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

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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.

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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. Fungal communities in these soils include representatives of all of the 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

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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 Gt 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 point (Fahnestock *et al.* 1998; Sturm *et al.* 2005). Winter respiration is hence critical to global carbon cycles and to predicting feedbacks to atmospheric CO_2 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 CO_2 to the atmosphere and to hence influence the Earth's climate.

In this review, we summarize molecular work describing the diversity and community structure of fungi – particularly 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 below-ground studies in the Arctic, rather than studies on above-ground sporocarps (Kobayasi & Kenkyujo 1967; Miller *et al.* 1973, 1982; Laursen *et al.* 1987, 2001). The 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.

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 1). Even Low Arctic soils at Toolik Lake in Alaska can remain frozen for 69 % of the year (Fig 1). Annual soil temperatures at 10 cm depth can range between −27 °C and 14 °C in the High Arctic and between −7 °C and 11 °C in the Low Arctic, compared to −2 °C and 22 °C in a temperate grassland (Fig 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 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 2A; Tarnocai 2009). During thaw, the underlying permafrost can

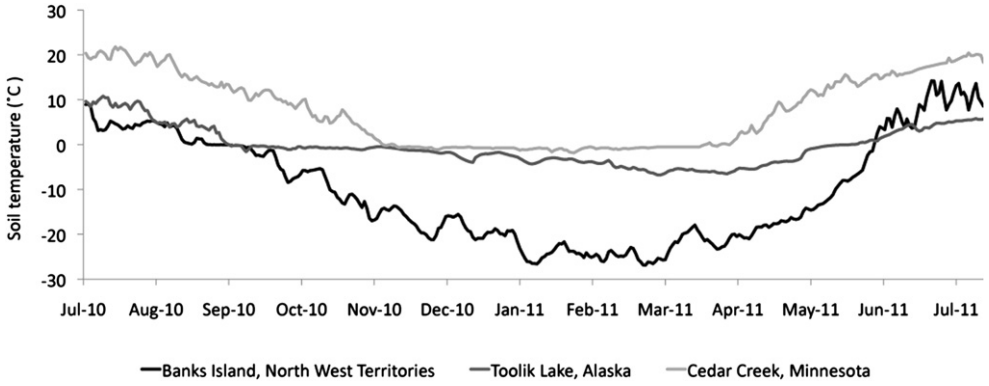

Fig 1 – Daily mean soil temperatures at 10 cm depth between July 2010 and 2011 on Banks Island in the North West Territories in the High Arctic, at Toolik Lake in Alaska in the Low Arctic and in temperate grassland at Cedar Creek, Minnesota. Data are from the Geophysical Institute Permafrost Laboratory and the Institute of Arctic Biology at the University of Alaska, Fairbanks, and from the Cedar Creek Ecosystem Science Reserve.

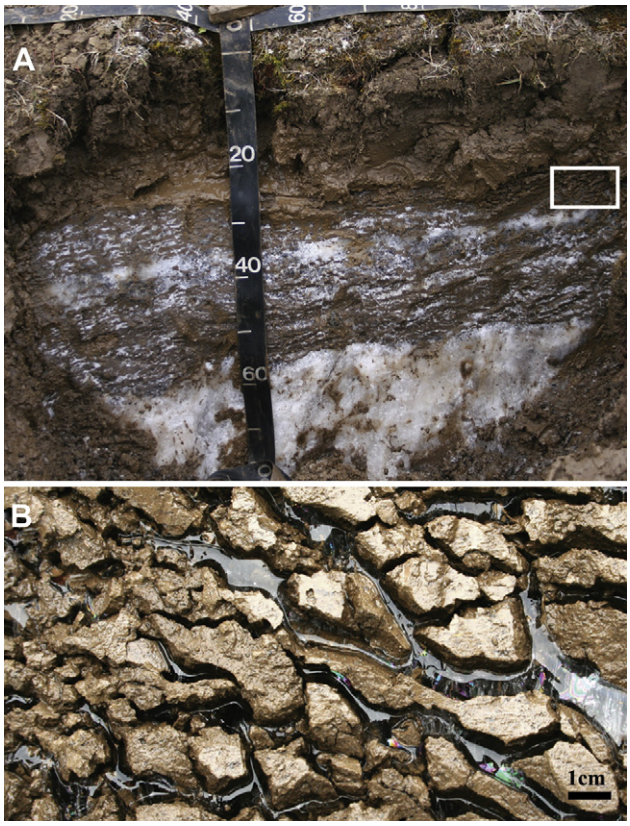

Fig 2 – (A) Soil profile at Isachsen on Ellef Ringnes Island in the High Arctic with permafrost at 25 cm depth, (B) ice lenses in permafrost from the rectangle marked in (A).

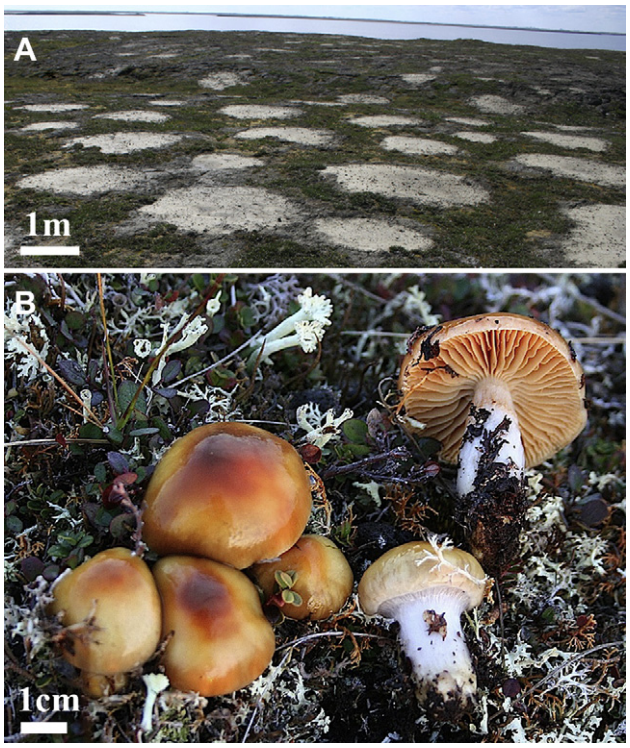

Fig 3 – (A) Patterned ground (frost boils) at Howe Island, Alaska, (B) *Cortinarius favrei*, a common Arctic basidiomycete, at Toolik Lake, Alaska.

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, thermal cracking, and the formation of needle ice and ice lenses (Fig 2B). These processes result in the mechanical movement of soil and the creation of microrelief, including patterned ground (Fig 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).

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 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 *Thelephora/Tomentella*, *Sebacina* and *Clavulina*. Other frequently recorded fungi include *Cenococcum geophilum* and dark septate endophytes (DSE), such as *Phialocephala fortinii* and *Cadophora finlandica* (Clemmensen & Michelsen 2006; Hrynkiewicz *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 on *Dryas integrifolia* and 179 OTUs on *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 4). However, neither of these studies achieved saturated sampling, and we hence still do not have a clear picture of whether or not soil fungal diversity alters at higher latitudes in the Arctic.

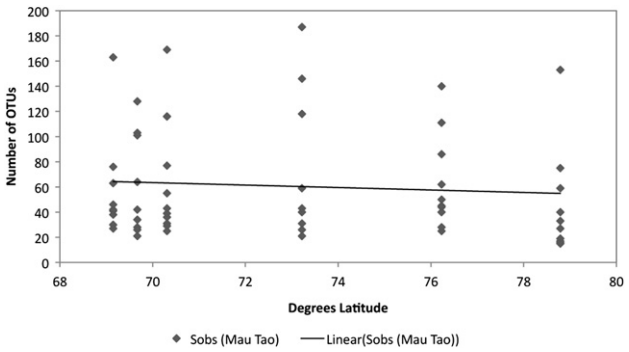

Fig 4 – Soil fungal OTU (species) richness (Mau Tao) along a latitudinal gradient through the North American Arctic (Timling *et al.* unpublished data). The presence of potential chimeras was reduced by excluding singletons from the data set. Linear regression showed no influence of latitude on OTU richness ($P = 0.54$).

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. In a relevant study, Geml *et al.* (2012) 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 phylotypes had been recorded outside of Svalbard. The same picture has emerged from studies of ectomycorrhizal root tips of *S. arctica* and *D. 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 be selected 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 *C. 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 *S. arctica* on Ellesmere Island in the High Arctic (Fujimura *et al.* 2008; Fujimura & Egger, this volume), 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 (see 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 *S. 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 type 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 harbour 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 Sebacinales (Basidiomycota) that were not restricted to specific hosts (Walker *et al.* 2011). Similar observations have been made for EMF on *D. integrifolia* and *S. 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 (see 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 basidomycetes (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.* in press).

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).

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 yr 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 dark-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 yr 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 yr of warming (Walker *et al.* 2006), and an increase in the abundance of *Betula nana* after 6–15 yr 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.* in press). When comparing the responses of above-ground 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 above-ground 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.* in press), and, in particular, a shift from tussock- to shrub-dominated tundra, is likely to alter soil fungal communities in favour 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 *B. 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 yr of warming, significant increases in EMF species diversity occurred, with changes in fungal community composition and structure associated with *B. 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 Cortinaricaeae 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 yr 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.* in press) 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.

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 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, this volume). 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. Kcat/Km, 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 °C do not function well at >20 °C (Hoshino *et al.* 2009). These tradeoffs make the widespread distribution of dominant Arctic fungi in habitats at lower latitudes particularly puzzling.

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; see also 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 the 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 of the 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 genetics 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 snow pack.

The 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 the fungal communities inhabiting Arctic soils.

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