clones/OTUs assigned to various taxonomic levels across patterned-ground features (PGFs) and between patterned-ground features (bPGFs) in five subzones in the North American Arctic. A plus (+) indicates the presence of clones/OTUs. Numbers in parentheses represents clones and OTUs (in bold) at each taxonomic level. OTU assignment was based on BLAST –search of NCBI.

Table S4.3 NCBI BLAST-matches and distribution of 1834 OTUs (7834 clones) in patterned-ground features (PGFs) and between patterned-ground features (bPGFs) along the NAAT. We included 99 sites (60 zonal mesic, 29 dry, 10 wet) at 6 locations (ER= Ellef Ringnes Island, PP= Prince Patrick Island, BI= Banks Island, HI= Howe Island, FB= Franklin Bluffs, HV= Happy Valley) across 5 bioclimatic subzones (A-E) in the North American Arctic. (†) We updated the taxonomic information (marked as italic names) for some OTUs, when matches had either incomplete or different taxonomic assignments. We based our adjustments on the most recent assignments of the particular taxa, using NCBI and Index Fungorum.

Table S4.4 Geographic distribution of NCBI matches to the 50 most abundant OTUs observed in patterned-ground ecosystems along the NAAT. Only the top matches at the species level (90% coverage of the ITS1-5.8S-ITS2 region with similarities of $\geq 97\%$) were included. We selected one match from each of the first five different studies, unless a study comprised sequences from a wide geographic range, beyond US-state level.

Table S4.5 Indicator species of zonal mesic patterned-ground ecosystems for the five bioclimatic subzones (A-E) along the NAAT.

Table S4.6 Indicator species for zonal mesic PGF and bPGF along the NAAT.

Table S4.7 Correlations coefficients (r^2) for variables in the NMDS ordination of zonal mesic sites along the NAAT.

Table S4.8 Spearman Rank Correlation coefficients of Thawing Degree Days (TDDair) and pH and most abundant fungal families in zonal mesic sites along the NAAT.

Correlation coefficients in bold are statistically significant ($P \leq 0.05$) following Holm adjustment for multiple tests.

CHAPTER 5 Conclusions

In my dissertation research, I examined the diversity, distribution and key drivers of fungal communities associated with two abundant shrubs (*Dryas integrifolia*, *Salix arctica*) and with patterned-ground features along a bioclimatic gradient across the North American Arctic. My findings are based on the use of molecular methods and multivariate analyses to identify and describe fungal communities and their distribution patterns. The work on EMF (Ch.3) was based on the combined efforts of two research initiatives that collected 326 shrubs (*D. integrifolia* and *S. arctica*) throughout the Canadian and North American Arctic and morphotyped and sequenced their root tips. The work on the fungal soil communities (Ch.4) was based on the collaboration with the Biocomplexity of Patterned Ground (BPG) project that studied the interaction of cryoturbation, vegetation, soils, and biogeochemistry in the self-organization of patterned-ground ecosystems along the NAAT (Walker et al. 2008b). The extensive environmental data sets generated by the BPG allowed me to correlate fungal communities with environmental factors and plant communities in order to determine the key drivers of fungal communities in the Arctic. I have generated the largest molecular-biodiversity dataset in existence for Arctic fungi and came to the following major conclusions:

5.1. Fungal diversity

As in other terrestrial biomes, fungi are abundant in Arctic soils. Despite the harsh environment of the Arctic, all major functional groups of fungi and nearly all currently described fungal phyla were recorded. The two major phyla that occurred in soils and on shrub roots were Ascomycota and Basidiomycota. The use of molecular methods revealed a great abundance and diversity of many inconspicuous fungi, such as *Thelephoraceae* and *Sebacinaceae* that were not seen with previous methods. Fungal communities of both soils and shrubs were dominated by a few species-rich families. Ectomycorrhizal families (*Thelephoraceae*, *Inocybaceae* and *Cortinariaceae*) that were dominant on roots were also dominant in the soil. Other families that were dominant as ectomycorrhizae on roots, but not in soils, were *Sebacinaceae* and *Pyronemataceae*. Lichens belonging to the *Verrucariaceae* and the functionally diverse *Helotaceae* and *Herpotrichiellaceae* were the most abundant and diverse families found in soils. This suggests that a few species-rich fungal families seem to be well adapted to arctic conditions. Furthermore, my results show that EMF community structure on roots is not perfectly mirrored in soils. In order to profile structures of total fungal communities in soils, one must include not only bulk soils *per se*, but also roots.

My studies clearly demonstrate that fungal diversity in the Arctic is higher than was estimated. In both studies, roots (242 OTUs) and soils (1834 OTUs from all sites/1267 OTUs from mesic sites), I did not saturate diversity at zonal sites across the five bioclimatic subzones. Instead, extrapolations of species richness estimates suggest fungal

diversity to be 2-5 times higher than what I observed. Currently, there are >4350 fungal species (1750 lichen, >2600 non-lichenized fungi) described for the Arctic (Dahlberg et al. 2013). Following recent extrapolations from the boreal forest, fungi could outnumber vascular plants by 17:1 (Taylor et al. 2013). With approximately 2200 vascular plants in the Arctic (Dahlberg et al. 2013), there could be 37,400 non-lichenized fungi based on the 17:1 ratio. The number for lichens (ca. 1750 species) is believed to be well-established for the Arctic (Dahlberg et al. 2013). I found about 1058 OTUs of non-lichenized and 209 lichenized fungi in six zonal mesic sites, which represents ca. 3% of the total estimated species richness of the Arctic. This enormous gap highlights not only the need to include all habitats in the study of fungal biodiversity the Arctic, but also to apply Next Generation Sequencing technologies to reach saturation of diversity.

5.2. Fungal distribution

The dominant fungal OTUs of root associated EMF and soil fungi were widely distributed in and beyond the Arctic, encompassing different continents, biomes and host plants. This suggests that dominant fungi in the Arctic are mainly generalists with wide ecological amplitudes. Furthermore it suggests that these fungi are excellent dispersers that do not display continental scale endemism, in contrast to fungi from lower latitudes. This parallels findings from Antarctica, where most fungi are considered to be cosmopolitans with only a few endemic taxa (Bridge & Spooner 2012). Overall, this suggests that the most abundant fungi found in polar regions are generalists. However, caution is advised, because we applied a cutoff of 97% similarity across the ITS region to

distinguish fungal species, which might not be applicable across all fungi. Therefore, future studies, that use a cutoff above 97 % in the ITS region and that include phylogenies of multiple loci, such as rBP2 and EF1a, as well as population genetics, might detect fungal species or populations that are endemic to polar regions.

Fungi in the Arctic do not follow the classic latitudinal diversity gradient. Fungal species richness did not decline in root associated EMF or among soil fungi. Nevertheless, in soils, overall species richness was maintained by the opposing patterns of two functional groups. While lichens increased with latitude, mycorrhizal fungi (including all mycorrhizal types) decreased. The increase in lichens parallels aboveground observations (Dahlberg et al. 2013, Kade et al. 2005, Vonlanthen et al. 2008). Further research is needed to investigate the decrease in mycorrhizal fungi, using Next Generation Sequencing.

I found that fungal communities on zonal sites are structured at two scales: a regional scale across subzones and at a local scale across patterned-ground features. At the regional scale, fungal communities (EMF and soil fungi) varied among subzones. Interestingly subzone E, which was the only acidic site/subzone, harbored very distinct fungal communities in soils; the majority of these taxa are also found in boreal forests. This parallels the observations of some Russian geobotanists who consider the southern 'hypoarctic' to be floristically largely a treeless extension of the Northern Boreal zone (Yurtsev 1994). With warming climate, such high proportions of shared taxa between subzone E and the boreal forest might facilitate treeline expansion into the tundra. At the

local scale, fungal communities within (PGF) versus between the patterned- ground features (bPGF) differed across all bioclimatic subzones, with the exception of subzone A. Fungal communities varied not only at the phylogenetic, but also at the functional guild level. The seemingly bare PGFs were characterized by ascomyceteous lichens and saprotrophs, while the vegetated bPGFs were dominated by basidiomyceteous EMF and by pathogens belonging to both phyla. The distinction of functional guilds suggests different ecosystem functions among these features. Finally, the high spatial heterogeneity of fungal communities in PGFs and bPGFs contributes to a high local alpha diversity across the arctic bioclimatic gradient.

5.3. Key drivers of fungal communities in the Arctic

Fungal communities are largely driven by the same factors that drive plant communities of patterned-ground ecosystems across the bioclimatic gradient in the North American Arctic. In particular, climate (temperature and precipitation), pH, plant productivity and plant communities are correlated with fungal communities across the bioclimatic gradient. Host plant identity only marginally affected the associated EMF communities of *D. integrifolia* and *S. arctica*, suggesting that this symbiosis is less specific than are many at lower latitudes. Disturbance caused by cryoturbation and frost heave affects plant communities, soils and the microclimate of patterned-ground features at the local scale. Soil fungal communities of patterned ground were strongly correlated with both local abiotic environment (especially temperature) and plant communities, with which they interact as symbionts and decomposers.

5.4. Future work

My dissertation research is the first description of fungal communities in patterned-ground features and on two principal shrubs in the Arctic that extends across all five bioclimatic subzones in the North American Arctic. Analogous studies are needed to validate the observed patterns. Therefore, future research should include the establishment of comparable transects across the five bioclimatic subzones in other regions of the Arctic (see Walker et al. 2012) as well as the application of high throughput sequencing technologies. Only such technologies have the potential to saturate diversity and to document total biodiversity. Furthermore, the coldest bioclimatic subzone A, which is also the smallest and most unique subzone, is endangered by climate change. Subzone A covers about 2% of the Arctic and occurs only in coastal regions where it depends on the presence of multi-year sea ice (Walker et al. 2008a). This makes it also the most sensitive subzone to climate change, especially the reduction in multi-year sea ice. Our study site in Isachsen on Ellef Ringness has undergone dramatic changes since we conducted our research (V. Romanovsky, personal communication). Photos taken in summer of 2006 and 2012 show that the 10x10 m Biocomplexity research grid has subsided due to thawing permafrost, creating a new much more irregular and varied surface. Reduction in sea ice extent and increasing temperatures are causing warming and degradation of permafrost. So far, these changes have affected the microtopography that is one of the key features for plant establishment in the High Arctic. Due to the reduced species richness in vascular plants and animals, this ecosystem

is considered to be ‘simpler’ than that found in the Low Arctic. Documenting and assessing such dramatic changes across soil physical, hydrological, biogeochemical properties, as well as changes in vegetation and microbes, including fungi and bacteria, will provide us with insights about the functioning of the ecosystem.

5.5. A fungal ‘outlook’

Fungal communities are directly and indirectly affected by plant communities. Plants have direct effects in their roles as hosts for mycorrhizal fungi, epi- and endophytes, and pathogens and by providing substrate for decomposer fungi. Plants also have indirect effects by affecting microclimates for fungi. Soils can be warmer in winter because vegetation traps snow and insulates the soil, and cooler in summer because of shading and insulation. Especially for lichens, vegetation cover decreases light and therefore decreases lichen abundance. Although abiotic factors are confounded with biotic (vegetation) factors, changing climate (with greening of the Arctic) will certainly affect fungal community structures at various scales. At the microscale of small patterned-ground features (PGF, bPGFs), a warmer climate would lead to the colonization of PGFs by vascular plants, as can be seen in the southern part of the bioclimatic gradient, where PGFs are usually masked by vegetation. Furthermore, there is evidence that PGFs facilitate the establishment of alder (*Alnus*) in the Low Arctic of Siberia (Frost et al. 2013). It is most likely that the establishment of alder in these nutrient poor features is facilitated by its symbionts (*Frankia* and EMF). In turn, alder establishment is likely to increase nitrogen pools (Uliassi & Ruess 2002). The colonization of PGFs by vascular

plants causes a suite of changes that would affect their fungal communities. For example, lichen cover would decline due to shading, as has been observed in tundra warming experiments (Walker et al. 2006). A lessening of frost heave would reduce the physical disturbance of the soil and could allow fungi of the long-distance exploration types, which are ubiquitous in the Low Arctic and Boreal forest (e.g., *Leccinum*) to establish beyond their current distributions. A build-up of organic matter would benefit decomposer fungi. Depending on the colonizing host-plants, fungal symbionts as well as pathogens may establish. Finally, an increased build-up of organic matter and increased plant cover and height would affect the thermal and hydrological regime of the PGFs, which could select for particular fungi. These biophysical changes will affect the functioning of patterned-ground ecosystems. At a regional scale across subzones, a changed climate will facilitate the expansion of certain plant groups, such as shrubs or trees, all of which form symbioses with EMF. Experimental warming of tundra demonstrated a shift of EMF communities toward increased abundance of *Cortinarius spp.* (Deslippe et al. 2011). Hence, the spread of shrubs *per se* will extend the distribution of EMF, while the increased temperatures will favor EMF that are currently found in the low Arctic and respond well to warmer soils, such as *Cortinarius*, *Russula*, and *Lactarius*. Furthermore, EMF (Ch. 2) along the NAAT gradient, which provides an analog to climate warming, supported the notion that EMF community structure will change in concert with climate.

‘Much work remains to be accomplished before any ‘final’ listing can be constructed.’ (Laursen & Chmielewsky 1980). Nearly three and half decades after ‘The First International Symposium on Arcto-Alpine Mycology’- this statement, referring to the documentation of fungal biodiversity, is still true. Based on extrapolations using molecular methods, fungal species richness surpassed previous estimations several times over the last one and half decades and completing their species descriptions is estimated to take several thousand years (reviewed in Blackwell 2011). While the majority of fungi still remain to be discovered and described, we are challenged to decipher the potential effects of a changing climate on fungi. By documenting high diversity combined with tremendous variation in community composition across the Arctic that is strongly related to plants, soils and climate, my dissertation research provides a first peek through a frosty window that is now only beginning to clear.

After we crossed the Brooks Range, a wide majestic landscape devoid of trees appeared. This land stretching north, the arctic tundra, was ruled not just by cold and permafrost, but also by summer warmth. It appeared harsh and beautiful as well as quiet and patient. It reminded me of paintings by Casper David Friedrich (the 'painter of stillness'), who painted the wide-open landscapes of northern Germany with which I was so familiar. The grandeur of this landscape made me feel elementary and small, but I was drawn to it through some strange invisible webs. Devoid of trees, shrubs were the only woody plants, and they got smaller and smaller the farther north one went, until they disappeared completely. Like in the pine forests, mushrooms grew close to woody plants (shrubs), with which they formed a symbiosis. Although usually much smaller, sometimes these mushrooms were taller than the shrub with which they were associated. This world seemed mysterious. DNA, the blueprint of life, provided the key to peek into the below-ground world of roots and mycelia. It seemed that fungi were one of the hidden rulers of this world. There were many more fungi in the soil than appeared on the surface. Fungi and plants that depended on each other appeared not to be very selective in the Arctic. These fungi were travelers like me, spanning distances across oceans and landmasses. Compared to plants, though, they seemed out of order; their number of species did not decline with latitude. It seemed that they had found their niche in the harsh environment of the Arctic. From the pine forests to the arctic tundra, fungi never failed to surprise me. And they have many more surprises that will provide many more adventures to come.

(Ina Timling)

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