




# Soil Fungal Responses to Warming in Polar Regions

**A thesis submitted to Cardiff University for the degree of**

**Doctor of Philosophy**

**by**

**Marta Misiak, MSc., BSc. (Hons)**

**December, 2018**

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## Statement of contribution

The contributions of colleagues to the work presented in this thesis are detailed below:

Chapter 2: Will Goodall-Copestake collaborated on molecular analysis consisting of cloning and identification of fungi extracted from the soil. Dr Kevin Newsham and his team conducted all the fieldwork in Antarctica (setting up of the experiment, treatment application and sample collection). Dr Kevin Newsham and his fieldwork assistants set up the experiment in Arctic in 2014.

Chapter 3: Dr Kevin Newsham provided the sequences used for primer design for Q-PCR analysis for Antarctic *P. pannorum*. Will Goodall-Copestake collaborated on primer design for the Q–PCR analysis. Dr Kevin Newsham provided soil fungal DNA extracts used for Q-PCR analysis.

Chapter 4: Dr Kevin Newsham provided *Pseudogymnoascus pannorum* isolates used for growth rate and enzyme analysis.

Chapter 5 and 6: Will Goodall-Copestake collaborated on primer design for the Q–PCR analysis.

Chapter 2, 3 and 5: Paul Giessler run all the C and N soil analyses.

## Summary

Polar regions are subjected to rapid climate change, with increased air temperatures and precipitation being predicted during future decades. Rising temperatures and precipitation will have an effect on saprotrophic soil fungi, microbes key to nutrient cycling and decomposition processes that are dominant in polar soils owing to their abilities of remaining physiologically active at low temperatures and water availabilities. Here, a combination of field warming experiments and laboratory experiments are used to investigate the effects of warming, water and nutrient availability on the abundance, growth and enzyme activities (cellulase, chitinase, acid and alkaline phosphatase and leucine aminopeptidase) of a range of saprotrophic fungi in Arctic and Antarctic soils. In a five-year-long maritime Antarctic field experiment, the abundance of *Pseudogymnoascus pannorum* DNA was reduced in soil warmed with open top chambers (OTCs) that had been enriched with nutrients. Laboratory experiments confirmed the inhibitory effect of warming to > 21 °C on the growth and enzyme activities of *P. pannorum*, but only when water was not a limiting factor. In contrast, in an Arctic field experiment, OTCs and watering had no effects on the abundance of DNA of seven Arctic soil fungal taxa after three years of treatment. The growth of five Arctic taxa (*Acremonium* sp., *Isaria* sp., *Leptosphaeria* sp., *Phialocephala* sp. and *Mortierella* spp.) was increased by warming to > 21 °C, with only that of *P. pannorum* being inhibited by warming to 24 °C. Warming did not affect the enzyme activities of Arctic fungi, with activities mainly being influenced by changes in water potential. The research here suggests that the growth and enzyme activities of Antarctic *P. pannorum* may be inhibited by future warming arising from continued greenhouse gas emissions to the atmosphere, but that Arctic saprotrophic soil fungi appear to be more resilient to environmental changes.

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# 1 Introduction

Globally, soils contain *ca*. 1500 Pg ($1.5 \times 10^{15}$ kg) of organic carbon. This is the largest pool of the element in terrestrial ecosystems (Scharlemann *et al.* 2014), is approximately 2–3 times the amount of carbon present in the Earth's atmosphere as gases (principally carbon dioxide, $CO_2$), and is three times the amount of organic carbon contained in global vegetation (Batjes and Sombroek 1997, Tarnocai *et al.* 2009). A third of the planet's terrestrial organic carbon is sequestered in polar regions (Zimov *et al.* 2006), predominantly in frozen, biologically inactive soil known as permafrost, which remains continuously frozen for more than two years (Eugster *et al.* 2000).

Global warming is leading to the thawing of permafrost, opening this vast organic carbon pool to biological processes. As permafrost melts, increased soil respiration is likely to have a positive feedback on the Earth's climate by accelerating $CO_2$ release to the atmosphere (Koven *et al.* 2011). Soil respiration is affected by abiotic (chiefly temperature and water availability) and biological (soil microbial activity) factors (Tirri *et al.* 1998). Terrestrial productivity is directly linked to the activity of the soil community, as soil microbes are an essential part of the carbon and other nutrient cycles (Sinsabaugh *et al.* 1991, Aon *et al.* 2001, Barrios 2007, Wallenstein 2008). The primary decomposer organisms, known as saprotrophs, are the bacteria and fungi (Singh and Gupta 1977, Swift *et al.* 1979), and in polar regions, these micro-organisms – especially the fungi - are the key drivers of decomposition (Davis 1980, Aon *et al.* 2001, Schadt *et al.* 2003, Arenz *et al.* 2006), with the contribution of invertebrates to decomposition being limited. In terms of abiotic factors, water availability has an important influence on the activities and composition of microbial communities, and has been identified as the major driver of terrestrial ecosystem processes in cold regions (Doeksen and Drift 1963, Convey *et al.* 2014). It is therefore of considerable importance to understand how soil processes in polar regions are driven by fungi (Alias and Suhaila 2008), and how the activities of these microbes are likely to be affected by changes in abiotic factors brought about by environmental change (Oechel *et al.* 1993, IPCC 2014).

## 1.1 Polar regions

Polar regions, areas bounded by the Arctic and Antarctic Circles at latitudes of 66.33° N and 66.33° S, respectively, are characterized by sub-zero temperatures for much of the year, low precipitation, limited water availability, and large seasonal variations in solar radiation, phenomena known as polar day and polar night (Melick *et al.* 1994, Burn 1996, Anisimov 2007, Prach *et al.* 2010). Although the Arctic and Antarctic share common characteristics, Antarctica is a landmass covered almost entirely by ice that is surrounded by ocean. Its climate is strongly influenced by the Antarctic Circumpolar Current, an ocean current that circulates clockwise around the continent, excluding warmer ocean waters from lower latitudes. In contrast, the Arctic mostly consists of frozen ocean surrounded by land. Owing to its connection with land masses at lower latitudes, human impacts are higher in the Arctic than in the mostly pristine Antarctic environment.

Owing to low solar radiation input, annual mean surface air temperatures in both polar regions vary between -60 °C and 0 °C in continental Antarctica (Comiso 2000), and between -7 °C and -2 °C in the Arctic (Førland *et al.* 2011). Even in summer, surface air temperatures are close to or below 0 °C in many regions (Rigor *et al.* 2000), with mean summer surface air temperatures of -15 °C to 3 °C on the Antarctic Peninsula and 2 °C to 5 °C on Svalbard in the High Arctic (Comiso 2000, Rigor *et al.* 2000, Førland *et al.* 2011, van Wessem 2015). The high net loss of radiation in most months contributes to cold and dry air at the land surface, and hence polar regions are often classified as cold deserts. Precipitation is typically very low and falls mostly as snow in the winter season (Anisimov 2007). Owing to their cold, arid environments, both polar regions are highly susceptible to environmental changes. Prolonged periods of warming or cooling across the freezing point threshold have a substantial impact on the physical environment and the biota of polar regions. Plant cover in the Antarctic, and at higher latitudes in the Arctic, is very sparse, with areas of bare, patterned ground, caused by frost heave processes, being frequent. Although soils are typically colder than those at lower latitudes, during the summer months at high latitudes, where bare soil surfaces are directly exposed to solar radiation, temperatures of dry soils often reach 15–20 °C for several hours around solar noon, with diurnal variations in temperature of up to 20 °C (Convey *et al.* 2018).

## 1.2 Climate change

Climate change, defined by the Intergovernmental Panel on Climate Change (IPCC 2007), as "a change in climate over time, whether due to natural variability or as a result of human activity", and its consequences on global ecosystems has been the subject of numerous scientific studies (for summaries use ACIA 2004, IPCC 2014). Changes in climate are driven by changes in atmospheric concentrations of greenhouse gases ($CO_2$, methane and nitrous oxide), aerosols in the atmosphere, solar radiation and land surface characteristics that affect the energy equilibrium of the Earth's climate system. Human activities, including fossil fuel combustion, land use change (such as large-scale deforestation) and agriculture, have been responsible for a significant increases in greenhouse gases concentrations in the atmosphere since the Industrial Revolution (IPCC 2007, IPCC 2014). Owing to the burning of fossil fuels, global $CO_2$ concentration in the atmosphere has increased from 280 ppm in pre-industrial times to > 400 ppm at present, while the concentration of methane, mostly originating from agriculture, has doubled to *ca*. 1800 ppb over the same time period (Ojima *et al.* 1994, NOAA 2018). These gases trap heat radiated from Earth within the atmosphere, causing the greenhouse effect, and resulting in an average global air temperature increase of 0.78 °C per decade (IPCC 2013), and predicted ocean temperature increases of 0.4 – 1.1 °C between 1990 and 2025 (Hansen *et al.* 2006, IPCC 2007, Turner *et al.* 2016).

Climate change disturbs the balance between incoming and outgoing energy on Earth, contributing to increased frequencies of severe weather events. Warming of the oceans leads to higher assimilation of $CO_2$ by autotrophs and the consequent acidification of the oceans, influencing marine biota, and especially coral reef ecosystems (Hoegh-Guldberg 2007, Spalding and Brown 2015). In the last half of the 20$^{th}$ Century, temperature increases in the Antarctic have been several times faster than the global average (Figure 1.1; Hansen *et al.* 2006, Adams *et al.* 2009, IPCC 2014, Turner *et al.* 2014), and in the Arctic, temperatures continue to rise (Huang *et al.* 2017). These increased rates of warming in polar regions arise from a phenomenon known as polar amplification (Figure 1.2), caused by several regional positive feedback processes that

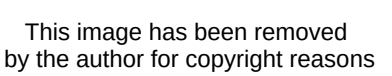



*Figure 1.1 Mean annual surface air temperature anomalies (in °C) for 2001–2005 relative to 1951–1980 (from Hansen* et al*. 2006).*

do not occur elsewhere on Earth (Hansen *et al.* 2006, Adams *et al.* 2009, Serrezze and Barry 2011, Cohen *et al.* 2014, Jeffries *et al.* 2014, Turner *et al.* 2014).

Because polar regions are predominantly covered by ice or snow, the thawing of this frozen water alters the surface albedo, with more of the underlying darker land surface being exposed to solar radiation, which results in faster solar heat absorption (Anisimov *et al.* 2007, Jeffries *et el.* 2014). As a result of this process, surface temperatures rise, resulting in increased permafrost thaw depth (Eugster *et al.* 2000, Guglielmin *et al.* 2014). Warming trends in polar regions are linked to glacial retreat, ice shelf calving and collapse, and reductions in ice and snow cover (ACIA 2004, Anisimov *et al.* 2007, Turner *et al.* 2009, Cook *et al.* 2010, Serrezze and Barry 2014, Turner *et al.* 2014, Cook *et al.* 2016). Ice cover in the Arctic Ocean, which

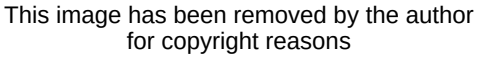



*Figure 1.2 Polar amplification. Physical changes at the Earth's surface are more pronounced at high latitudes, with the effect being greater in the Arctic than in the Antarctic (image adapted from ACIA 2004, Antarctic image taken from shutterstock.com).*

is strongly correlated with surface temperature, is declining rapidly at a rate of 15 – 17 % per decade (Comiso 2012, Stroeve *et al.* 2012). The ten lowest minimum Arctic sea ice extents have been recorded in the last decade, with the minimum, recorded in 2012, being 45 % lower than the 1981–2010 mean (NSIDC 2016). Antarctic Peninsula sea ice cover has also reduced by 40 % in the last 25 years (Stammerjohn *et al.* 2008). Sea ice cover in East Antarctica is, however, currently increasing in area by 1.3 % per decade, which is a combined effect of ozone layer loss, altered wind circulation patterns and intensification of the freshwater cycle around Antarctica (Zhang 2007, Turner 2009, Liu and Curry 2010, Turner *et al.* 2014).

Mean annual near surface air temperature on the western Antarctic Peninsula increased by 2.8 °C in the second half of the 20$^{th}$ Century, with most of this increase being associated with warming in the winter months (Marshall *et al.* 2006, Turner *et al.* 2014). The western and northern Antarctic Peninsula experienced the highest temperature increases in the winter season (0.54 °C per decade between 1951 and 2011; Turner *et al.* 2014), with the eastern side of the Antarctic Peninsula warming by 0.39 °C per decade between 1946 and 2011 during the summer and autumn (Turner *et al.* 2014). Although this regional trend has temporarily paused since the late 1990s, it is predicted to continue under moderate greenhouse gas emission scenarios (Bracegirdle *et al.* 2008, Bracegirdle and Stephenson 2012, Turner *et al.* 2016).

The Arctic is warming faster than any other place on Earth (ACIA 2005, IPCC 2007). This is likely due to loss of sea ice, and changes in atmospheric and oceanic circulation (Miller *et al*. 2010, Isaksen *et al.* 2016). Temperature records from 1911 show positive annual and seasonal (spring, summer and autumn) temperature increases (Førland *et al.* 2011), with particularly high temperature increases being observed in winter towards the end of the 20$^{th}$ Century (Førland *et al.* 2011). On Svalbard in the High Arctic, annual air temperatures during 1981–2010 were 2.1 °C higher than the 1961–1990 mean, with winter and summer temperatures increasing by 3.4 °C and 1 °C, respectively (Førland *et al.* 2011). There was a decrease in the number of cold days (temperature (T) < -10 °C) and an increase in the number of warm days (T > 5 °C) each year (Gjelten *et al.* 2016). The number of extreme winter warming events in the Arctic, including warm weather episodes and occasional intense rainfall, has increased in the last 50 years, leading to a higher number of melt days, specifically 9.2 days decade$^{-1}$ on Svalbard and 3–7 days decade$^{-1}$ on the Arctic mainland (Norway, Sweden and Finland

north of the Arctic Circle, at 66.33 °N, Gjelten *et al.* 2016). Predictions suggests that the number of warming events, compared to 1985–2014, will double in the Arctic and triple on Svalbard during the 21st Century (Vikhamar-Schuler *et al.* 2016).

## 1.3 Climate change impacts on the fauna and flora of polar regions

Increased temperature is affecting terrestrial habitats across polar regions, with the ranges of organisms adapted to cold temperatures shrinking, and species from lower latitudes becoming more prevalent (Vincent *et al.* 2011). The fauna of polar ecosystems is changing in response to climate warming. In Antarctica, climate change affects sea ice extent, and hence krill biomass, with its subsequent effects on higher level marine predators. For example, climate change in Antarctica affects seal population sizes owing to its influence on the animal food supply, habitat availability and reproductive rates (Forcada *et al.* 2012). Adélie penguin populations have also contracted in their distribution and moved poleward, while Gentoo and Chinstrap penguins have expanded their range southward (Forcada and Trathan 2009). In the Arctic, climate change threatens polar bear populations, which rely on sea ice cover for breeding, hunting and migration (Hunter *et al.* 2010). Shrinking sea ice cover affects the behaviour of bears, which spend more time feeding at ground-nesting bird colonies (Hamilton *et al.* 2017) that do not provide sufficient nutrition (Dey *et al.* 2016).

Climate warming is also affecting plant communities and populations, both in tundra ecosystems of the Arctic (Walter *et al.* 2002, Bellard *et al.* 2012, Elmendorf *et al.* 2012) and the Antarctic (Robinson *et al.* 2003, Convey *et al.* 2006, Robinson *et al.* 2018). Vegetated soils in the Antarctic are dominated by sparse tundra type vegetation, notably grass-herb fellfield, and moss and lichen communities (Bliss 1979, Day *et al.* 2008). The two native Antarctic flowering plant species, *Colobanthus quitensis* and *Deschampsia antarctica,* represent the climax vegetation of the maritime Antarctic (Day *et al.* 2008), with increases in the abundances of both species, most probably arising from increases in surface air temperatures, having been recorded in recent decades (Fowbert and Smith 1994, Grobe *et al.* 1997).

Tundra ecosystems are also frequent in the Arctic, with shrub-sedge tundra, tussock dwarf-shrub tundra and wet graminoid-moss tundra occurring in the Low Arctic, and barren polar semi deserts, dominated by mosses and lichens, occurring in the High

Arctic (Chapin *et al.* 2012). Shrubification, a significant increase in the abundances and biomasses of shrub and woody plant species, has occurred widely across Arctic tundra in recent decades (Figure 1.3; Myers-Smith 2011, Mod and Luoto 2016).

Although significant increases in the ranges and sizes of plant populations have been recorded in both the Arctic and Antarctic over recent decades (Fowbert and Smith 1994, Myers-Smith 2011), it is not yet clear how these changes will affect overall ecosystem processes, as changes in belowground processes, and interactions between plants, soil microbes and nutrient cycling, are still not fully understood (Hill *et al.* 2011).


*Figure 1.3 Increase in plant biomass in Arctic tundra in recent decades (from Epstein* et al. *2012).*

## 1.4 Ecosystem processes in polar regions

Ecosystem processes include complex interactions between biotic and abiotic factors (Tirri *et al.* 1998) that involve the transfer of energy and nutrients within the ecosystem (Lyons *et al.* 2005). They include soil respiration, decomposition, nutrient cycling, primary production and community dynamics, and are driven by the soil environment, the most complex component of terrestrial ecosystems (Barrios 2007, Wallace 2007). Soil respiration, which generates $CO_2$, integrates all soil metabolic functions (Lundergårdh 1927). The evolution of $CO_2$ from soil is mainly driven by the catabolism of soil organic compounds by the microbial community, but is also affected by abiotic processes (i.e., the chemical oxidation of organic and inorganic compounds; Bunt and Rovira 1954, Singh and Gupta 1977, Chapin *et al.* 2002, Boyero *et al.* 2014). A simplified model of terrestrial ecosystem functioning shows that the decomposition

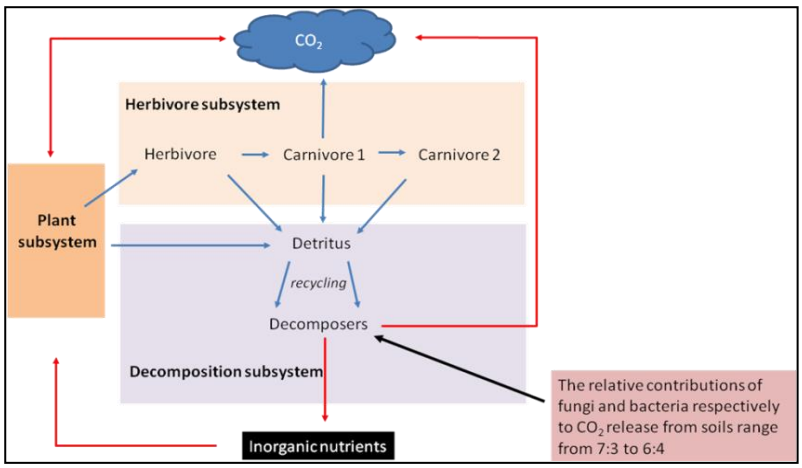


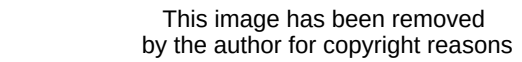

*Figure 1.4 General model of terrestrial ecosystem processes. Based on Fig. 1.10 in Swift, Heal and Anderson (1979) and data in Joergensen and Wichern (2008).*

subsystem recycles inorganic nutrients, and releases them to be absorbed by plants and other organisms (Figure 1.4).

The main abiotic factors that affect decomposition are the chemical composition of the material to be decomposed and the environmental conditions under which it breaks down, with temperature and water availability strongly affecting the process (Swift *et al.* 1979). Water is required by microbes for growth and for the breakdown of organic matter to enable transportation of chemical compounds. Sufficient soil moisture is crucial for decomposition and to support the process as soils warm (Aerts 2006), as it affects fungal and bacterial growth and enzyme production (Marin *et al.* 1995), and overall microbial diversity (Arenz *et al.* 2006, Newsham *et al.* 2016). When water is present in a frozen state, it is not accessible to micro-organisms and decomposition will be inhibited. Evaporative loss of water from soil surfaces in polar regions will not only slow decomposition processes, but will also lead to increased concentrations of ions at soil surfaces, such as the accumulation of chloride that occurs at soil surfaces in the McMurdo Dry Valleys in continental Antarctica (Barrett *et al.* 2009). At the other extreme, high water content can inhibit decomposition by restricting oxygen availability and reducing aerobic respiration (Flanagan & Vaum 1974, Rosswall & Hall 1974, Bunnell *et al.* 1977, Boddy 1986). Hence, in the Arctic and Antarctic, water is considered to be much more of a limiting factor for decomposition than temperature (Doeksen and Drift 1963, Convey *et al.* 2014).

In tundra environments, where most of the Earth's organic carbon has accumulated (Olson 1963, Tarnocai 2009), the rate of decomposition is low, compared to other, warmer ecosystems (Figure 1.5; Douglas and Tedrow 1959, Wanner 1970). Currently, there is a simplified, and unsubstantiated, expectation that climate warming and consequent increases in water availability in polar regions will affect the metabolic activities of organisms positively (Aerts 2006, Convey 2011, Deslippe and

Hartmann 2011, Pearson *et al.* 2013, Sistla *et al.* 2013), resulting in net $CO_2$ loss as soil temperatures rise (Cahoon *et al.* 2012).




As a consequence of these positive effects on catabolism, changes to the composition of soil communities are anticipated at the species level both in the Arctic (Semenova *et al.* 2015) and Antarctic (Ludley and Robinson 2008, Convey *et al.* 2014, Newsham *et al.* 2016).

*Figure 1.5 Decomposition rates in different ecosystems. Note that the rate of decomposition of organic matter is lowest in tundra. Figure adapted from Swift* et al. *(1979).*

## 1.5 Soil fungi

Fungi are the "living networks that maintain cycles of growth, death and decay in the ecosystem, interconnecting the lives of plants and animals in innumerable and often surprising ways" (Rayner 1993). These microbes, which are essential components of terrestrial biomes, play vital ecological roles in all soils as decomposers, pathogens of plants and animals, and as partners in symbioses such as the mycorrhizal and lichen mutualisms (Swift *et al.* 1979, Lynch and Leij 1995, Newsham *et al.* 2016). Despite the importance of fungi in terrestrial ecosystems, little is known about the majority of the estimated five million species present on Earth (Blackwell 2011, Hawksworth 2011), with only approximately 120,000 species of fungi having been described to date (Hawksworth and Lücking 2017).

Fungi dominate soil microbial communities in organic soil horizons in polar regions, with the biomass of fungi being up to 10 times greater than that of bacteria (Schmidt and Bölter 2002), and with $CO_2$ respired from fungal biomass almost always exceeding that respired from bacterial biomass (Joergensen and Wichern 2008; Fig. 1.4). Moreover, in permafrost, fungi are more resistant to hostile conditions than are bacteria, leading to greater fungal biomass in these soils (Steven *et al.* 2006). In polar regions, much mycological research has focused on the identification of fungi isolated from soil onto artificial growth media (Möller and Dreyfuss 1996, Blanchette *et al.* 2004,

Arenz *et al.* 2006, Alias and Suhaila 2008, Ludley and Robinson 2008, Krishnan *et al.* 2011, Ali *et al.* 2013). These studies have repeatedly isolated filamentous saprotrophic fungi such as species of *Pseudogymnoascus, Penicillium*, *Cladosporium, Phialophora, Phoma, Tetracladium* and *Mortierella*, along with many other genera that have wide distributions in polar regions and are key components of both Arctic and Antarctic soils (Table 1.1, Kurek *et al.* 2007, Singh *et al.* 2012). More recently, molecular methods, such as cloning (Lawley *et al.* 2004, Bridge and Newsham 2009), denaturing gradient gel electrophoresis (Arenz *et al.* 2006, Yergeau *et al.* 2007) and next generation sequencing (Leung *et al.* 2011, Ihrmark 2012, Dennis *et al.* 2012, Ji *et al.* 2015, Semenova *et al.* 2015, Cox *et al.* 2016, Newsham *et al.* 2016) have been applied to the study of soil fungi in polar regions. These studies have identified a wider range of fungi than those determined using conventional culturing, with chytrids, lichen forming fungi, and yeasts frequently occurring in clone and next generation sequencing libraries (Ji *et al.* 2015, Semenova *et al.* 2015, Cox *et al.* 2016, Newsham *et al.* 2016).

*Table 1.1 The most frequent fungal genera identified in Arctic and Antarctic soils[1].*

| **Fungal growth form or ecological role** | **Antarctic** | **Arctic** |
|---|---|---|
| lichenized | *Acarospora* | *Atla* |
| | *Polyblastia* | *Verrucaria* |
| | *Verrucaria* | |
| saprotrophs | *Acremonium* | *Acremonium* |
| | *Alatospora* | *Aspergillus* |
| | *Antarctomyces* | *Cadophora* |
| | *Aspergillus* | *Cladosporium* |
| | *Cladophialophora* | *Fusarium* |
| | *Cladosporium* | *Mortierella* |
| | *Fusarium* | *Nectria* |
| | *Glarea* | *Phialocephala* |
| | *Penicillium* | *Phialophora* |
| | *Phoma* | *Phoma* |
| | *Pseudeurotium* | *Pseudogymnoascus* |
| | *Pseudogymnoascus* | *Tetracladium* |
| | *Tetracladium* | *Thelebolus* |
| | | *Verticillium* |
| yeasts | *Cryptococcus* | *Cryptococcus* |
| | *Mrakia* | *Rhodotorula* |
| | *Rhodotorula* | |
| ectomycorrhiza | | *Cortinarius* |
| | | *Inocybe* |
| | | *Phialocephala* |
| | | *Sebacina* |

[1]*Data based on Bridge and Spooner (2012), Timling and Taylor (2012) and Timling* et al. *(2014)*

Although fungal representatives from all major phyla have been found in extremely cold environments (Zalar and Gunde-Cimerman 2014), there are some clear patterns of fungal distribution in polar soils. For example,

ectomycorrhizal fungi are widely dispersed across the Arctic, reflecting an abundance of suitable woody host plant species, with *Inocybe*, *Russula, Sebacina, Cortinarius*, *Hebeloma*, *Thelephora* and *Lactarius* being the most common genera found in soil (Timling *et al.* 2014). In contrast, ectomycorrhizas are not found in the Antarctic because of a lack of suitable host plant species (Newsham *et al.* 2009), although the DNA of these fungi can be polymerase chain reaction (PCR) amplified from soil as far south as 72 °S, many hundreds of kilometres from the nearest plant hosts in South America (Bridge and Newsham 2009). Similarly, sporocarp-forming Basidiomycota are frequent in the Arctic (Väre *et al.* 1992, Väre *et al.* 1997), but only occur very infrequently in maritime Antarctic, with an apparent southerly limit of *ca*. 67 °S on the Antarctic Peninsula (Pegler *et al.* 1980; K.K. Newsham pers. communication). Basidiomycete yeasts prevail in the soils of both polar regions. Yeasts are particularly well adapted to polar environments, and dominate soil fungal communities in very cold and dry habitats, such as the McMurdo Dry Valleys (Butinar *et al.* 2007, Zalar & Gunde-Cimerman 2014), possibly owing to their tolerance of high anion concentrations in soil (K.K. Newsham pers. observ.). Ascomycota, including lichenized and saprotrophic fungi, are dominant in polar soils (Zhang *et al.* 2016, Newsham *et al.* 2016). Of the ascomycetes, the genus *Pseudogymnoascus* (formerly *Geomyces* and *Chrysosporium*) is one of the most frequently isolated genera in cold regions (Krishnan *et al.* 2011, Ali *et al.* 2013) and, in a DNA-based survey, was the only genus to occur in all 29 fellfield soils sampled from a 1,650 km transect throughout almost the entire maritime Antarctic (Newsham *et al.* 2016). Lichen-forming fungi are also widespread and common in Arctic and Antarctic soils, and are dominated by members of the genus *Verrucaria* in both regions (Timling *et al.* 2014, Newsham *et al.* 2016). In addition to these patterns of occurrence of fungi in polar soils, high throughput sequencing of DNA extracted from Antarctic soil has shown that the range distributions of polar fungi vary between circumpolar and regionally endemic species, as well as bipolar and cosmopolitan fungi (Cox *et al.* 2016).

## 1.6 Extracellular enzymes

Micro-organisms secrete catabolic enzymes; extracellular hydrolases that breakdown complex polymers (such as cellulose) into smaller molecules which can then be absorbed and utilised intracellularly (Swift *et al.* 1979, Baldrian 2008). These enzymes

have central roles in the cycling of nutrients in ecosystems. For example, B-glucosidase and phosphomonoesterases are involved in the mineralization of carbon, nitrogen, phosphorus and sulphur compounds in soils (Taylor *et al.* 2002). The production of extracellular enzymes by soil fungi from lower latitudes has been studied because of their significance in the decomposition of soil organic compounds (Table 1.2; Bandick and Dick 1999, Sinsabaugh *et al.* 2008, Hiscox *et al.* 2010, A'Bear *et al.* 2014). Research on the enzymology of polar soil fungi has been limited, and comprehensive understanding of the effects of environmental factors on the enzyme activities of polar soil fungi is consequently lacking (Robinson *et al.* 2001, Robinson 2002, Ludley and Robinson 2008, Leung *et al.* 2011). Some studies have concentrated on determining the range of enzymes produced by polar soil fungi. For example, Singh *et al.* (2012) screened extracellular enzymes produced by Arctic soil fungi, and showed that *Aspergillus* sp., *Cladosporium* sp., *Emericella* sp., *Pseudogymnoascus pannorum*, *Phialophora* spp., *Preussia* spp. and *Xylaria* spp. produced amylase, cellulase, phosphatase or pectinase. Similarly, Krishnan *et al.* (2011) screened the enzymes secreted by mostly ascomycetes from Antarctic soil and showed amylase, cellulase and protease production by the isolates.

*Table 1.2 Enzymes commonly produced by soil fungi.*

| Enzyme function | Enzymes |
|---|---|
| phosphatases | acid phosphatase, alkaline phosphatase |
| glycosyl hydrolases | **L-glucosidase**, B-glucuronidase, L-fucosidase |
| cellulolytic | cellobiohydrolase, **B-glucosidase** |
| lipolytic | **esterase,** C-8 lipase, C-14 lipase |
| aminopeptidases | leucine amnopeptidase, valine aminopeptidase, cysteine aminopeptidase |
| chitinolytic | chitobiosidase, N-acetylglucosaminidase |
| hemicellulolytic | **B-galactosidase**, L-galactosidase, L-mannosidase |
| protease | trypsin, chymtrypsin |
| laccase | laccase |

*Information based on Baldrian* et al. *(2011) and Eichlerova* et al. *(2012). Note that the most frequent enzymes produced by the fungi studied here are marked in bold.*

Changes to soil enzyme activities could be an early warning of ecosystem stress to changing conditions, but few studies have assessed the effects of environmental factors, such as nutrient addition or temperature change, on the synthesis of enzymes by polar soil fungi (Aon *et al.* 2001). Wallenstein *et al.* (2008) showed that the production of

N-acetylglucosaminidase, B-glucosidase and peptidase by polar fungi was nitrogen limited. Krishnan *et al.* (2017) examined the effects of temperature manipulations on the enzyme activities of polar soil fungi, specifically the production of amylase and cellulase by *Pseudogymnoascus* spp*., Cosmospora* spp., and other unidentified fungi, and showed a negative correlation between fungal growth and fungal enzyme activity, suggesting that increased temperature stresses cells and affects their ecophysiology.

## 1.7 Psychrophily in polar soil fungi

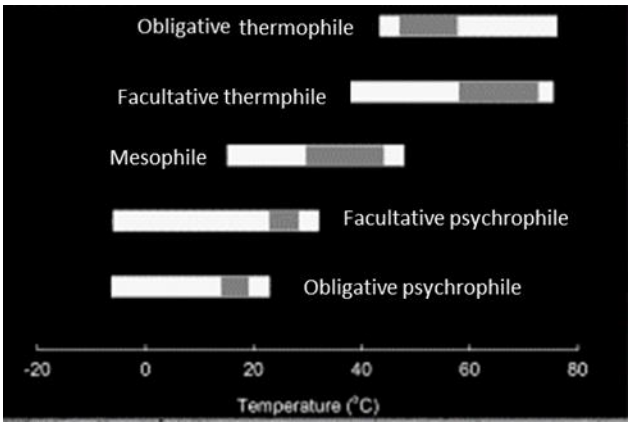



*Figure 1.6 Temperature ranges over which fungi and other microbes can grow. White bars define absolute temperature ranges, and grey inserts denote optimal ranges. Adapted from Kirstidali (2009).*

The majority of microbes in polar environments tend to be cold– adapted mesophiles with growth temperature ranges between 0 °C and 45 °C, and optimum growth temperatures of *ca.* 15–20 °C (Russell *et al.* 1990, Baross & Morita 1978). Some taxa of fungi endemic to polar regions may however be obligate psychrophiles. These fungi, such as the snow mould *Typhula ishikariensis* (Tojo and Newsham 2012), are capable of growing at 0 °C or below, and have maximum growth temperatures of *ca.* 20 °C, and optimum growth temperatures of *ca*. 15 °C or below (Figure 1.6; Robinson 2001, Zalar and Gunde- Cimerman 2014). With the upper cardinal growth temperatures for psychrophilic fungi likely to be exceeded in the natural environment (soil surface temperatures exceeding > 20 °C, Section 1.1), it is conceivable that the growth and activities of these psychrophilic soil fungi will be affected in the natural environment. This possibility has hitherto received no apparent attention, with the majority of studies concentrating on the effects of increases in temperatures at or close to freezing point as predictors for how polar soil microbes will respond to climate warming (Mikan *et al.* 2012). Furthermore, Convey *et al.* (2018) suggest that the minimum and maximum temperatures to which organisms are exposed may have significant impacts on their

physiologies, and yet these effects are not considered in studies of the effects of warming on polar ecosystems.

## 1.8 Thesis outline

Increases in temperature, water and nutrient availability associated with climate change in polar regions are likely to affect the growth and metabolic activities of saprotrophic fungi, microbes that are central to decomposition and nutrient cycling processes in soils. It is crucial to improve current understanding of the effects of climate change on polar soil fungi. The aim of the research in this thesis is to examine, through a series of field and controlled environment studies, the effects of warming, water and nutrient availability on saprotrophic fungal growth and enzyme activity in High Arctic (area above 75 °N, Dowdeswell 1995) and maritime Antarctic soils in order to predict how these microbes might respond to future climate change.

Field studies were carried out on established experiments. Chapter 2 describes these field experiments, the physicochemical characteristics of the soils and the methodologies used to study the fungi present in samples taken from them. Chapter 3 uses quantitative (Q-)PCR to investigate the main and interactive effects of warming and nutrient additions on the abundances of the widespread polar soil fungus *Pseudogymnoascus pannorum,* and total fungi in a long-term (five year) field experiment in the southern maritime Antarctic. A series of laboratory experiments are reported in Chapter 4 examining the growth and enzyme activity of *P. pannorum* at temperatures and water potentials encountered in the austral summer in the southern maritime Antarctic.

Chapter 5 uses Q-PCR to examine the effects of warming and water application on the abundances of seven key culturable soil fungal taxa in a three-year field warming experiment on Svalbard in the High Arctic. In Chapter 6, the main and interactive effects of elevated temperature and water availability on the hyphal extension rates and enzyme activities of 13 High Arctic soil fungal strains, including six taxa studied in Chapter 5, are examined in laboratory experiments. As in Chapter 4, these experiments simulated conditions encountered in High Arctic soils during summer. Finally, in Chapter 7, the experimental findings are synthesized, discussed in relation to other studies in the literature, and proposals are made for new avenues of research.

# 2 Methods

## 2.1 Description of Antarctic field site

The maritime Antarctic field site was located at Mars Oasis (Two Step Cliffs) on the south-eastern coast of Alexander Island, off the western Antarctic Peninsula (71° 52, 4' S, 68° 15, 00' W, Figure 2.1). This site has been described in detail by Convey and Smith (1997), Bridge and Newsham (2009) and Newsham *et al.* (2010). It consists of an upper and lower terrace, with the upper terrace formed of sandstone and mudstone, and the lower terrace being composed of till, fluvial and lacustrine sediments with very limited moss cover (Sugden and Clapperton 1981, Newsham *et al.* 2010).

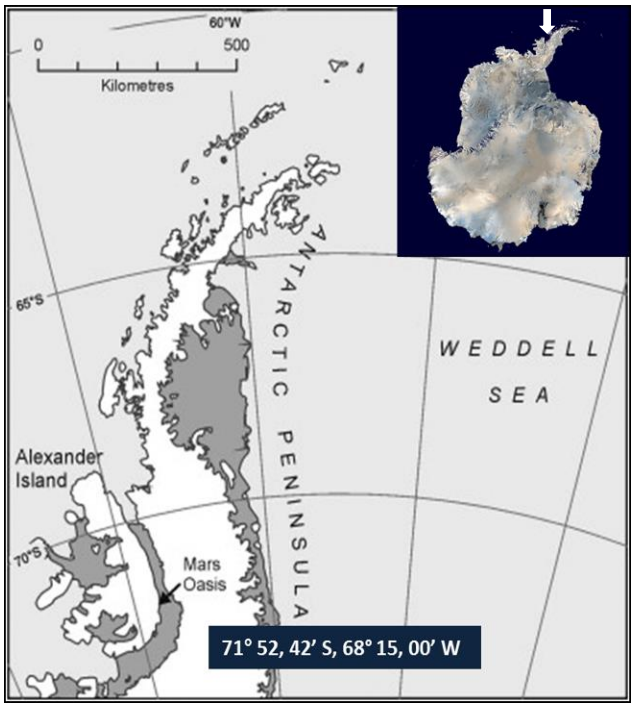



*Figure 2.1 Location of the Antarctic field site at Mars Oasis on Alexander Island, western Antarctic Peninsula.*

The climatic characteristics of the site are amongst the most extreme of the maritime Antarctic, and in some respects approach those of continental Antarctic Dry Valleys ecosystems (Convey and Smith 1997). Mean annual air temperature at Mars Oasis is - 10 °C and mean June temperature is -22 °C. During December and January, the mean air temperature at the site is *ca.* 1 °C (Bridge and Newsham 2009, Dennis *et al.* 2012).

### 2.1.1 Soil warming experiment

A long term soil warming experiment was set up on the lower terrace at Mars Oasis in 2007, on a level, homogeneous expanse of exposed soil (Figure 2.2). Sixty-four circular plots (1 m diameter) were established over a 17 m x 17 m area, to which warming and three nutrient amendment treatments (simulating inputs of plant matter to soil) were applied in factorial combination (Dennis *et al.* 2013). Soil in half of the plots was warmed using open top chambers (OTCs), which increased soil temperature by an

average of *ca*. 2 °C during summer. Open top chambers are commonly used as a means of warming plants and soils in remote regions with no reliable power supplies (Bokhorst *et al.* 2011, 2013). Nutrient amendments, applied at concentrations of 2.0 mg C $g^{-1}$ dry wt soil, consisted of powdered glucose, glycine or powdered tryptic soy broth (Difco, USA) mixed into soil with a sterile spoon to *ca*. 20 mm depth. Unamended soil, to which substrates were not added, was also mixed with a sterile spoon to *ca*. 20 mm depth. The experimental design resulted in eight combinations of treatments, each replicated eight times (Appendix 1).

Soil temperatures were recorded in three control plots and three OTC plots at a depth of *ca*. 10–50 mm, using temperature loggers (TinyTag Plus 2 TGP-4027, Gemini Data Loggers Ltd. Chichester, UK). Hourly temperature data were collected (Figure 2.3). Soil samples were initially gathered in November 2007, prior to start of the experiment, and then annually, in December, between 2009 and 2012. Using sterile spoons, soil from each plot was placed into a clean tube and frozen at -20 °C prior to transport to the UK and storage at the same temperature until further processing.

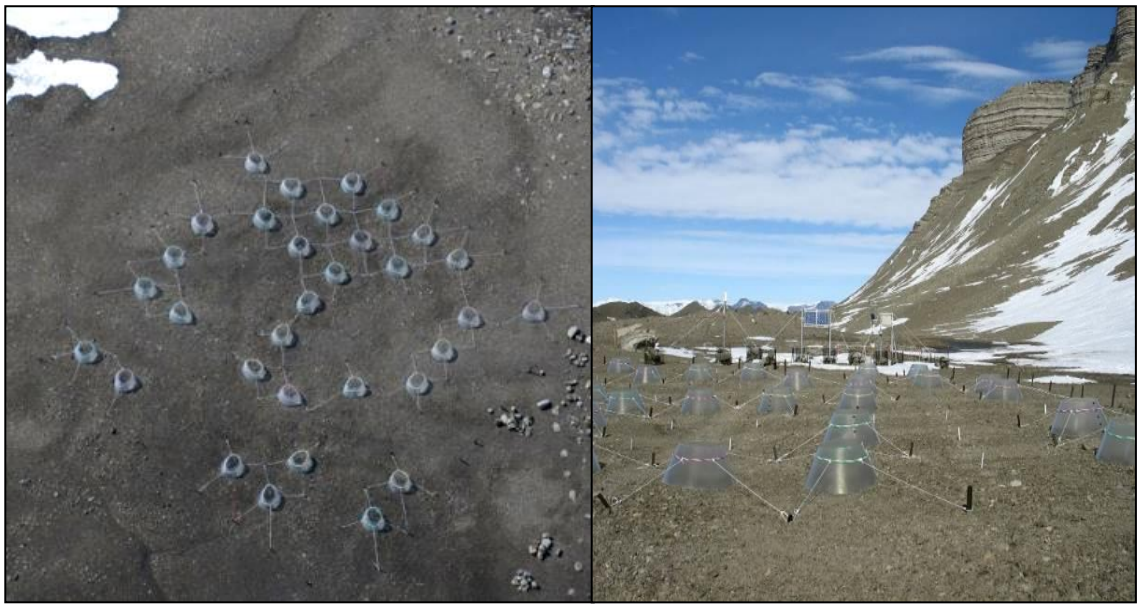


*Figure 2.2 The Mars Oasis field warming experiment; an aerial view of the experiment, which was located on an extensive and level area of fluvial till (left), and the open top chambers that were used to warm the soil (right).*

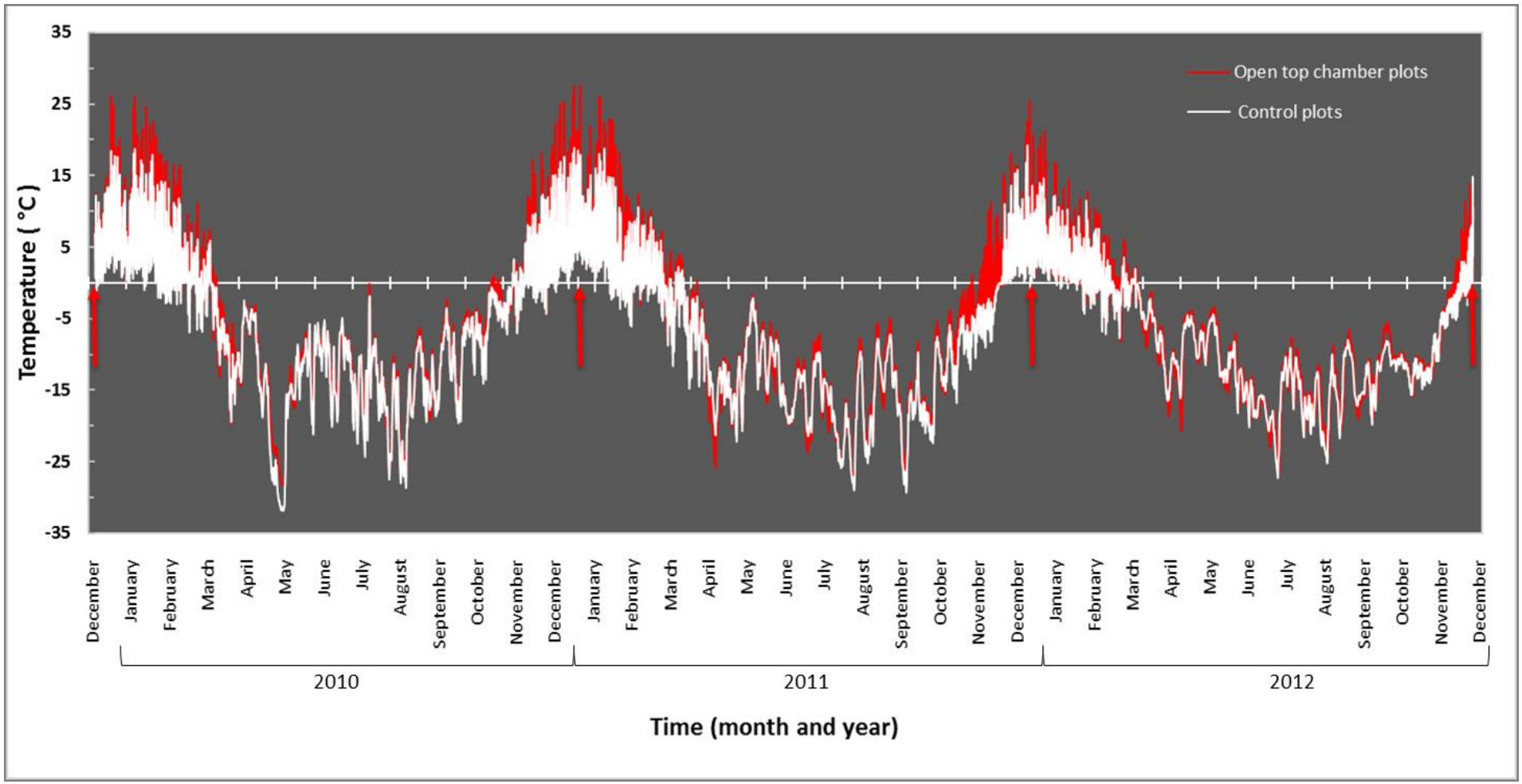



*Figure 2.3 Soil temperatures recorded hourly at the Mars Oasis experimental site between December 2009 and December 2012. Temperatures were recorded at a depth of* ca. *1 – 5 cm using Tiny Tag Plus 2 TGP-4017 data loggers in control (white line,* n=*3) and warmed (open top chamber, red line*, n=*3) plots. Soil samples were collected annually at the beginning of December (arrows).*

## 2.2 Description of High Arctic field site

The Arctic field site was located on Svalbard at Kvadehuken on the Brøggehalvøya Peninsula, approximately 11 km north-west of Ny-Ålesund (78° 58.002' N, 11° 28.446'; Figure 2.4) in an area exposed to very low anthropogenic impacts. The site is a polar desert (CAVM Team, 2003) located on a coastal terrace (Figure 2.5). It has a mean July air temperature of 5 °C and a mean annual precipitation of 385 mm water equivalent (Førland *et al.* 1997, Brossard *et al.* 2002). It is a typical High Arctic tundra habitat, with frost boils of soil surrounded by dolomite limestone rock. The soils around the boils are colonised by *Salix polaris*, *Bistorta vivipara*, *Saxifraga oppositifolia*, a range of mosses and lichens, and soil microbial crusts.

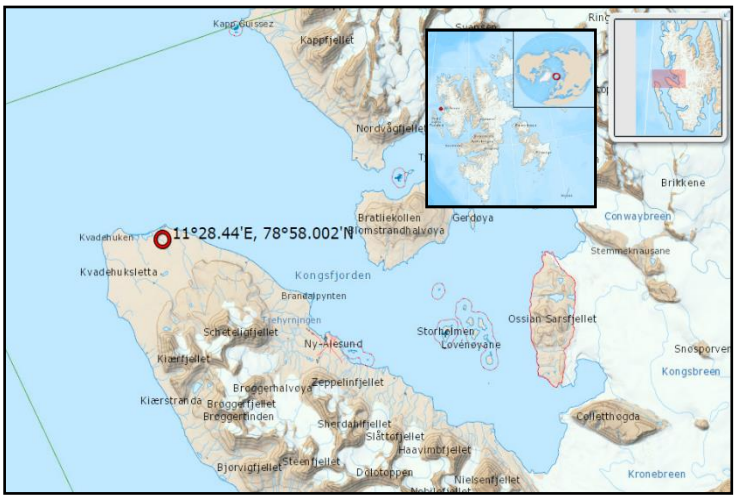



*Figure 2.4 Location of the High Arctic field site on Svalbard, where a soil warming experiment using ITEX open top chambers was established in 2014. Map taken from https://toposvalbard.npolar.no.*

*Figure 2.5 Location of the soil warming experiment on the Brøggerhalvøya Peninsula on Svalbard. The ecosystem is a typical High Arctic polar desert habitat with patterned ground (inset) located on a coastal terrace (arrow).*

### 2.2.1 Soil warming experiment

A long-term soil warming experiment was set up at the site in September 2014. As in the experiment at Mars Oasis, OTCs were used to elevate the soil surface temperature (0–5 cm) by *ca.* 1–2 °C during the summer. Since the 1990s, OTCs have been used by the International Tundra Experiment programme (ITEX, Henry and Molau 1997) to investigate the effects of warming on terrestrial ecosystems (e.g., Welker *et al.* 1993, Wookey *et al.* 1993). Forty-eight plots, arranged in three blocks, were established over periglacial patterned ground (Figure 2.6). Two treatments, warming and water addition, were applied in a fully factorial combination, resulting in four treatment types (control plots, warmed plots, watered plots, and warmed and watered plots), each replicated 12 times over three blocks (Appendix 1).

The OTCs used at the experiment had a basal diameter of 1.2 m, an aperture diameter of 0.75 m and a height of 0.4 m (Marion *et al.* 1997). The water treatment was delivered by the application of 1 L of deionised water onto each frost boil at the beginning and end of each growing season (usually mid-June and late August – early September). In late August or early September 2014, 2015 and 2016, temperature loggers (TinyTag Plus 2 TGP-4017, Gemini Data Loggers Ltd., Chichester, UK) were inserted into the soil at a depth of *ca*. 0–5 cm in four chambered plots and four unchambered plots to log soil temperature (Figure 2.7). Soil sampling took place at the beginning of September 2014 and in late August 2015, 2016 and 2017. Soil samples (*ca*. 15 g) were collected from a depth of 0–5 cm using sterile spoons.

*Figure 2.6 The High Arctic soil warming experiment. Forty-eight plots were established over soil polygons in September 2014. Open top chambers provided a warming treatment, whilst deionised water was applied to simulate increased precipitation, resulting in four treatment types, each replicated 12 times over three blocks.*

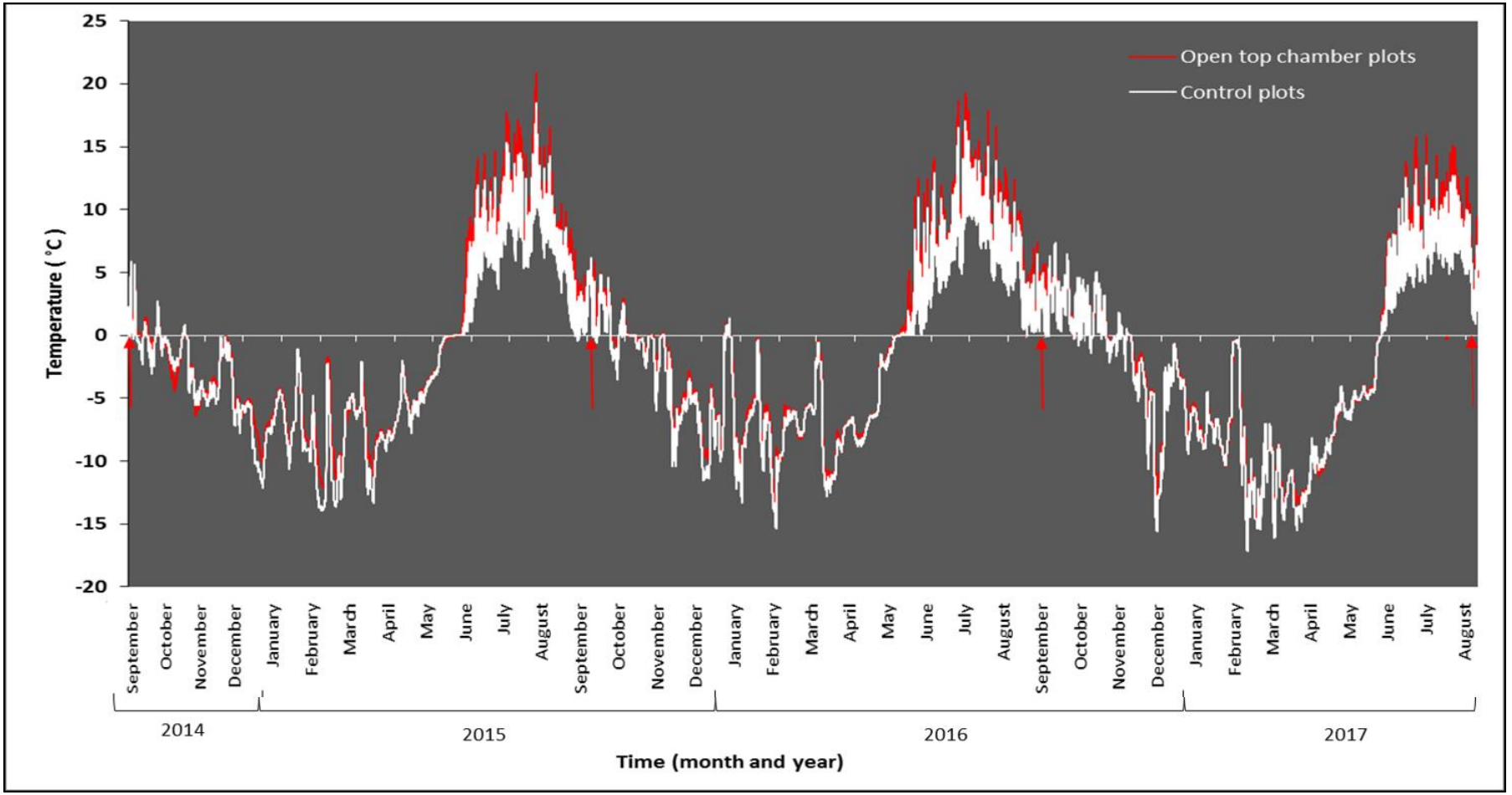



*Figure 2.7 Soil temperatures recorded hourly at the High Arctic warming experiment between September 2014 and August 2017. Temperatures were recorded at a depth of* ca. *0 – 5 cm using Tiny Tag Plus 2 TGP-4017 data loggers in control plots (white line,* n=*4) and warmed (open top chambers) plots (red line,* n=*4). Soil samples were collected annually at the end of August or the beginning of September (arrows).*

Each soil sample was placed into a sterile tube and frozen at -20 °C as soon as possible (normally within 4–8 h), prior to transport to the UK (under cool conditions, *ca*. 0 °C) normally lasting for 30 h, and then storage at -20 °C until further processing.

## 2.3 Soil characteristics

Soils were characterized by measuring pH, moisture, organic matter concentration, and total carbon and nitrogen concentration prior to the warming experiments being deployed in 2007, at the Antarctic site and 2014, at the Arctic site. A sample of soil from each of the plots was used for these analyses ($n$ = 64 for Antarctic soils and $n$ = 48 for Arctic soils, unless specified otherwise). For the pH measurements, approximately 1 g of fresh soil was weighed into 15 ml plastic centrifuge tubes and topped up with deionised water (1:2.5 ratio). The suspensions were shaken vigorously by hand and left for 10 min to settle before the pH was measured using a Thermo Scientific Orion meter (Paisley, United Kingdom). Soil moisture (expressed as a percentage of oven dry weight) was measured by calculating the change in weight of approximately 200 mg of 2 mm-sieved soil after drying at 105 °C for 24 h in preweighed aluminium foil packets. Soil organic matter was determined by loss on ignition, measuring the change in mass of the dried soil used for moisture determinations after ashing in a muffle furnace for 2 h at 550 °C (Allen 1989). Total carbon and nitrogen concentrations were determined in dried soil encapsulated in tin using a CE-440 Elemental analyzer (EIA – Exeter Analytical, Inc. Coventry, UK) with acetanilide standards.

Biological water availability of the soil was determined by preparing wetting and drying soil moisture curves, and measuring the water potential (Ψ) of the soil at 18 °C using a WP4C dewpoint potentiometer (Decagon Devices, Pullman, USA), following the manufacturer's directions. Between three and five replicate soil samples were used for preparing each curve. Homogenised soil was dried in an oven at 60 °C for 24 h and passed through a 2 mm sieve before being placed into metal cups. For the wetting curves, soil was mixed with different volumes of deionised water to obtain a range of soil moisture concentrations (1–10 % for Antarctic soils and 1–50 % for Arctic soils) and was left to equilibrate for 24 h at 15 °C before Ψ was measured. For the drying curves,

soil samples were mixed with deionised water to achieve a range of soil moisture concentrations (1–50 %) and left to equilibrate as described above. Measurements of soil Ψ were recorded and the mass of each sample was measured immediately afterwards. After the first measurement, each sample was air–dried for an hour and the measurements repeated. The measurements were taken until there was no further appreciable change in the mass of the soil (up to 48 h). The samples were then placed in a drying oven at 60 °C for 24 h. The mass of oven dried soil was used to determine the moisture concentration of the soil at each reading.

Soil at Mars Oasis had a pH of 7.3–8.3, a soil moisture content of *ca*. 2 %, a low organic matter concentration (*ca*. 3 %), and low total carbon and nitrogen concentrations (*ca.* 0.25 % and *ca*. 0.02 %, respectively). Biological water availability (expressed as Ψ) varied between -1.5 and -10 MPa for soil moisture concentrations of 2–8 %, and decreased to <-70 MPa for a soil moisture concentration of *ca*. 1 %, based on the soil moisture curves (Appendix 2). Further details of Mars Oasis soil chemical parameters can be found in Newsham *et al.* (2010) and Dennis *et al.* (2012, 2013). The High Arctic soil had a mean pH of 8.37, an organic matter concentration of 3.14 % dry weight and a moisture concentration of 14.2 % dry weight. Biological water availability varied between 0 and -2 MPa for moisture concentrations of 5–50 %. Water availability decreased sharply for soil moisture concentrations below 4 % (Appendix 2).

## 2.4 Fungal species isolation, identification and quantification

The only fungus isolated from the Antarctic soil was *Pseudogymnoascus pannorum*, the most widespread and frequent fungal species detected in Antarctic soil by conventional isolation methods (e.g., Arenz and Blanchette 2011) and by 454 pyrosequencing (Newsham *et al.* 2016). A range of fungal species was isolated and identified from High Arctic soil using conventional microbiological and PCR-based methods.

### 2.4.1 Conventional soil fungal isolation and identification

To isolate fungi, soil samples from the High Arctic soil warming experiment were defrosted overnight at 4 °C. A sterile microspatula was used to dispense soil (1 – 1.5 m

dry mass) onto Czapek-Dox agar medium with reduced sucrose (1 g $L^{-1}$) and Rose Bengal (1:15,000) to slow the growth of rapidly-spreading fungi, and to suppress the growth of bacteria (Appendix 3). The soil was evenly dispersed across the surface of the medium in 90 mm diameter Petri dishes using a sterile spreader. Soil dilutions ($1 \times 10^{-1}$, $1 \times 10^{-2}$, and $1 \times 10^{-3}$ concentrations) in sterile water were also prepared, with 100 µl of each dilution being spread on the surface of Czapek-Dox agar medium with Rose Bengal and reduced sucrose in 90 mm diameter Petri dishes, as described above. All of the dishes were put into plastic ziplock bags and incubated at 4 °C, and were checked for fungal growth at one month and nine months after inoculation. Morphologically distinct colonies were subcultured onto half-strength Potato Dextrose Agar medium (PDA, Oxoid, Basingstoke, UK) and kept at < 15 °C.

### 2.4.2 Fungal identification based on DNA extracted from cultures

Morphologically distinct colonies were molecularly identified based on sequencing of Internal Transcribed Spacer (ITS) regions of ribosomal DNA, the standard barcoding region for fungi (Schoch *et al.* 2012). Genomic DNA from fungal cultures was extracted with Extract-N-AmpTM Plant PCR kits (Sigma Aldrich, Gillingham, UK). A 5 to 7 mm disk of fungal hyphae, scrapped of the cellophane disc layered on top of half-strength Potato Dextrose Agar medium, was placed in 100 µl of extraction solution and incubated at 95 °C for 10 min, followed by the addition of 100 µl of dilution solution. The ITS1-5.8S-ITS2 region of ribosomal DNA was amplified with ITS1F (5'- CTTGGTCATTTAGAGGAAGTAA-3') and ITS4 (5'-TCCTCCGCTTATTGATATGC-3') primers (White *et al.*, 1990, Gardes and Bruns, 1993). Polymerase chaing reactions consisted of 4 µl of 5 × buffer, 0.2 µl of each primer (10 nMolar primer stock solution), 0.1 µl of 5 u Taq DNA polymerase and 1 µl of template DNA in a final volume of 20 µl. The PCR reactions were run in a PCR Max Thermocycler (AlphaCycler 4, PCR Max, Stone, UK) for 35 cycles (95 °C for 60 s, 54 °C for 60 s and 72 °C for 60 s), with an initial denaturation of 95 °C for 5 min and a final extension at 72 °C for 7 min. The PCR products were separated by agarose gel electrophoresis and visualized after staining with GelRed to confirm that single products of the expected size had been amplified. They were then bidirectionally sequenced using Sanger sequencing by outsourcing to a commercial

facility (Source Bioscience, Cambridge, UK). De novo assembly of forward and reverse sequences was completed using Geneious Version 8, with manual adjustments and base call editing when required. Consensus sequences derived from the assemblies (Appendix 4) were checked against existing sequences logged in GenBank using BLAST (https://blast.ncbi.nlm.nih.gov/Blast.cgi) with standard parameter search settings (nucleotide collection database and highly similar sequences).

### 2.4.3 Fungal identification based on DNA extracted from soil

In an attempt to characterise the soil fungal community, total fungal DNA was PCR-amplified from soil samples collected in September 2014 from the High Arctic warming experiment. A MoBio soil DNA extraction kit (Cambio Ltd, Cambridge, UK) was used to extract DNA from 10 samples of soil following the manufacturer's protocol, but with the bead-beating stage of the protocol being reduced from 10 to 7.5 min. Fungal ITS regions were amplified by PCR using ITS1F and ITS4 primers under the same conditions as described in Section 2.4.2. The PCR products were cloned using a TOPO TA cloning kit (Invitrogen by Thermofisher, Hemel Hempstead, UK) according to the manufacturer's protocol. Fungal PCR products were cloned into a PCR 4–TOPO vector, by mixing PCR product with the vector for 5 min at room temperature. Clones were transformed into competent *Escherichia coli* cells by adding 2 µl of the cloning reaction into OneShot Chemically Competent *E. coli* cells, which were gently mixed and incubated on ice for 5 min. The cells were then heat-shocked for 30 s at 42 °C and 250 µl of S.O.C. medium, provided with the kit, was added to the cells. The cells were shaken at 37 °C for 1 hour. Cell suspensions (10–50 µl) were spread onto selective Luria Broth medium containing 50 µg $ml^{-1}$ kanamycin and incubated overnight at 37 °C. Fifty plaques derived from cells that had positively incorporated vector–containing fungal DNA were randomly picked for PCR amplification with M13F (5'-GTAAAACGACGGCCAG-3') and M13R (5'- CAGGAAACAGCTATGAC-3') primers under the same conditions as described in Section 2.4.2. The PCR products were separated by agarose gel electrophoresis and visualized after GelRed staining to confirm that inserts of the correct size had been cloned. These PCR products were then outsourced for Sanger sequencing and edited as described in Section 2.4.2. BLAST searches of GenBank were conducted as described in

Section 2.4.2 and sequences checked against GenBank using the BLAST search as described in Section 2.4.2 as well. The resulting BLAST hits returned mostly *Polyblastia* and *Verrucaria* spp. matches.

### 2.4.4 Quantitative PCR analysis

The effects of the treatments on the abundances of soil fungal taxa were determined using Q-PCR analyses. Soil from each of the 64 plots of the Antarctic experiment sampled in 2007, 2009, 2010, 2011 and 2012 (*n* = 320), and from each of the 48 plots of the High Arctic experiment that were sampled in 2014 and 2017 (*n* = 96) were used for DNA extractions. Soil from the Antarctic was passed through a sterile 2 mm sieve to homogenize the sample and to remove larger stones prior to extractions. For the High Arctic soils, which were much wetter, roots and larger stones were removed with tweezers under sterile conditions. Approximately 1–1.5 g of fresh soil from each plot was used for DNA extractions using a DNeasy Power Soil Kit (Qiagen, Manchester, UK) following the manufacturer's protocol, but with the bead-beating stage of the protocol being reduced from 10 min to 7.5 min. The concentration of total DNA was estimated using a Nanodrop One spectrophotometer (ThermoFisher Scientific, Hemel Hampstead, UK). The DNA extracts were stored at -30 °C prior to Q-PCR analyses.

Quantitative-PCR was used to amplify fragments of the ITS2 region of ribosomal DNA of specific fungal genera. Seven pairs of primers were used for Q-PCR reactions (Table 2.1). The primers were designed, based on sequences obtained from fungal strains isolated from Arctic and Antarctic soil, using Geneious v8 (Silkeborg, Denmark) to generate PCR products of 100–200 bp in length. Quantitative-PCR assays were designed for *Pseudogymnoascus* spp. (in both soils) and for *Mortierella* spp., *Cadophora* spp., *Phialocephala* spp., *Leptosphaeria* spp., *Tetracladium* spp. and Dothideomycete species. The primers developed for *Cadophora* spp. also amplified the DNA of a closely- related *Phialocephala* spp., and so were used to estimate the abundances of both genera. Following Trabelsi *et al.* (2009) and Seear *et al.* (2010, 2012), the primers were checked for specificity using fungal DNA extracted from cultures isolated from the soil. The ITS86F/ITS4 primer set (De Beeck *et al.* 2014) was also used to measure total fungal DNA concentrations in soil (Table 2.1).

Amplification of DNA was performed in 48-well plates using an Eco 48 Machine (PCRMax Limited, Witford, UK) and SYBR Green chemistry (Agilent Technologies, Manchester, UK). Quantitative-PCR conditions were as follows: initial denaturation at 95 °C for 10 minutes, followed by 35–40 cycles of 95 °C for 30s, 59 °C for 30 s and 72 °C for 30 s, followed by generation of melting curves to verify amplification specificity (Appendix 5). Reactions contained 0.5 µl of template DNA, 5 µl of SYBR Green Master Mix (Agilent Technologies, Manchester, UK), 0.2 µl of each primer (10 nMolar stock primers solution each) and 4.1 µl nuclease-free water. All samples were run in duplicate or triplicate along with a no-template control (Juardo *et al.* 2008, Seear *et al.* 2012). Standard curves were prepared for each primer pair using a dilution series of gDNA extracted from fungal cultures. For standard curves, either sterile soil DNA extracts spiked with fungal DNA of specific genera or PCR amplified products were used. Efficiency of PCR was calculated as E= 10 (-1/slope) – 1. Quantification was based on

*Table 2.1 Primers used for Q-PCR analysis of fungal DNA concentrations in soil.*

| **Primer name** | **Sequence (5' to 3')** | **Primer direction** | **Target taxon** |
|---|---|---|---|
| Pseudogymnoascus_Fr | GTCATTACAACCCTCAAGCTCAG | forward | *Pseudogymnoascus* |
| Pseudogymnoascus_Rv | GCGAGAAGAATTACTACGCTCG | reverse | *Pseudogymnoascus* |
| Mortierella_Fr | AGCATGCTTGTTTGAGTATCAGTAA | forward | *Mortierella* |
| Mortierella_Rv | AGCTCAGAGAAAAGTCCAGCT | reverse | *Mortierella* |
| Dothideomycete_Fr | TGCCTGTTCGAGCGTCATTTA | forward | Dothideomycete |
| Dothideomycete_Rv | GGTCTGCTGAAGTCGCTTG | reverse | Dothideomycete |
| Leptosphaeria_Fr | GCCTGTTCGAGCGTCATTTG | forward | *Leptosphaeria* |
| Leptosphaeria_Rv | TGGATGCCAACAATCATGACAA | reverse | *Leptosphaeria* |
| Cadophora_Fr | CGTCATTATAACCACTCAAGCTCTC | forward | *Cadophora* and *Phialocephala* |
| Cadophora_Rv | GCGAGGAGTATTACTACGCGTA | reverse | *Cadophora* and *Phialocephala* |
| Tetracladium_Fr | ATGCCTATTCGAGCGTCATTATC | forward | *Tetracladium* |
| Tetracladium_Rv | TACGCTTAGAGACCGACAGC | reverse | *Tetracladium* |
| ITS86F | GTGAATCATCGAATCTTTGAA | forward | Total fungal DNA |
| ITS4 | TCCTCCGCTTATTGATATGC | Reverse | Total fungal DNA |

threshold cycle values ($C_T$), which are an indicator of detectable PCR product. Concentrations of DNA were compared with the standard values and expressed in units of ng $g^{-1}$ dwt soil based on the number of amplicon copies. The estimation of PCR efficiencies and quantifications were carried out using the PCRMax Eco Study software Version 5.2.12.0 (PCRMax Limited, Witford, UK).

## 2.5 Fungal isolates used in laboratory experiments

In total, 16 fungal isolates, three from Antarctic soil and 13 from Arctic soil, were used in the controlled laboratory experiments described in Chapters 4 and 6 to determine the effects of warming and water availability on fungal physiology, extension rate and enzyme activity (Table 2.2).

## 2.6 Laboratory warming experiments – temperature and water manipulations

The 16 fungal isolates listed in Table 2.2 were used in controlled environment experiments to investigate the physiological responses of polar soil fungi to environmental manipulations simulating summer field conditions. The growth (mycelial extension) rate and extracellular enzyme activities of the isolates were measured in response to different temperature cycles and water availabilities. Rather than growing isolates on standard media (*cf.* Krishnan *et al.* 2017), which provide nutrients in excess of those available in the field, they were instead grown in Petri dishes on weak soil extract medium (SEM). Method adapted from Robinson *et al*. (2004) was used to prepare SEM. Soil was mixed with natural mineral water (1:2.5 ratio). After settling, the supernatant was collected, filtered and mixed with sucrose (1 g $L^{-1}$), $KH_2PO_4$ (0.02 g $L^{-1}$), yeast extract (0.01 g $L^{-1}$) and polyethylene glycol (PEG) 8000 (ChemCruzer) prior to autoclaving for 15 min at 121 °C.

The PEG 8000 was used to alter the water potential of the media, following the equation of Michel (1983). This osmoticant was specifically chosen as it alters the matric potential of the medium, and is hence representative of biologically available water in

*Table 2.2 List of fungal isolates used in laboratory studies, with NCBI database matches.*

| Polar region | Identity | GenBank information | |
|---|---|---|---|
| | | Similarity (%) | Coverage (%) |
| Arctic | *Acremonium* sp. | 91 | 96 |
| Arctic | *Cadophora* sp. | 94 | 98 |
| Arctic | Dothideomycete | 99 | 99 |
| Arctic | *Isaria farinosa* | 99 | 100 |
| Arctic | *Leptosphaeria* sp. | 98 | 92 |
| Arctic | *Mortierella* sp. | 100 | 98 |
| Arctic | *M. alpina* | 99 | 99 |
| Arctic | *M. polygonia* | 99 | 100 |
| Arctic | *M. verticilliata* | 99 | 100 |
| Arctic | *Phialocephala lagerbergii* | 100 | 98 |
| Arctic | *Pseudogymnoascus pannorum* | 99 | 99 |
| Arctic | *Pseudogymnoascus* sp. | 99 | 100 |
| Arctic | *Pseudogymnoascus* sp. | 99 | 100 |
| Antarctic | *P. pannorum* | 99 | 100 |
| Antarctic | *P. pannorum* | 99 | 100 |
| Antarctic | *P. pannorum* | 99 | 100 |

soil. This is in contrast to other approaches, in which osmoticants such as salt and glycerol are added to media to alter water availability (e.g. Marin *et al.* 2004). Changes in chemical composition using salt and glycerol alter solute water potential, which is not truly representative of the way in which water acts in the environment, especially in soil (Magan and Lynch 1986). By adding different concentrations of PEG 8000 to the SEM (*ca*. 386, 541, 687 and 980 g $L^{-1}$), the Antarctic isolates were exposed to environmentally realistic water potentials of -1.06 MPa, -3.6 MPa, -6 MPa and -10.8 MPa, approximately equivalent to soil moisture contents of 8%, 5%, 3% and 2% in the field, respectively (Appendix 2). By including PEG 8000 in SEM at different concentrations (334, 358 and 419 g $L^{-1}$), the Arctic isolates were exposed to water potentials of -0.2 MPa, -0.6 MPa and -1.6 MPa, which approximated to soil moisture contents of 15 %, 8 % and 5 % in the field, respectively (Appendix 2).

Each 90 mm diameter Petri dish was prepared by adding a circle of sterile Gardman capillary matting to its base, followed by a sterile circle of black polyester. PEG 8000-enriched SEM was then added to the dish and a sterile circle of Natureflex 28 NP cellophane was placed on top of the polyester (Magan 1988, Ramirez *et al.* 2004). A 5 mm diameter plug of each fungal isolate, growing on half strength PDA medium, was then carefully placed in the centre of the cellophane. For fungal isolates that produced copious dry spores (e.g., *Pseudogymnoascus* and *Isaria* spp.), spore suspension plates were prepared 16 h before the dishes were inoculated by adding 2 ml of deionised water to a fungal colony, rubbing the surface of the colony with a sterile spreader, and then spreading 0.2 ml of the water onto half strength PDA medium, which was incubated at *ca*. 15 °C. The 5 mm diameter plugs were then cut from these suspension plates.

The Petri dishes were sealed with Parafilm and placed into sealed ziplock bags for 3–12 weeks. They were incubated in controlled environment cabinets (Sanyo, Versatile Environmental Test Chamber, MCR–350) at three different temperature cycles, representative of field conditions during summer (2–18 °C, 2–21 °C and 2–24 °C for the Antarctic isolates and 5–18 °C, 5–21 °C and 5–24 °C for the Arctic isolates; Figure 2.8). The mean temperatures at the 2–18 °C, 2–21 °C and 2–24 °C cycles were 10.1 °C, 12.0 °C and 12.9 °C, and those at the 5–18 °C, 5–21 °C and 5– 24 °C cycles were 11.4 °C, 13.1 °C and 14.3 °C, respectively. A total of 729 dishes were prepared, 585 for the Arctic isolates and 144 for the Antarctic isolates, with four or five replicates per isolate in each treatment.

### 2.6.1 Extension rate analyses of fungal isolates

To investigate the responses of hyphal extension rates to a range of water potentials and temperature cycles, images of each culture were captured using a digital camera (Canon SX700HS) from a height of 20 cm. Images were taken weekly between 1 and 12 weeks, except for rapidly-growing *Mortierella* spp., for which images were taken twice a week for up to 3–4 weeks. The first image of each colony was taken after growth was visible around the inoculation point. A linear scale was included in each

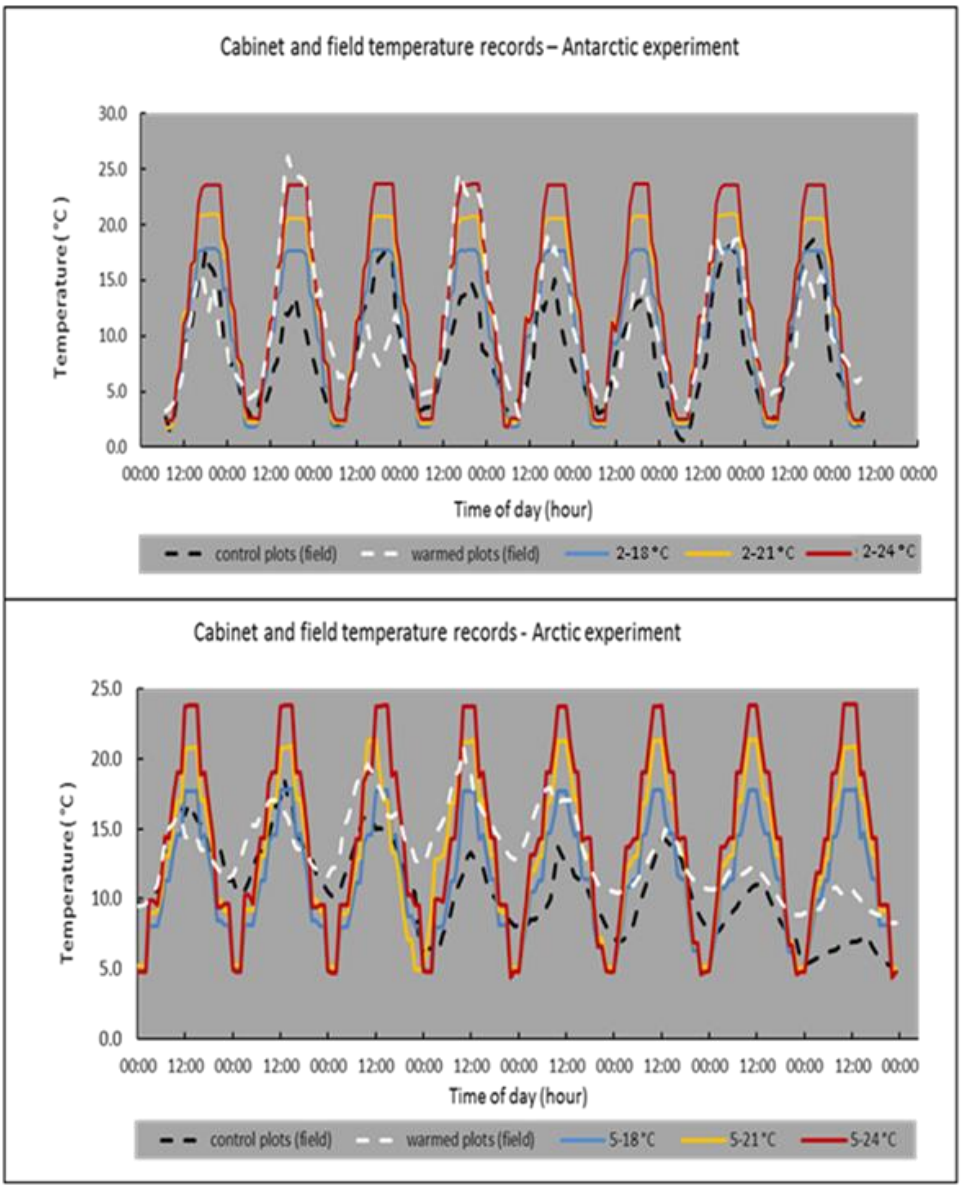



*Figure 2.8 Temperatures measured in the field and applied in the laboratory. Black and white lines show soil surface temperatures measured over eight days during summer in the Antarctic (top) and High Arctic (bottom) in control plots and warmed plots, respectively. Blue, yellow and red lines show temperatures in controlled environment cabinets under the 2–18 °C, 2–21 °C and 2–24 °C temperature cycles, respectively for Antarctic experiment, and 5–18 °C, 5–21 °C and 5–24 °C temperature cycles, respectively, for Arctic experiment.*

image. The surface area of each colony was measured using ImageJ Version 1.49v (National Institute of Health, Bethesda, Maryland, USA), and its extension rate was determined in the linear growth phase from regressions between time and surface area (Appendix 6). The slope of the regression line ($r^2 > 0.95$) was used as an estimate of extension rate, measured in $mm^2\ d^{-1}$.

### 2.6.2 Enzyme assays

#### *2.6.2.1 Screening for extracellular enzyme production by fungal isolates*

The API ZYM test (bioMerieux, Basingstoke, UK) was used to identify the extracellular enzymes secreted by the 16 fungal isolates. This is a semi-quantitative test based on a colorimetric method that is designed for the identification of 19 enzymes commonly produced by micro-organisms (Appendix 7). Enzyme extracts of fungal cultures, maintained on half strength PDA medium, were prepared by placing eight 5 mm diameter plugs, cut from the margins of fungal colonies, into 4 ml of 0.85 % NaCl solution. Solutions were incubated at 4 °C on a shaker for 100 min. Enzyme extract (65 µl) was then loaded onto the strips and incubated in the dark at 37 °C for 4 h. After incubation, one drop of ZYM A reagent and one drop of ZYM B reagent were added simultaneously. The strips were left on the bench, away from direct light, and the colour allowed to develop. Each strip was read within 2 hr and scored according to the colorimetric scale provided with the kit. Exemplar API ZYM kit test results are shown in Appendix 8.

#### *2.6.2.2 Enzyme extracts preparation*

Enzymes were extracted from each fungal colony in the laboratory growth experiments described in Chapters 4 and 6. At the end of the experiment, the cellophane on which the hyphae were growing was submerged upside down in 4 ml of 10 mM phosphate buffer at pH 7.2, and was shaken in the dark at 7 °C for 2 h. The extract solutions were collected into two 2 ml capacity Eppendorf tubes and stored at -80 °C prior to analysis.

#### *2.6.2.3 Enzyme activity analysis using p-nitrophenol assays*

Following the methods of Alam *et al.* (2009), the activities of five enzymes (N-acetyl-βD-glucosaminidase, B-glucosidase, acid and alkaline phosphatase, and leucine aminopeptidase) were measured using p-nitrophenyl substrates (Table 2.3). Enzyme kit (API ZYM kit) test results indicated that these enzymes were secreted by the

*Table 2.3 List of enzymes measured in this study and enzymes assay conditions used.*

| Enzyme | Substrate[1] | Concentration (mM) | Buffer's pH[2] | Target molecules[3] |
|---|---|---|---|---|
| alkaline phosphatase | 2-napthyl phosphate | 5 | 8 | phosphoesters |
| acid phosphatase | 2-napthyl phosphate | 5 | 6 | phosphoesters |
| β-glucosidase | 2-napthyl-βD-glucopyranoside | 5 | 6 | cellulose |
| N-acetyl-βD-glucosaminidase | 1-napthyl-N-acetyl-βD-glucosamide | 5 | 6 | chitin |
| leucine aminopeptidase | L-leucyl-2 napthylamide | 2.5 | 6 | polypeptides, specifically leucine |

[1] Information based on API ZYM Kit datasheet (bioMerieux)

[2] 10 mM sodium acetate buffer

[3] SIB ExPASy Bioformatics Resources Portal Database (https://www.expasy.org/)

isolates. The p-nitrophenol enzyme assay is a colorimetric method, in which the liberation of p-nitrophenol is detected by measuring optical density at 405 nm. In the presence of a specific enzyme, the p-nitrophenyl substrate is converted into p- nitrophenol, resulting in a change in the colour of solutions to yellow. Extracellular enzyme activities were measured in 96–well microplates, using three technical replicates and four to five biological replicates. Enzyme extract (40 µl) was mixed with 40 µl of enzyme substrate and made up to 100 µl with 10 mM sodium acetate buffer (pH 6 or pH 8, see Table 2.3).

Samples were incubated at 37 °C for 0.25–2 hr along with appropriate controls. The reactions were stopped by adding 10 µl of 1 M sodium bicarbonate solution to each well and absorbance readings were recorded immediately using a microplate reader (Sunrise, TECAN, Austria) at a wavelength of 405 nm. Attempts were also made to measure esterase and lipase activities, but due to technical difficulties and high background levels of absorbance, the results were discarded.

Total enzyme activity, estimating the overall amount of enzyme secreted by hyphae, was calculated from p-nitrophenol standard calibration curves and expressed as µmol of p-nitrophenol released per hour. The calibration curve ranged from 0– 1.5 µmol $ml^{-1}$ of p-nitrophenol. Transformation of 1 µmol of p-nitrophenol $hr^{-1}$ $\mu g^{-1}$ of protein was also calculated. This value, known as specific enzyme activity, is a more

accurate measure of enzyme activity as it takes into account the amount of protein present in the sample. Protein content was measured using a Micro BCA Protein Assay Kit (ThermoFisher, Hemel Hampstead, UK). Protein assays were prepared in microplates in triplicate per sample by adding 100 µl of working solution (BCA reagents A, B and C, mixed in a 25:24:1 ratio, respectively) to 100 µl of sample. Microplates with samples and controls were incubated at 37 °C for 2 h, cooled and absorbance measured at 562 nm. Protein concentration was calculated from an albumin calibration curve (range 0– 200 µg $ml^{-1}$) and used for specific enzyme activity calculations. Colony biomass was determined by washing hyphae onto pre-weighed filter paper and drying overnight at 105 °C before reweighing. Total and specific enzyme activities were subsequently expressed per dry biomass of fungal colony.

## 2.7 Statistical analyses

All statistical analyses were conducted in R (version 1.0.136; R Development Core Team, 2015) or Minitab 17 (version 17.2.1). Main and interactive effects of treatments on concentrations of fungal DNA derived from Q-PCR analyses and on colony extension rates, and the total and specific enzyme activities derived from laboratory experiments were determined using GLMs. Details of statistical analyses are included in each Chapter.

# 3 The effects of warming and nutrient amendment on the abundances of *Pseudogymnoascus* spp. and total fungi in Antarctic soil

## 3.1 Introduction

Prior to the late 1990s, the maritime Antarctic was the most rapidly warming region in the Southern Hemisphere, with temperature increases of *ca*. 0.2– 0.4 °C decade$^{-1}$ having been recorded since the 1950s (Convey *et al.* 2011, Turner *et al*. 2014). Although this regional warming trend has slowed since the turn of the millennium (Turner *et al.* 2016), further warming in the region, similar in magnitude to that which occurred in the latter half of the 20$^{th}$ Century, is predicted under moderate greenhouse gas emissions scenarios by 2100 (Bracegirdle *et al.* 2008, Bracegirdle and Stephenson 2012, Turner *et al.* 2016). Rising air temperatures in the maritime Antarctic have had widely-publicised effects in the physical environment, such as the collapse of ice shelves and the retreat of glaciers (Vaughan *et al.* 2003, Cook *et al.* 2010, Cook *et al.* 2016). The biota of maritime Antarctic ecosystems has also responded to warming, with studies recording expansions in populations of *Deschampsia antarctica* and *Colobanthus quitensis*, the two native Antarctic vascular plant species, during recent decades (Fowbert and Smith 1994, Grobe *et al.* 1997). These expansions in the sizes of plant populations are likely to increase nutrient inputs to soils, with potentially positive effects on soil microbial biomass (Hill *et al.* 2011, Royles *et al.* 2013, Dennis *et al.* 2013).

Temperature increases in the Antarctic arising from climate change also have the potential to influence fungal communities. For example, air temperature is thought to be one of the most important factors affecting the diversity of fungi in the soils of maritime Antarctica, with increased fungal species richness being recorded in soils from warmer habitats on a 1,650 km climatic transect through the region (Newsham *et al.* 2016). Furthermore, a previous study found that more fungal DNA was present in two maritime Antarctic soils that had been warmed for three years with OTCs than in control soils (Yergeau *et al.* 2012). Although it is much more difficult to predict field responses from controlled environment experiments, laboratory studies also point to

possible effects of rising temperatures on the abundances of soil fungi. Many of these studies have focused on *Pseudogymnoascus* (formerly *Geomyces* and *Chrysosporium*) *pannorum*, one of the most commonly isolated fungi from Antarctic soils (Arenz and Blanchette 2011, Krishnan *et al.* 2011, Ali *et al.* 2013), and the most frequent taxon detected in the DNA-based survey of Newsham *et al.* (2016). Several studies that have cultured *P. pannorum* in the laboratory at a range of constant temperatures have found that it is able to grow at 4–25 °C, but that its optimum growth temperature is *ca*. 15 °C (Krishnan *et al.* 2011, Krishnan *et al.* 2012). It is, therefore, classified as a psychrophilic fungus (see Section 1.7 for definition; Azmi and Seppelt 1995, Krishnan *et al.* 2012, Hughes *et al.* 2013). Of all fungi isolated from soil on King George Island in the maritime Antarctic, *Pseudogymnoascus* was the most frequent genus, and had the highest enzyme activity at 4 °C, suggesting that it is an ecologically important saprotrophic fungus in Antarctic soils (Krishnan *et al.* 2012). As warming continues in the maritime Antarctic (Bracegirdle *et al.* 2008, Bracegirdle and Stephenson 2012), it is plausible that psychrophilic fungi such as *Pseudogymnoascus* spp. will be increasingly exposed to temperatures exceeding their optima for growth (*ca*. 20 °C; Section 1.7; Longton and Holdgate 1967, Convey *et al*. 2018), potentially affecting their abundance, growth and activities. The extent of these potential effects on the abundance of the taxon in soil have hitherto not been investigated in the field.

Here, quantitative (Q-)PCR is used to measure the abundance of *Pseudogymnoascus* spp. DNA in soil sampled from a field experiment at Mars Oasis in the southern maritime Antarctic. Quantitative-PCR, which is highly specific, can be used to compare across different taxonomic levels using small samples (Heid *et al.* 1996, Tellenbach *et al.* 2010). It has been successfully used for the estimation of fungal biomass, for example in roots (Tellenbach *et al.* 2010), and as a proxy for the biomass of the aquatic leaf-associated fungus *Alatospora pulchella*, showing a high sensitivity and reliable quantification down to 9.9 fg DNA in environmental samples (Feckler *et al.* 2017). It has also been used to determine the abundances of arbuscular mycorrhizal fungi in soil (Thonar *et al.* 2012), and of *Pseudogymnoascus destructans*, a pathogen causing white-nose syndrome in bats, in hibernacula (Shuey *et al.* 2014, Verant *et al.* 2016). By using Q-PCR, the effects of warming with OTCs on the

concentrations of genomic DNA of *Pseudogymnoascus* and total fungi in soil at Mars Oasis are measured. In addition, because expanding plant populations will enhance nutrient inputs to maritime Antarctic soils (Hill *et al.* 2011), Q-PCR is also used here to investigate the main effects, and interactive effects with warming, of three artificial growth substrates on the abundances of *Pseudogymnoascus* spp. and total fungi in soil. It is hypothesised that:

1) warming will increase the abundances of genomic DNA (gDNA) of *Pseudogymnoascus* species and total fungi in Antarctic soil,
2) growth substrates will increase the abundances of gDNA of *Pseudogymnoascus* spp. and total fungi in Antarctic soil, and
3) warming and growth substrates will interact to influence the abundances of gDNA of *Pseudogymnoascus* spp. and total fungi in Antarctic soil.

## 3.2 Methods

### 3.2.1 Warming experiment and sampling

The soil warming experiment at Mars Oasis on the south-eastern coast of Alexander Island, western Antarctic Peninsula, is described in Section 2.1.1. Briefly, the experiment consisted of 64 plots to which a factorial combination of warming (with OTCs) and growth substrates (glucose, glycine and tryptic soy broth) were applied for five years. Samplings for the experiments described below took place on 27 November 2007, 10 December 2009, 2 December 2010, 21 December 2011 and 9 December 2012 by placing soil from each plot (0–2 cm depth), sampled with a sterile spoon, into a sterile 50 ml capacity falcon tube. The soils were kept at *ca*. -3 °C for up to 24 h before being returned to Rothera Research Station, where they were frozen at -20 °C, prior to transport to the UK and subsequent storage at the same temperature.

### 3.2.2 Q-PCR analyses

The Q-PCR analyses that were used are described in detail in Section 2.4.4. Briefly, soil samples were defrosted and total DNA was extracted from 1.1 g fwt of soil using a

MoBio Soil DNA Extraction kit (Section 2.4.3). Q-PCR was then used to estimate the concentrations of gDNA of *Pseudogymnoascus* spp. and total fungi by amplifying ITS regions of DNA using the Pseudogymnoascus_Fr/Pseudogymnoascus_Rv and ITS86F/ITS4 primers (Table 2.1).

### 3.2.3 Statistical analyses

Concentrations of gDNA were corrected to $ng^{-1}$ dry weight soil and were log(*n*+1)- transformed prior to analysis to improve data distribution. General Linear Models (GLMs) were used to test for the main and interactive effects of OTCs, growth substrates and year on the concentrations of gDNA of *Pseudogymnoascus* spp. and total fungi in soil. Significant effects were further investigated using one way Analysis of Variance (ANOVA) and Tukey's multiple comparisons tests. Pearsons' correlations were used to determine associations between concentrations of fungal DNA in soil and soil moisture concentration.

## 3.3 Results

### 3.3.1 *Pseudogymnoascus* spp.

There was no significant difference in the concentration of *Pseudogymnoascus* spp. gDNA between warmed and unwarmed plots before the OTCs were installed in 2007, with most gDNA concentrations being below the limit of detection for that year. In 2009–2012, GLMs anlysis showed that OTCs had an overall negative effect on the concentration of *Pseudogymnoascus* spp. gDNA in soil ($F_{1, 251} = 7.90$, $P = 0.005$), but that the effect differed between years, with a significant year × OTC interaction being recorded (Table 3.1). Depending on the substrate applied, growth substrates either had no effect on, or increased, the concentration of *Pseudogymnoascus* spp. gDNA in soil that had not been warmed with OTCs, with glucose application having no overall effect, but glycine and TSB having significant main effects on the concentration of gDNA of the fungus in unchambered soil (Table 3.1, Appendix 9). Although there was no overall main effect of glucose on *Pseudogymnoascus* spp. gDNA concentrations in soil, in 2009, glucose application to unchambered soil increased the abundance of

*Table 3.1 Outputs from General Linear Models testing (three way ANOVA) for treatment effects on the abundance of* Pseudogymnoascus *spp. DNA in Mars Oasis soil.*

| Factor | *F -value*$_{1, 251}$ | *P-value* |
|---|---|---|
| Year | 4.34 | 0.005 |
| OTC | 16.86 | ↓<0.001 |
| glucose | 0.24 | 0.627 |
| glycine | 117.58 | ↑<0.001 |
| TSB | 74.11 | ↑<0.001 |
| Year x OTC | 3.03 | 0.030 |
| Year x glucose | 0.26 | 0.855 |
| Year x glycine | 5.32 | 0.001 |
| Year x TSB | 5.34 | 0.001 |
| OTC x glucose | 0.81 | 0.370 |
| OTC x glycine | 23.61 | <0.001 |
| OTC x TSB | 1.08 | 0.299 |
| Year x OTC x glucose | 0.12 | 0.951 |
| Year x OTC x glycine | 1.14 | 0.333 |
| Year x OTC x TSB | 1.27 | 0.286 |

*Note that log (n+1) transformation was used prior to statistical analyses. Bold* P values *indicate significant treatment effects, and ↑ or ↓ indicate increasing or decreasing, respectivaely, trends in response to treatments.*

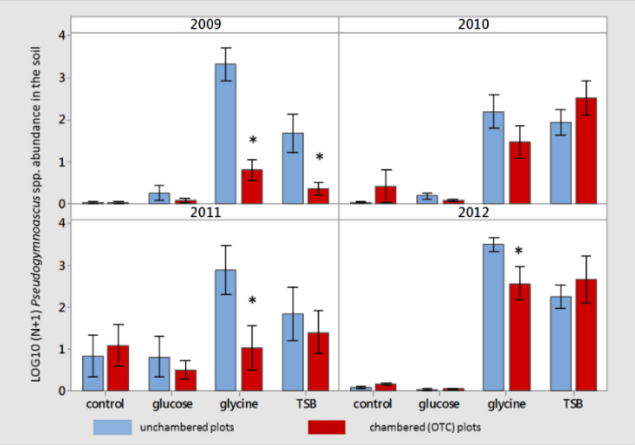



*Figure 3.1 Effects of warming with OTCs and three growth substrates (glucose, glycine and TSB) on the concentration of* Pseudogymnoascus *spp. gDNA in soil at Mars Oasis over four years. Blue bars denote plots with no temperature manipulation and red bars denote warmed plots. Values are means of eight values ± SEM. Asterisks indicate means differing at* P *< 0.05.*

*Pseudogymnoascus* spp. gDNA by 39 times (Appendix 9, Figure 3.1). In the same year, TSB and glycine amendment in unchambered soils increased the abundance of *Pseudogymnoascus* spp. gDNA by three to four orders of magnitude, compared with unamended soils (Appendix 9, Figure 3.1). Glucose application also increased the concentration of DNA of the fungus in 2010 by six times in unchambered soil, with glycine and TSB amendment increasing the abundance of *Pseudogymnoascus* spp. gDNA by three orders of magnitude in the same year, compared to control soil (Appendix 9, Figure 3.1). In 2011, the application of glycine to soil increased the concentration of gDNA of the fungus by 36 times, with this substrate eliciting a four orders of magnitude increase in gDNA concentration in 2012 (Appendix 9, Figure 3.1). Amendment with TSB also led to a similar three order of magnitude increase in the concentration of *Pseudogymnoascus* spp. gDNA in 2012 (Appendix 9, Figure 3.1).

Over all samplings, there was a highly significant OTC × glycine interaction on the concentration of *Pseudogymnoascus* spp. gDNA in soil (Table 3.1). This was caused by the significantly lower concentrations of *Pseudogymnoascus* spp. gDNA that were recorded in chambered soil to which the substrate had been applied, compared with unchambered soil that had received the substrate (Figure 3.1). In 2009, 2011 and 2012, concentrations of gDNA of the fungus were reduced by 21–99 % in glycine-amended soil sampled from OTCs, relative to glycine-amended soil from unchambered plots (Figure 3.1). Similarly, although the OTC × TSB, or the year × OTC × TSB interactions, were not significant (Table 3.1), in 2009, the concentration of *Pseudogymnoascus* spp. gDNA in chambered soil to which TSB had been applied was 99 % lower than in unchambered soil that had received the substrate (Figure 3.1).

### 3.3.2 Total fungi

There was no significant difference in the concentration of total fungal gDNA between chambered and unchambered plots before the OTC were installed in 2007. The concentration of total fungal gDNA in nutrient-amended soil increased over the duration of the experiment (Appendix 9). General Linear Models indicated that OTCs had no overall effect on the concentration of total fungal DNA measured in soil, but that growth substrates had a positive effect on the abundance of total fungal DNA (Table 3.2). Over the duration of the experiment, TSB was the only substrate that had a main effect on the concentration of total fungal DNA in soil (Table 3.2). In 2009, glycine and TSB application increased soil fungal DNA concentration by 164 and 144 times, respectively, relative to unamended soil (Appendix 9 and 10). In the following year, glucose application doubled the concentration of total fungal DNA in soil, with glycine and TSB leading to two to three orders of magnitude increases in fungal DNA concentrations (Appendix 9). Similarly, in 2011 and 2012, glucose amendment led to 14 and 66 fold increases in soil fungal DNA concentrations, respectively, with glycine and TSB application resulting in between 140 to 385 fold higher concentrations of fungal DNA in soil, relative to unamended plots (Appendix 9).

As for the Q-PCR analyses on *Pseudogymnoascus* spp., there were significant interactive effects of OTCs and substrates on the concentrations of total fungal DNA in soil (Table 3.2). GLMs indicated significant OTC x glycine and OTC x TSB interactions on total fungal DNA concentrations in soil (Table 3.2). As for *Pseudogymnoascus* spp., these two-way interactions were caused apparently by lower concentrations of total fungal DNA in soil that had received substrates and had been warmed with OTCs than those to which the substrates had been applied, but had not been warmed. In 2009, the concentrations of all fungal DNA in chambered soil that had received glycine and TSB were both 99 % lower than in unchambered soil that had received the substrates (Figure 3.2, Appendix 9). Similar effects were observed in 2010 and 2012, when the concentrations of total fungal DNA were 74 % and 21 % lower in chambered soil that had been amended with glycine than in unchambered soil that had received the substrate (Figure 3.2, Appendix 9). In addition to these two-way interactions, there was also a significant year × OTC × TSB interaction, indicating that the interactive effects of OTCs and TSB application on total fungal DNA concentration varied between years (Table 3.2), most probably owing to the significant reduction in total fungal DNA concentrations in chambered, TSB-amended soil relative to unchambered, TSB-amended soil in 2009, and an absence of effects in 2010–2012 (Figure 3.2).

*Table 3.2 Outpust from General Llinear Model (three way ANOVA) testing for treatment effects on the abundance of total fungal DNA in Mars Oasis soil. Note that log (n+1) transformation was used prior to statistical analyses. Bold P values indicate significant treatment effects (P < 0.05), and ↑ or ↓ indicate increasing or decreasing, respectively, main effects of treatments.*

| Factor | $F_{1,\ 248}$ | *P* |
|---|---|---|
| Year | 2.24 | 0.166 |
| OTC | 0.82 | 0.463 |
| Substrate | **19.95** | **↑0.021** |
| Glucose | 6.00 | 0.187 |
| Glycine | 6.75 | 0.063 |
| TSB | **19.87** | **↑0.029** |
| | | |
| Year x OTC | 2.05 | 0.273 |
| Year x substrate | **8.71** | **<0.001** |
| Year x glucose | 4.53 | 0.123 |
| Year x glycine | **62.27** | **0.003** |
| Year x TSB | 5.85 | 0.090 |
| OTC x glucose | 0.77 | 0.455 |
| OTC x glycine | **18.64** | **0.023** |
| OTC x TSB | **27.77** | **0.013** |
| | | |
| Year x OTC x glucose | 0.18 | 0.913 |
| Year x OTC x glycine | 1.21 | 0.309 |
| Year x OTC x TSB | **3.39** | **0.019** |

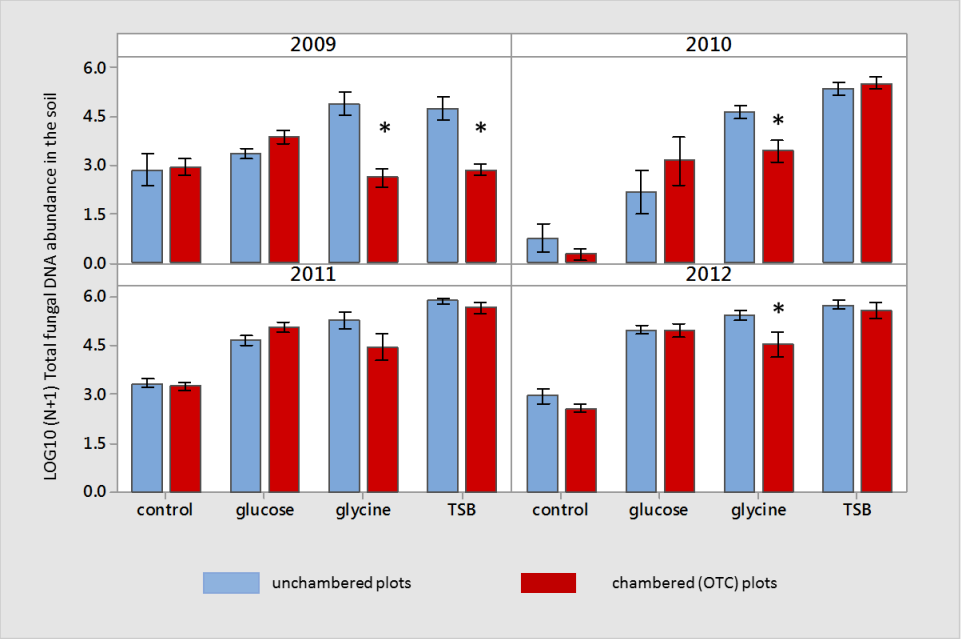



*Figure 3.2 Effects of warming with OTCs and three growth substrates (glycine, glucose and TSB) on the concentrations of total fungal DNA in soil at Mars Oasis over four years. Blue bars denote plots with no temperature manipulation and red bars denote warmed plots. Values are means of eight values ± SEM. Asterisks indicate values differing at* $P < 0.05$ *based on ANOVA test.*

### 3.3.3 Soil moisture

One way ANOVA showed that soil moisture concentration varied significantly between years ($F_{3, 252} = 35.91$, $P < 0.001$). It was highest in 2012 (5.8 %), intermediate in 2010 (4.6 %) and 2009 (3.2 %), and lowest in 2011 (2.2 %; Figure 3.3). There was no significant difference in soil moisture concentration between warmed and unwarmed plots before the OTCs were installed in 2007. General Linear Model test indicated a marginally significant effect of OTCs on soil moisture concentration ($F_{1, 252} = 2.00$, $P = 0.055$), decreasing it by 15 %, from 3.8 % to 3.3 %, over all years. Growth substrates had no main effects on soil moisture concentration ($P > 0.05$). In soil to which glycine had been applied, there was however an increase in soil moisture, resulting in a 57 % increase in soil moisture concentration in chambered plots relative to unchambered plots in 2010 ($F_{1, 16} = 15.33$, $P = 0.002$, Figure 3.3). The same effect was recorded in glucose-amended soils in 2009, with a 12 % higher moisture concentration in chambered soil compared with unchambered soil in this year ($F_{1, 16} = 2.66$, $P = 0.048$, Figure 3.3).

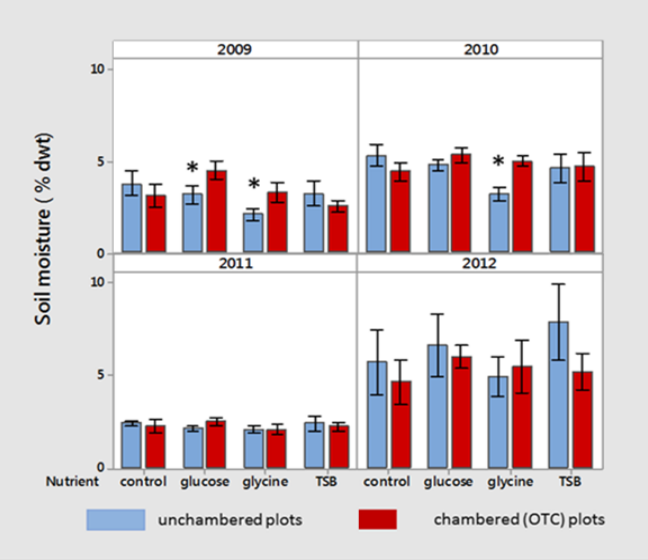



*Figure 3.3 Soil moisture concentration (percentage of dwt soil) at the warming experiment in 2009–2012. Values are means of eight replicates and bars show SEM. OTC, open top chamber; TSB, tryptic soy broth. Blue and red bars represent unchambered and chambered plots, respectively. Asterisks denote significantly different soil moisture concentration between the plots based on ANOVA analysis.*

Pearson's correlations showed that the concentration of *Pseudogymnoascus* spp. gDNA in soil was not associated with soil moisture concentration ($r$ = 0.048, $P$ = 0.451). There was also no association between the concentration of total fungal DNA in soil and moisture concentration ($r$ = 0.084, $P$ = 0.194), but the concentrations of DNA of *Pseudogymnoascus* spp. and total fungi in soil tended to be higher in drier years.

## 3.4 Discussion

Contrary to Hypothesis 1, the observations here indicate that warming with OTCs decreases, not increases, the abundance of *Pseudogymnoascus* spp. gDNA in an Antarctic soil. This is believed to be the first demonstration of warming in the natural environment having negative effects on the growth of an Antarctic soil fungus, with previous studies usually pointing towards positive effects of rising temperatures in polar regions on soil microbial abundances and activities (Clemmensen *et al.* 2006; Yergeau *et al.* 2012, Chen *et al.* 2015). However, such effects are not unprecedented, with warming in the Low Arctic giving rise to reductions in fungal abundances in soil (Allison and Treseder 2008, Deslippe *et al*. 2010, Christiansen *et al*. 2017), and reductions in the richness of ectomycorrhizal, ericoid mycorrhizal and lichenized fungi in response to warming in Canadian Arctic (Geml *et al*. 2015). It is possible that the main effect of OTCs on the abundance of *Pseudogymnoascus* spp. gDNA in soil arose from soil surface temperatures in chambered plots frequently exceeding 20 °C during summer (Figure 2.3), which, as indicated by previous laboratory studies (Kerry 1990, Azmi and Seppelt 1997, Hughes *et al*. 2003, Krishnan *et al*. 2017), most probably inhibited the

growth of this psychrophilic fungus. The possibility cannot, however, be discounted that other abiotic factors that are influenced by OTCs, such as soil moisture (Bokhorst *et al*. 2011), concentrations of which were reduced in OTCs by 15 %, but increased in chambered, glucose- or glycine amended soils by 12–59 %, may also have influenced the growth and activity of *Pseudogymnoascus* spp. in soil.

The data strongly support Hypothesis 2 that the growth substrates would increase the abundance of gDNA of *Pseudogymnoascus* spp. and total fungi in soil at Mars Oasis. Previous studies have found that adding glucose, glycine, ammonium chloride and TSB, also at rates of 2.0 mg C $g^{-1}$ dwt soil, increases the concentrations of ester linked fatty acid (ELFA) markers for total microbes in Antarctic soils (Hopkins *et al*. 2008, Dennis *et al*. 2013), but this is the first report of nutrient amendment specifically increasing the abundances of fungi in Antarctic soil. Carbon addition to soil, in the form of glucose, had little or no effect here on the abundance of *Pseudogymnoascus* spp. gDNA or total fungal gDNA. Carbon and nitrogen addition, in the form of glycine and TSB (which added an additional 0.58 and 0.2 mg N $g^{-1}$ dwt soil, respectively; Benhua *et al*. 2014), however increased the abundance of *Pseudogymnoascus* spp. and total fungal DNA by three to four orders of magnitude. This indicates that, unlike bacteria - some of which are able to fix nitrogen from the atmosphere (Güsewell and Gessner 2009) - fungi inhabiting Mars Oasis soils are nitrogen, not carbon, limited. The data recorded in this study suggest that increased concentrations of organic nitrogen in Antarctic soils (Hill *et al.* 2011), arising from expanding plant populations in warmer habitats (Fowbert and Smith 1994, Grobe *et al*. 1997), could lead to substantial increases in fungal abundances in soil. Given that fungi are known to have positive effects on the cycling of carbon and nitrogen in soils (Swift *et al*. 1979), it is likely that these apparent increases in fungal biomass in soil with higher nutrient inputs could lead to accelerated mineralisation rates of these nutrients.

In support of Hypothesis 3, significant interactive effects of growth substrates and warming were recorded on the abundance of fungal gDNA in soil, with interactions between OTCs and glycine being recorded for *Pseudogymnoascus* spp. and total fungal gDNA. In a response similar to that recorded for bacterial DNA in warmed, nutrient amended soils at Mars Oasis (Dennis *et al*. 2013), these interactions were owing to

21– 99 % reductions in the abundance of fungal DNA in chambered soil to which glycine or TSB had been applied, compared with unchambered soil that had received the substrates. As with the main effect of OTCs on the abundance of *Pseudogymnoascus* spp. gDNA discussed above, it is likely that soil surface temperatures exceeding 20 °C may have inhibited the growth of psychrophilic fungi such as *Pseudogymnoascus* in soil at Mars Oasis, but it is unclear why these effects were restricted to soils that had been amended with glycine or TSB. It is possible that the substantial three to four orders of magnitude increases in the abundance of fungal gDNA in soils that received these substrates enabled GLMs to detect treatment effects of OTCs. Although OTCs tended to dry soil (see above), in two of the four soils in which significant interactive effects occurred in the present study, soil moisture concentrations were higher in chambered soil that had received glycine than in unchambered, glycine-amended soil. This suggests that moisture concentration may have influenced fungal responses to warming, with previous studies similarly indicating interactions between warming with OTCs and water availability on fungal abundances and activities (Deslippe *et al*. 2010, Geml *et al*. 2015).

Although warming of the maritime Antarctic has been reported to have halted since the turn of the millennium (Turner *et al*. 2016), climate models simulations using moderate greenhouse gas emissions indicate 2–4 °C increases in near surface air temperatures by the end of the 21$^{st}$ Century across the region (Bracegirdle *et al*. 2008, Bracegirdle and Stephenson 2012). The observations in this study suggest that reductions may occur in the growth rate of *Pseudogymnoascus* spp*.* and other psychrophilic fungi at the surfaces of maritime Antarctic soils due to raising soil temperatures. Temperatures of soils at Mars Oasis already reach 19 °C during summer (see Figure 2.3). With climate warming resuming in the region, the negative effect of temperature increase on the growth rates of the psychropilic fungi will be most pronounced in soils with freely available water. Inhibitory effects of warming on the abundances of *Pseudogymnoascus* spp*.,* a frequent saprotrophic fungus in soils of cold regions (Duncan *et al*. 2008, Arenz and Blanchette 2009, Bridge and Spooner 2012, Reynolds and Barton 2014), might be expected to deleteriously affect the mineralization rates of nutrients in soils. For example, *Pseudogymnoascus* spp. are known to be

important in the decomposition of cellulose and chitin in soils, the two most frequent polymers in terrestrial ecosystems (Rinuado 2006), with reductions in its activities thus having potentially widespread effects.

An important caveat to the observations here is that the three to four orders of magnitude increases observed in the abundance of fungal gDNA in response to glycine and TSB amendments, and the 21–99 % reductions in the abundance of *Pseudogymnoascus* spp. gDNA in response to warming with OTCs, might not be a realistic representation of fungal biomass changes in soil. Q-PCR, as with other nucleic acid-based methods, has artefacts associated with it (Prosser 2015), notably the over-estimation of fungal biomass owing to increased ITS copy numbers of amplified gene regions (Berdal *et al.* 2008, Tellenbach *et al*. 2010, Taylor *et al.* 2016, Feckler *et al.* 2017). Sequence read abundance is hence only an estimate of fungal abundance in the natural environment (Lindahl *et al*. 2013). The data reported in this study are however strongly suggestive of substantial changes to fungal biomass in warmed and nutrient-amended Antarctic soils.

## 3.5 Conclusion

The results highlight the deleterious effects of warming with OTCs in the natural environment on the abundance of *Pseudogymnoascus* spp., a widespread Antarctic soil fungi, and of total fungi in a southern maritime Antarctic soil. When growth substrates - and in particular glycine - are applied to soil, these negative effects of OTCs on fungal DNA abundances are exacerbated, suggesting that access to nutrients or water may have significant interactive effects with warming on the growth of the *Pseudogymnasocus* spp.

# 4 The *in vitro* effects of elevated temperature and altered water availability on the growth and extracellular enzyme activities of Antarctic *Pseudogymnoascus pannorum*

## 4.1 Introduction

Antarctic terrestrial ecosystems are characterised by low temperatures, high UV radiation, frequent freeze-thaw cycles, and low water and nutrient availability, making them harsh environments for resident biota (Morita 1975, Beyer and Bölter 2000, Ruisi *et al*. 2007, Hassan *et al.* 2016). In Antarctica, soils are usually low in nutrients (Beyer and Bolter 2000), are characterised by an absence of insects and higher animals, and are dominated by a wide range of micro-organisms consisting of Archaea, bacteria, fungi and algae, and limited numbers of invertebrates such as springtails (Collembolla) and mites (Acari; Margesin and Miteva 2011, Convey 2013). Micro-organisms are important components of decomposition processes in all soils, including those of the Antarctic, and play a key role in food webs and biogeochemical processes, especially in the cycling and mineralization of nutrients, such as carbon, nitrogen and phosphorus (Swift *et al*. 1979, Rosa *et al*. 2010, Tedersoo *et al.* 2014, Krishnan *et al*. 2018). They decompose complex organic compounds, such as chitin (present in fungal cell walls and arthropod exoskeletons) and cellulose (present in plant cell walls), the two most frequent polymers present in the natural environment (Rinuado 2006).

Despite fungi being frequent in the soils of cold regions (Onofri *et al*. 2007, Yergeau *et al.* 2007, de Graaff *et al.* 2010, Tedersoo *et al*. 2014), and being the dominant decomposers of organic matter in these soils, most of the research on these microbes in Antarctica has focused on bacteria and yeasts, rather than filamentous fungi. Antarctic mycological research has hitherto mostly concentrated on the phylogeny of fungi present in soils and plants (e.g., Onofri *et al*. 2000, Arenz *et al*. 2006, Newsham and Bridge 2010, Arenz *et al*. 2011), little is known about fungal diversity in these habitats and how these microbes influence ecosystem functioning (Duncan *et al.* 2008, Krishnan *et al*. 2011, 2016 and 2018, Held and Blanchette 2017). The fungi encountered in Antarctica colonize a variety of different substrata, and are able to withstand a wide

range of environmental conditions (Ruisi *et al.* 2007). They are able to survive owing to a range of adaptations, survival strategies and physiological mechanisms that enable them to grow and function at low temperatures and water availabilities (Robinson 2001, Gunde-Cimerman *et al*. 2003, Ruisi *et al*. 2007, Margesin and Miteva 2011, Cararasco *et al*. 2012, Loperena *et al*. 2012, Krishnan *et al*. 2016, Tsuji *et al*. 2016).

Climate change has had significant effects on maritime Antarctic ecosystems over the previous few decades (IPCC 2007, Bracegirdle *et al*. 2008). Although warming of the Antarctic Peninsula has slowed since 2000, it is predicted to resume later this century under moderate greenhouse gas emission scenarios (Bracegirdle *et al*. 2008, Turner *et al.* 2016), with projected temperature increases of 2.4 °C over the next century and an increase in net precipitation being predicted to have significant impacts on this highly sensitive polar region (IPCC 2007, Bracegirdle *et al*. 2008, Shanhun *et al*. 2012, Nielsen and Wall 2013). It is logical to assume that rising temperatures will enhance the metabolism of resident biota, the growth of which is normally restricted by the low temperatures and water availabilities encountered in Antarctic soils. Given that fungi in the soils of the Antarctic are mostly psychrophilic or cold-adapted mesophiles (Morita 1975, Robinson 2001), as temperatures rise in the region, those inhabiting soil surfaces may be frequently exposed to temperatures exceeding their growth optima (Longton and Holdgate 1967). Climate change thus has the potential to inhibit the growth and metabolism of these microbes. Furthermore, given that increased temperatures in maritime Antarctic soils will result in the mobilisation of water (Bokhorst *et al.* 2007, Singh *et al*. 2010), the impacts of increased bioavailability of water may be an important factor determining the responses of fungi to climate change in these soils (Kennedy 1993).

Enzyme assays are a useful method to assess microbial community function to answer questions related to soil decomposition and biogeochemical processes. Two approaches exist to screen for a range of enzymes produced by microbes, the culture based method, involving growing isolates on solid media, and the metagenomic approach. Both have been used in recent studies identifying enzyme activities of Antarctic fungi (for example Duncan *et al*. 2006, Krishnan *et al*. 2011, Carassco *et al*. 2012, Krishnan *et al*. 2016). There is an interest in the enzymes produced by cold

adapted microbes due to their potential use in biotechnological processes and industry (Hankin and Anagnostakis 1975, Margesin and Feller 2010, Singh *et al*. 2010, Krishnan *et al*. 2018). Ecological research is however required to understand how enzyme activities are affected by environmental changes and what effects they are likely to have on soil processes in polar regions. Antarctic fungi produce a range of extracellular enzymes (ECEs) that decompose complex organic molecules and contribute towards carbon, nitrogen and phosphorus turnover in soil (Burns 2002, Allison and Jastrow 2006). The ECEs are secreted into the environment to break down complex molecules into smaller compounds, which are absorbed for immediate use by the fungus (Sinsabaugh *et al*. 1993, Burns and Dick 2002, Allison 2006, Krishnan *et al*. 2016). The activities of enzymes depend on their stability and the environmental matrix into which they are secreted (Hanking and Anagnostakis 1975, Allison 2006). The ECEs most commonly produced by Antarctic soil fungi include amylase, cellulase, chitinase, glucosidase, invertase, lipase, pectinase, protease, tannase and xylanase, urease, RNase, DNase and phosphatase (Fenice *et al*. 1997, Duarte *et al*. 2017). The production of these ECEs is an expression of microbial activity and function, and is affected by external environmental factors, such as temperature and water availability (Sinsabaugh *et al*. 2013).

*Pseudogymnoascus pannorum* is one of the most frequent fungi recorded in maritime Antarctica (Newsham *et al*. 2016) and is thought to play a significant role in decomposition and nutrient cycling in the soils of the region (Arenz *et al*. 2006, Duncan *et al*. 2008, Arenz and Blanchette 2009, Blanchette *et al*. 2010, Rosa *et al.* 2010, Arenz and Blanchette 2011, Hayes 2012, Krishnan *et al*. 2016). It produces a range of ECEs, *viz*. amylase, cellulase, chitinase, endoglucanase, glucanase, lipase, phosphatase, protease, protease and urease, which metabolize a wide range of compounds present in cold soils (Fenice *at el*. 1997, Arenz *et al*. 2006, Duncan *et al.* 2006, Hayes 2012, Krishnan *et al*. 2018). There is however, a shortage of information on the effects of environmental changes, such as increases in temperature and changes to water availability, on the activity of *P. pannorum* in the natural environment. Because of their effects on enzyme kinetics, substrate availability and binding, temperature and water are dominant factors controlling soil enzyme activities (Arnosti *et al*. 2014). Hence, these abiotic factors are

likely to affect the growth and enzyme activity of *P. pannorum* in Antarctic soils (Waldrop *et al*. 2010, Arnosti *et al*. 2014).

When testing for the potential effects of climate change on the growth of Antarctic *P. pannorum* in controlled laboratory experiments, diel temperature oscillations have not been applied, despite the fact that daily cycles in temperature, which strongly affect Antarctic soils (Longton and Holdgate 1967), are known to affect key ecosystem processes (Dang *et al*. 2009). Furthermore, previous ecophysiological studies on Antarctic *P. pannorum* have not controlled water availability by altering matric water potential, which is known to strongly influence fungal growth (Wynn- Williams 1990, Robinson 2001) and have used artificial media that are rich in nutrients on which to grow isolates (Hughes *et al.* 2003, Krishnan *et al.* 2011). These previous studies have hence resulted in incremental advances in knowledge of how environmental changes will affect the function and role of *P. pannorum* in Antarctic soils (Hughes *et al*. 2013, Krishnan *et al.* 2011, 2016, 2018, Tajuddin *et al*. 2018).

In Chapter 3, the abundance of *Pseudogymnoascus* spp*.* DNA was found to be lower in warmed, nutrient-amended soils in the natural environment. In the present chapter, under controlled conditions, the growth and enzyme activities of *P. pannorum* grown on soil extract medium are examined and the influenced of water availability and warming, applied using environmentally-realistic oscillating temperatures. Using data reported in Chapter 3, it is hypothesised that:

1) warming to > *ca*. 20 °C decreases the growth rate and enzyme activities of *P. pannorum,*

2) altered water availability influences the growth rate and enzyme activities of *P. pannorum,* and

3) warming interacts with water availability to determine the growth rate and enzyme activities of *P. pannorum.*

## 4.2 Materials and methods

### 4.2.1 Fungal isolates and experimental design

Three isolates of *P. pannorum* were used in this study (Table 4.1, Figure 4.1). The isolates, identified by analyses of ITS region sequences, were isolated at 4 °C from Mars Oasis soil collected in 2009 (Section 2.1.1, 2.4.1 and 2.4.2), and were maintained on half strength potato dextrose agar medium at 4 °C. They were exposed on cellophane film overlaying soil extract medium (SEM; see Section 2.6) to three daily temperature cycles using incubators (2–18 °C, 2–21 °C and 2–24 °C), mimicking natural *in situ* temperature changes at soil surfaces at Mars Oasis during the summer, and likely future temperature increases arising from climate warming (see Figure 2.8). The three isolates were also grown at four water potentials (-10.8 MPa, -6.0 MPa, -3.6 MPa and -1.06 MPa), mimicking water availabilities in dry (2 % moisture) through to wet (8 % moisture) conditions, characteristic of Mars Oasis soil during summer (Section 2.3). The water potential of the SEM was manipulated using polyethylene glycol 8000, which alters matric potential ($\Psi_m$; Section 2.6). Temperature cycles and water availability treatments were applied factorially, resulting in 12 treatment combinations. Each treatment was replicated four times for each of the three isolates (Section 2.6).

*Table 4.1 Identification of* Pseudogymnoascus pannorum *isolates used in this study based on NCBI BLAST comparisons of ITS regions. See Section 2.4.2 for details of methods.*

| Isolate | Species | Identity (%) | Coverage (%) | Reference matches |
|---|---|---|---|---|
| Isolate 1 | *P. pannorum* | 99 | 100 | KP411571, LC085196 |
| Isolate 2 | *P. pannorum* | 99 | 100 | KP411571, LC085196 |
| Isolate 3 | *P. pannorum* | 99 | 98 | KP411571, LC085196, KP714598 |

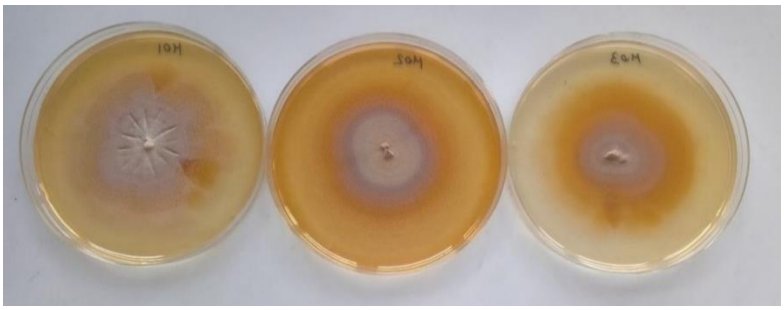


*Figure 4.1 The* Pseudogymnoascus pannorum *isolates used in the laboratory experiment investigating the effects of water and temperature manipulations on hyphal extension rates and enzyme activities.*

### 4.2.2 Extension rate analyses

Colonies of *P. pannorum* were exposed to the treatments for 11 weeks, approximating to the length of a growing season at Mars Oasis (Figure 2.3). Once the extension of the colony was visible beyond the inoculation point, an image was taken weekly with a digital camera from a height of 20 cm. The colony's surface area was measured using ImageJ software (Version 1.49v, National Institute of Health, Bethesda, Maryland, USA). The extension rate of each colony was determined using the slope value from regressions between time and colony surface area in the linear growth phase (Section 2.6.1, Appendix 6).

### 4.2.3 Extracellular enzyme assays

Colorimetric assays were used to measure the concentrations of extracellular enzymes in extracts from colonies. The extracts were prepared at the end of the experiment by placing the cellophane film on which the colonies were growing upside down into 4 ml of 10 mM phosphate buffer in a 90 mm diameter Petri dish for 2 hours at 7 °C, with gentle shaking. The concentrations of the five most frequent enzymes (acid and alkaline phosphatase, chitinase, cellulase and leucine aminopeptidase) produced by the three isolates were measured using p-nitrophenol assays, following the methods of Alam *et al*. (2009). Enzyme activities were calculated as µmol p-nitrophenol released (U) from standard curves constructed from known concentrations of p-nitrophenol. Total protein concentration was also measured, using a BCA protein kit and following the manufacturer's protocol. Further details of enzyme analyses are given in Section 2.6.2.

Enzyme activities were expressed in four ways, *viz*., enzyme activity per colony (EAC; U $hr^{-1}$ $colony^{-1}$), specific enzyme activity per colony (SEAC; U $hr^{-1}$ $colony^{-1}$ mg $protein^{-1}$), enzyme activity per biomass (EA; U $hr^{-1}$ mg $hyphae^{-1}$) and specific enzyme activity per biomass (SEA; U $hr^{-1}$ mg $hyphae^{-1}$ mg $protein^{-1}$). Response ratios were calculated in order to interpret how the measures of enzyme activity responded to temperature cycles more easily. For each fungal isolate at each water potential, two response ratios were calculated, the first by dividing the mean enzyme activity at 2– 21 °C by that at 2–18 °C, and the second by dividing the mean enzyme activity at 2– 24 °C by that at 2–18 °C. The values were $\log_{10}(n+1)$ transformed for graphical

visualisation. Transformed response ratio values of > 0.33 hence indicated positive effects of the 2–21 °C or 2–24 °C temperature cycles on enzyme activities, relative to the 2–18 °C temperature cycle, and transformed values of < 0.033 indicated negative effects.

### 4.2.4 Statistical analyses

All statistical analyses were performed either in the R statistical package or MINITAB 17 (version 17.2.1). General Linear Models were used to test for the main and interactive effects of temperature cycles and water potentials on extension rates, protein concentrations and the four measures of enzyme activity described above. Obvious outliers were removed from the dataset prior to statistical analyses. For enzyme data, negative values were treated as zero, indicating no enzyme production. Data were $\log_{10}(n+1)$-transformed prior to analyses. One way ANOVA and Tukey's multiple comparisons tests were used to determine significant differences between treatment levels. Enzyme activities were analysed separately for each enzyme and isolate.

## 4.3 Results

### 4.3.1 Extension rates

The extension rates of the three *P. pannorum* strains were significantly affected by isolate ($F_{2, 142}$ = 17.09, $P < 0.001$), matric water potentials ($\Psi_m$; $F_{3, 142}$ = 120.88, $P < 0.001$) and temperature cycles ($F_{2, 142}$ = 28.55, $P < 0.001$). All of the interaction terms (isolate × temperature, isolate × $\Psi_m$, temperature × $\Psi_m$ and isolate × temperature × $\Psi_m$) were significant ($P < 0.005$, Table 4.2). The extension rate of isolate 2 (2.21 ± 0.53 mm$^2$ day$^{-1}$) was faster than that of isolates 1 and 3 (1.51 ± 0.23 and 1.00 ± 0.18 mm$^2$ day$^{-1}$,

*Table 4.2 Output from General Llinear Model (three way ANOVA analysing for main and interactive effects of isolate, temperature cycles (2–18 °C, 2–21 °C and 2– 24 °C) and matric water potentials (-10.8 MPa, - 6.0 MPa, -3.6 MPa, -1.06 MPa) on the hyphal extension rates of three* P. pannorum *isolates. Bold* P *values indicate statistically significant effects (*P *< 0.05).*

| Factor | $F_{2, 142}$ | *P* |
|---|---|---|
| isolate | 17.09 | **< 0.001** |
| T | 28.55 | **< 0.001** |
| $\Psi_m$ | 120.88 | **< 0.001** |
| Isolate x T | 4.15 | **0.004** |
| Isolate x $\Psi_m$ | 3.65 | **0.002** |
| T x $\Psi_m$ | 10.82 | **< 0.001** |
| Isolate x T x $\Psi_m$ | 2.11 | **0.022** |

*Abbreviation: $\Psi_m$, water potential, T, temperature cycle*

respectively). The mean extension rate of all three isolates was fastest at -1.06 MPa (3.50 ± 0.66 mm$^2$ day$^{-1}$), and, relative to this $\Psi_m$, decreased by 45 % at -3.6 MPa, 81 % at -6.0 MPa and 93 % at -10.6 MPa (Figure 4.2). Across all three isolates, the fastest extension rate was recorded at the 2–18 °C temperature cycle (mean colony extension rate 2.53 ± 0.56 mm$^2$ day$^{-1}$), with extension rates reducing by 46 % and 66 % under the 2–21 °C and 2–24 °C temperature cycles, respectively (Figure 4.2). Decreasing $\Psi_m$ and warming negatively affected the extension rate of *P. pannorum*, accounting for the highly significant temperature × $\Psi_m$ interaction ($F_{6, 142}$ = 10.82, $P$ < 0.001).

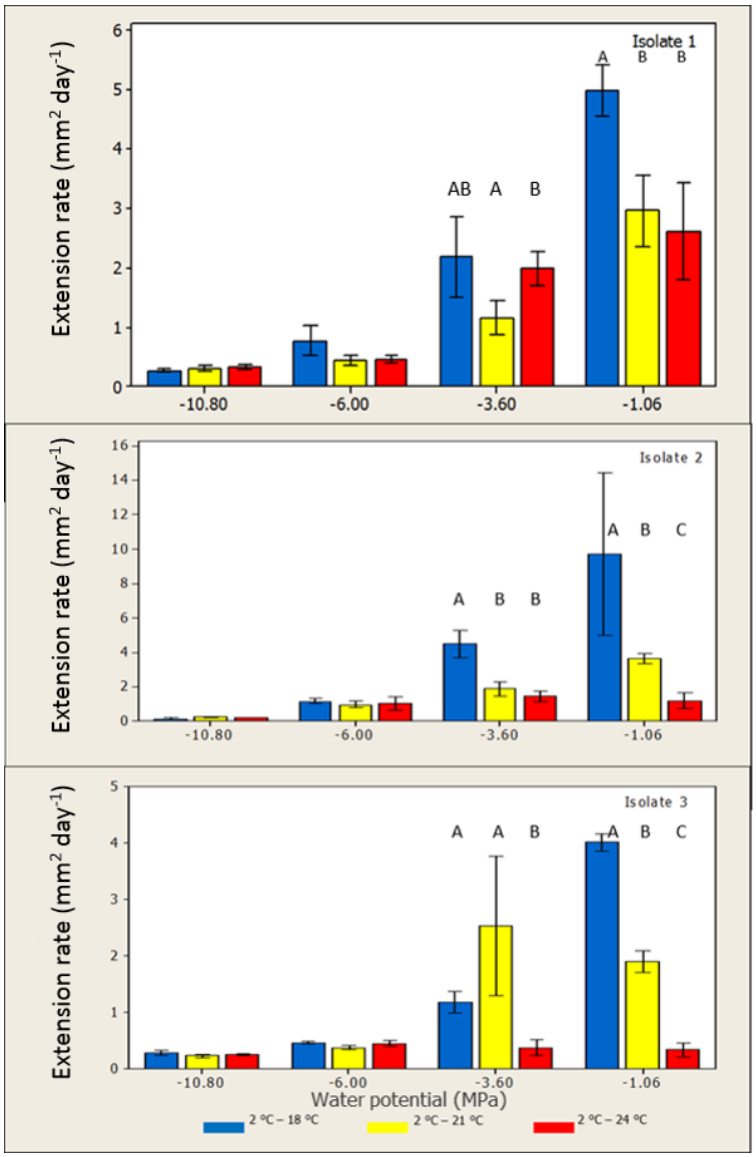



*Figure 4.2 Extension rates of* P. pannorum *at different temperature cycles and water potentials in the laboratory experiment. Values are means of four replicates ± SEM. Note that the y-axes of the three graphs are scaled differently. Differently superscripted letters indicate significant (P < 0.05) differences between treatments based on one way ANOVA test.*

As extension rates differed significantly between the isolates (Table 4.2), the effects of temperature cycles and water potentials were examined separately. Those of all three *P. pannorum* isolates were significantly affected by $\Psi_m$ and temperature cycle (all $P$ < 0.05, Table 4.3). The extension rate of isolate 1 was reduced by 51 %, 84 % and 92 % at -3.6 MPa, -6.0 MPa and -10.8 MPa, respectively ($F_{3, 47}$ = 50.08, $P$ < 0.001), compared with its mean extension rate at -1.06 MPa (3.60 ± 0.46 mm$^2$ day$^{-1}$,

Figure. 4.2). That of isolate 2 was reduced by 46 %, 78 % and 96 % at these water potentials ($F_{3, 46}$ = 5.98, *P* = 0.002), relative to its mean extension rate at -1.06 MPa (4.84 ± 1.79 mm$^2$ day$^{-1}$). Similarly, the extension rate of isolate 3 declined by 39 %, 79 % and 88 % at each of the three water potentials ($F_{3, 45}$ = 128.22, *P* < 0.001), compared with its mean extension rate at - 1.06 MPa (2.08 ± 0.46 mm$^2$ day$^{-1}$). Warming had a negative effect on the extension of the *P. pannorum* isolates. Compared with mean extension rates at the 2– 18 °C temperature cycle (2.04 ± 0.53, 4.12 ± 1.51 and 1.48 ± 0.39 mm$^2$ day$^{-1}$ for isolates 1, 2 and 3, respectively), the 2–21 °C and 2–24 °C temperature cycles reduced the mean extension of *P. pannorum* isolates by 40–77 % (Figure 4.2). Temperature increases only affected the extension of the fungus at - 1.06 MPa and - 3.6 MPa (Figure 4.2). At -1.06 MPa, the 2–21 °C and 2–24 °C cycles significantly slowed the colony extension rate of isolate 1 by 40 % and 47 %, respectively, compared with its mean extension rate at 2–18 °C (4.97 ± 0.46 mm$^2$ day$^{-1}$, $F_{2, 11}$ = 4.57, *P* = 0.048). At the same water potential, that of isolate 2 was reduced by 68 % and 80 % at 2–21 °C and 2– 24 °C, respectively, relative to its extension rate at 2–18 °C (5.12 ± 1.47 mm$^2$ day$^{-1}$). Also at -1.06 MPa the extension rate of isolate 3 was reduced by 52 % and 91 % at 2 – 21 °C and 2– 24 °C, respectively, compared with that at 2–18 °C (4.01 ± 0.16 mm$^2$ day$^{-1}$; Figure 4.2). At - 3.6 MPa, an increase in temperature to 2–21 °C decreased the extension rates of isolate 1 by 44 % and isolate 2 by 61 %, compared with their mean extension rates at the 2–18 °C temperature cycle. At the same $\Psi_m$, an increase in temperature to 2–24 °C reduced the extension rates of isolates 2 and 3 by 68 %, compared with their mean extension rates at the 2–18 °C temperature cycle.

*Table 4.3 General Linear Model (two-way ANOVA) outputs analysing main and interactive effects of temperature and water potential on the hyphal extension rates of three* P. pannorum *isolates. Bold text indicates significant (*P< *0.05) values, superscribed numbers are degrees of freedom.*

| | | ***P. pannorum* isolate** | | |
|---|---|---|---|---|
| **Treatment** | **Statistics** | **1$_{6, 47}$** | **2$_{6, 46}$** | **3$_{6, 45}$** |
| T | *F* | 6.14 | 4.38 | 18.48 |
| | *P* | **0.005** | **0.020** | **<0.001** |
| $\Psi_m$ | *F* | 50.08 | 5.98 | 28.22 |
| | *P* | **<0.001** | **0.002** | **<0.001** |
| T x $\Psi_m$ | *F* | 2.98 | 2.04 | 13.13 |
| | *P* | **0.020** | 0.086 | **<0.001** |

*Abbreviations: T, temperature cycle; $\Psi_m$, matric water potential*

### 4.3.2 Protein concentration

Protein concentration was significantly affected by temperature ($F_{2, 142}$ = 40.82, $P$ < 0.001) and $\Psi_m$ ($F_{3, 142}$ = 354.58, $P$ < 0.001). It did not vary significantly between isolates, but was affected by the interactions between isolate and temperature ($F_{4, 142}$ = 3.64, $P$ = 0.008), and isolate and $\Psi_m$ ($F_{6, 142}$ = 3.85, $P$ < 0.001). The $\Psi_m$ × temperature interaction also had a significant effect on protein concentration ($F_{6, 142}$ = 5.47, $P$ < 0.001). Mean protein concetration was highest at - 1.06 MPa (0.20 ± 0.01 mg ml$^{-1}$) and decreased by 55 %, 68 % and 79 % at - 3.6 MPa, -6.0 MPa and - 10.8 MPa, respectively. Mean protein concentration was 31 % higher at the 2–21 °C temperature cycle compared to the 2–18 °C cycle. At the 2–24 °C cycle, however, protein concentration was reduced by 11 % compared with the 2– 18 °C cycle (mean 0.09 ± 0.01 mg ml$^{-1}$), and by 32 % compared with the 2–21 °C cycle (mean 0.12 ± 0.01 mg ml$^{-1}$, $F_{2, 142}$ = 4.36, $P$ = 0.015; Table 4.4, Figure 4.3).

*Table 4.4 General Linear Model (two way ANOVA) outputs analysing main and interactive effects of temperature and water potential on protein concentration in extracts from three* P. pannorum *isolates. Bold text indicates significant (P < 0.05) values; superscribed numbers are degrees of freedom.*

| | | Statistics | |
|---|---|---|---|
| **Isolate** | **Treatment** | $F_{2, 47}$ | $P$ |
| | T | 14.43 | **<0.001** |
| 1 | $\Psi_m$ | 202 | **<0.001** |
| | T x $\Psi_m$ | 5.89 | **<0.001** |
| | T | 20.47 | **<0.001** |
| 2 | $\Psi_m$ | 142 | **<0.001** |
| | T x $\Psi_m$ | 13.37 | **<0.001** |
| | T | 14.78 | **<0.001** |
| 3 | $\Psi_m$ | 75 | **<0.001** |
| | T x $\Psi_m$ | 3.09 | **0.015** |

*Abbreviations: T, temperature; $\Psi_m$, matric water potential*

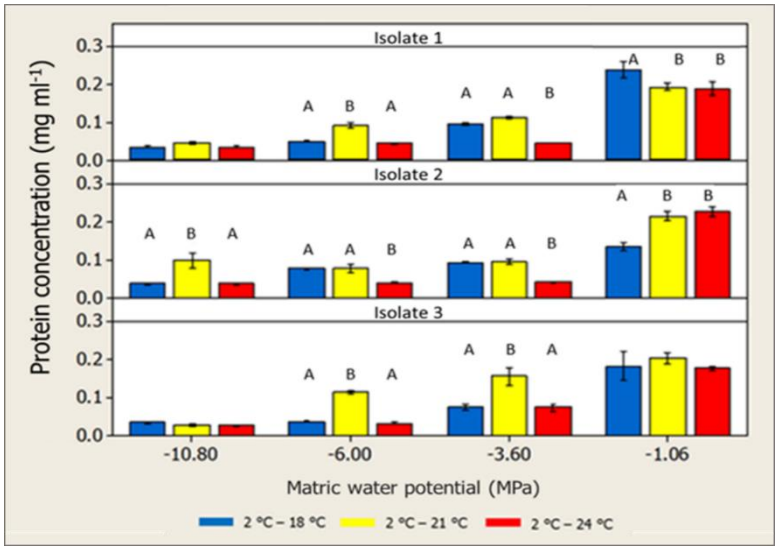



*Figure 4.3 Protein concentration (mg ml$^{-1}$) in extracts from three* P. pannorum *isolates at different temperature cycles and water potentials in the laboratory experiment. Values are means of four replicates ± SEM. Different letters indicate significant (P < 0.05) differences between treatments based on one way ANOVA.*

### 4.3.3 Extracellular enzyme activities

Enzyme activity expressed per colony (EAC) differed significantly between enzymes ($F_{4, 715}$ = 3610.24, $P$ < 0.001) and isolates ($F_{2, 715}$ = 25.01, $P$ < 0.001). The effect of temperature cycle ($F_{2, 715}$ = 261.91, $P$ < 0.001) and $\Psi_m$ ($F_{2,715}$ = 2205.22, $P$ < 0.001) significantly affected EAC, as did all of the 2-, 3- and 4-level interaction terms (Table 4.5). Cellulase had the highest EAC (66.95 ± 5.73 U $hr^{-1}$ $colony^{-1}$), followed by chitinase (10.75 ± 0.91 U $hr^{-1}$ $colony^{-1}$) and leucine aminopeptidase (7.21 ± 0.72 U $hr^{-1}$ $colony^{-1}$). Phosphatases had the lowest EAC values, with alkaline and acid phosphatase enzyme activities of 2.22 ± 0.28 U $hr^{-1}$ $colony^{-1}$ and 0.49 ± 0.07 U $hr^{-1}$ $colony^{-1}$, respectively. Enzyme activity expressed per colony at 2–21°C decreased by 20 % compared with the 2–18 °C temperature cycle (17.95 U $hr^{-1}$ $colony^{-1}$), and by 29 % compared with the 2– 24 °C cycle. The EAC decreased at lower water potentials, by 56 %, 78 % and 99 % for -3.6 MPa, -6.0 MPa and -10.8 MPa, respectively, compared with -1.06 MPa (42.12 ± 5.0 U $hr^{-1}$ $colony^{-1}$; $F_{3, 718}$ = 120.49, $P$ < 0.001).

Specific enzyme activity per colony (SEAC) showed similar trends to EAC, varying significantly between enzymes ($F_{4, 715}$ = 3794.14, $P$ < 0.001) and isolates ($F_{2, 715}$ = 35.49, $P$ < 0.001), and being significantly affected by temperature cycle ($F_{2, 715}$ = 439.06, $P$ < 0.001) and $\Psi_m$ ($F_{3, 715}$ = 3818.65, $P$ < 0.001). All interaction terms, at all levels, were significant (Table 4.5). SEAC decreased by 27 %, 47 % and 93 % at -3.6 MPa, -6.0 MPa and -10.6 MPa, relative to -1.06 MPa (86.24 ± 0.36 U $colony^{-1}$ mg $protein^{-1}$). The SEAC was also affected by temperature cycle, being 4 % and 13 % lower in colonies incubated at 2–18 °C and 2– 24 °C, relative to 2-21 °C.

As for EAC and SEAC, the overall enzyme activity expressed per biomass (EA) varied significantly between enzymes ($F_{4, 701}$ = 267.78, $P$ < 0.001) and isolates ($F_{2, 701}$ = 559.12, $P$ < 0.001). The effects of $\Psi_m$ ($F_{3, 701}$ = 1197.08, $P$ < 0.001) and temperature cycle ($F_{2, 701}$ = 48.32, $P$ < 0.001) were significant, as were all interaction terms at all levels ($P$ < 0.001, Table 4.5), except for the $\Psi_m$ x isolate interaction. Isolate 1 had the highest values of EA (17.91 ± 2.90 U $hr^{-1}$ mg $biomass^{-1}$). As for the other two measures of enzyme activity, EA was highest for cellulase (43.32 ± 4.69 U $hr^{-1}$ mg $biomass^{-1}$), and was lowest for the two phosphatases. At -10.8 MPa, mean EA (7.26 ± 2.35 U $hr^{-1}$ mg of $biomass^{-1}$) was 34 %, 45 % and 63 % lower than at -1.06 MPa,

- 3.6 MPa and -6.0 MPa, respectively. The 2–21 °C temperature cycle increased EA by 2.4 times, compared with the 2–18 °C treatment. In contrast, the 2–24 °C cycle reduced enzyme activity by 70 % and 87 %, compared with the 2–18 °C and 2–21 °C treatments, respectively.

Specific enzyme activity per biomass (SEA) showed similar trends to EA, and was significantly affected by enzyme ($F_{4, 701}$ = 396.31, $P$ < 0.001), isolate ($F_{2, 701}$= 360.68, $P$ < 0.001), temperature cycle ($F_{2, 701}$ = 45.67, $P$ < 0.001) and $\Psi_m$ ($F_{3, 701}$ = 1106.63, $P$ < 0.001). All interaction terms for SEA were highly significant ($P$ < 0.001), except that for $\Psi_m$ × isolate (Table 4.5). The highest values of SEA were recorded at -6.0 MPa (275.3 ± 40.5 U $hr^{-1}$ mg $biomass^{-1}$ mg $protein^{-1}$) and the lowest at -1.06 MPa (57.8 ± 10.1 U $hr^{-1}$ mg $biomass^{-1}$ mg $protein^{-1}$). Isolate 1 had the highest SEA values, compared with isolates 2 and 3 (217.8 ± 32.8 *vs*. 126.6 ± 19.1 and 164.0 ± 55.4 U $hr^{-1}$ mg $biomass^{-1}$ mg $protein^{-1}$, respectively). SEA values at 2– 24 °C were reduced by 56 % and 79 % compared with the 2–18 °C and 2–21 °C temperature cycles.

*Table 4.5 Outputs from General Linear Models (four way ANOVA) showing main effects of enzyme, isolate, temperature and matric water potential, and interactions between these factors, on the protein concentration, enzyme activities and specific enzyme activities of* P. pannorum. *Bold - treatments that were statistically significant (P < 0.05). Data were* $log_{10}$*(n + 1) transformed prior to analyses;* $F_{\text{degrees of freedom}}$.

| | EAC | | SEAC | | EA | | SEA | | Protein content | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Factor** | $F_{2, 715}$ | $P$ | $F_{2, 715}$ | $P$ | $F_{2, 701}$ | $P$ | $F_{2, 701}$ | $P$ | $F_{2, 143}$ | $P$ |
| E | 3610.24 | **< 0.001** | 3794.14 | **< 0.001** | 267.78 | **< 0.001** | 396.31 | **< 0.001** | | |
| I | 25.01 | **< 0.001** | 35.49 | **< 0.001** | 559.12 | **< 0.001** | 360.68 | **< 0.001** | 0.12 | 0.889 |
| T | 261.91 | **< 0.001** | 439.06 | **< 0.001** | 48.32 | **< 0.001** | 45.67 | **< 0.001** | 40.82 | **<0.001** |
| $\Psi_m$ | 2205.22 | **< 0.001** | 3818.65 | **< 0.001** | 1197.08 | **< 0.001** | 1106.63 | **< 0.001** | 354.58 | **<0.001** |
| I x E | 17.95 | **< 0.001** | 11.39 | **< 0.001** | 12.49 | **< 0.001** | 13.41 | **< 0.001** | | |
| T x E | 45.83 | **< 0.001** | 34.35 | **< 0.001** | 38.44 | **< 0.001** | 11.49 | **< 0.001** | | |
| T x I | 20.81 | **< 0.001** | 18.88 | **< 0.001** | 18.28 | **< 0.001** | 18.12 | **< 0.001** | 3.64 | **0.008** |
| $\Psi_m$ x E | 182.55 | **< 0.001** | 334.51 | **< 0.001** | 83.85 | **< 0.001** | 89.46 | **< 0.001** | | |
| $\Psi_m$ x I | 2.42 | **0.026** | 4.69 | **< 0.001** | 1.53 | 0.167 | 3.69 | 0.001 | 3.85 | **0.002** |
| $\Psi_m$ x T | 25.13 | **< 0.001** | 82.45 | **< 0.001** | 46.87 | **< 0.001** | 17.85 | **< 0.001** | 5.47 | **<0.001** |
| T x I x E | 5.31 | **< 0.001** | 6.61 | **< 0.001** | 4.94 | **< 0.001** | 4.07 | **< 0.001** | | |
| $\Psi_m$ x I x E | 11.64 | **< 0.001** | 6.01 | **< 0.001** | 5.56 | **< 0.001** | 9.29 | **< 0.001** | | |
| $\Psi_m$ x T x I | 9.07 | **< 0.001** | 12.11 | **< 0.001** | 4.68 | **< 0.001** | 6.23 | **< 0.001** | 6.51 | **<0.001** |
| $\Psi_m$ x T x E | 23.6 | **< 0.001** | 8.33 | **< 0.001** | 9.28 | **< 0.001** | 12.94 | **< 0.001** | | |
| $\Psi_m$ x T x I x E | 7.28 | **< 0.001** | 4.42 | **< 0.001** | 3.59 | **< 0.001** | 5.38 | **< 0.001** | | |

*Abbreviations: EAC, enzyme activity per colony, SEAC, specific enzyme activity per colony, EA, enzyme activity per biomass, SEA, specific enzyme activity per biomass, E, enzyme, I, isolate, T, temperature cycle, $\Psi_m$, matric water potential.*

### 4.3.3.1 Chitinase

There were significant temperature × $\Psi_m$ effects on chitinase EAC for all isolates of *P. pannorum* (all $F_{6, 47}$ = 59.04–125.77, $P$ <0.001; Appendix 11, Figure 4.4). For all

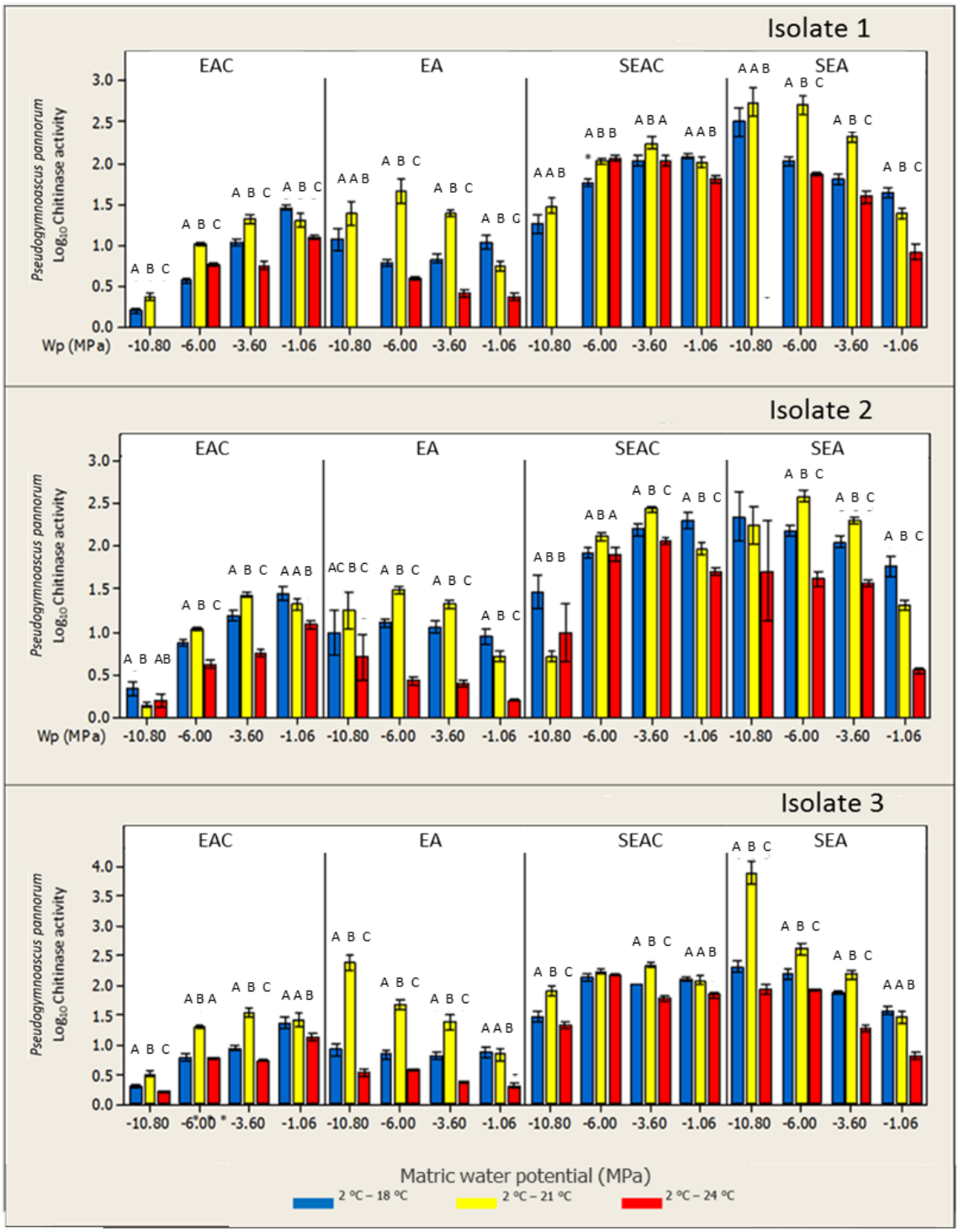



*Figure 4.4 Chitinase activities of three* P. pannorum *isolates at 2–18 °C, 2–21 °C and 2–24 °C temperature cycles and matric water potentials of -10.80, -6.00, -3.60 and -1.06 MPa. Values are means of four replicates ± SEM. EAC, enzyme activity per colony; SEAC, specific enzyme activity per colony; EA, enzyme activity per biomass; SEA, specific enzyme activity per biomass. Different letters indicate significant (P < 0.05) differences between treatments based on one way ANOVA. Data were log$_{10}$ transformed prior to statistical analyses.*

isolates, the 2–21 °C temperature cycle increased chitinase EA by *ca*. 1.5 – 2.5 times at $\Psi_m$ of ≤ - 3.6 MPa, and reduced it by *ca*. 15 % at -1.06 MPa, compared with the 2–18 °C temperature cycle. The 2–24 °C cycle reduced chitinase EA by *ca*. 55–60 % at -10.8 MPa, -3.6 MPa and -1.06 MPa, and by 10 % at -6.0 MPa, compared to the 2–18 °C temperature cycle (Figure 4.4). Similar trends were observed for SEAC, EA and SEA, with the difference that values for EA and SEA were higher than for EAC and SEAC (Figure 4.4).

Analyses of response ratios indicated that when colony biomass was taken into account, the 2–21 °C temperature cycle increased chitinase EA by 3–6 times at ≤ - 3.6 MPa, with this effect becoming more pronounced at lower water potentials (Figure 4.5). However, at the highest water potential (-1.06 MPa) there were 14–55 % decreases in EA under the 2–21 °C and 2–24 °C cycles, relative to the 2–18 °C cycle. The 2–24 °C temperature cycle reduced chitinase activity by *ca*. 70–90 % at all water potentials, relative to the 2–18 °C cycle. Similar trends were observed for SEAC, EA and SEA (Figure 4.5).

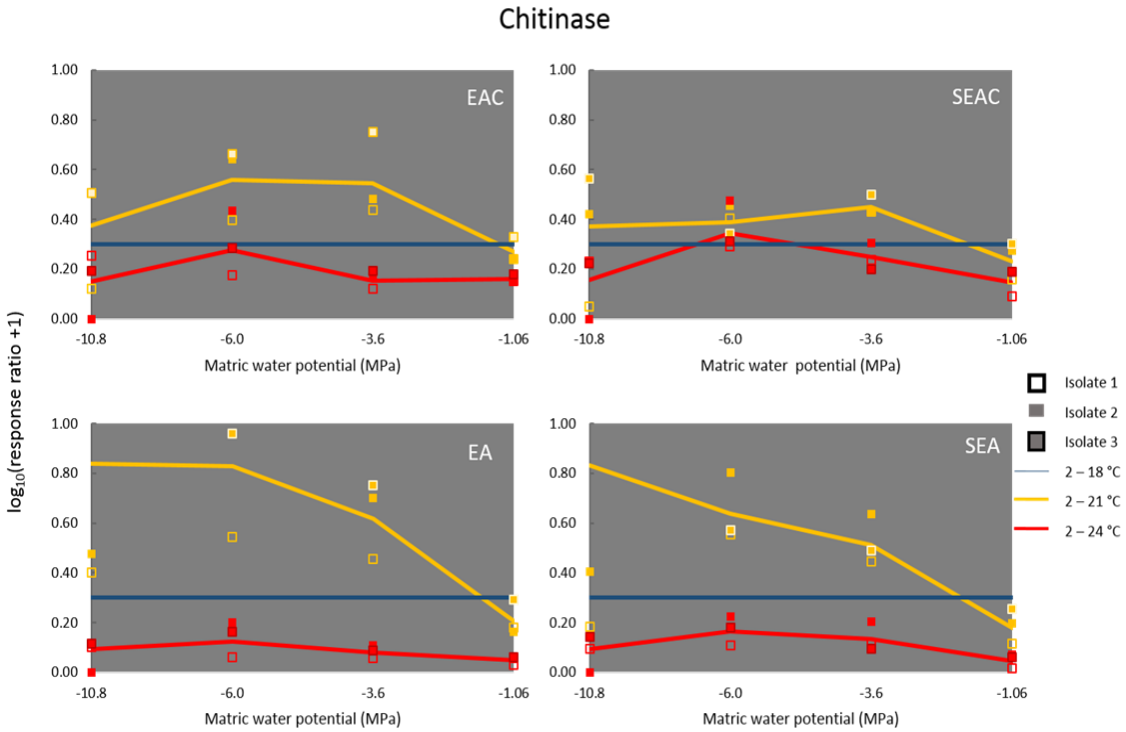



*Figure 4.5 $Log_{10}$ response ratios (relative to the 2 – 18 °C temperature cycle, denoted by blue line) of the chitinase activities of three* P. pannorum *isolates at 2–21 °C (yellow line) and 2–24 °C (red line) temperature cycles and at water potentials of -10.80, -6.00, -3.60 and -1.06 MPa. EAC; enzyme activity per colony, SEAC; specific enzyme activity per colony, EA; enzyme activity per biomass, SEA; specific enzyme activity per biomass.*

### 4.3.3.2 Cellulase

Cellulase EAC for all isolates was affected by $\Psi_m$ ($F_{3, 47}$ = 262.23–364.20, all $P < 0.001$). EAC was highest at -1.06 MPa (169.1–180.6 U hr$^{-1}$ colony$^{-1}$) and decreased to 0– 1.37 U hr$^{-1}$ colony$^{-1}$ with decreasing $\Psi_m$ (Figure 4.6). There was a significant

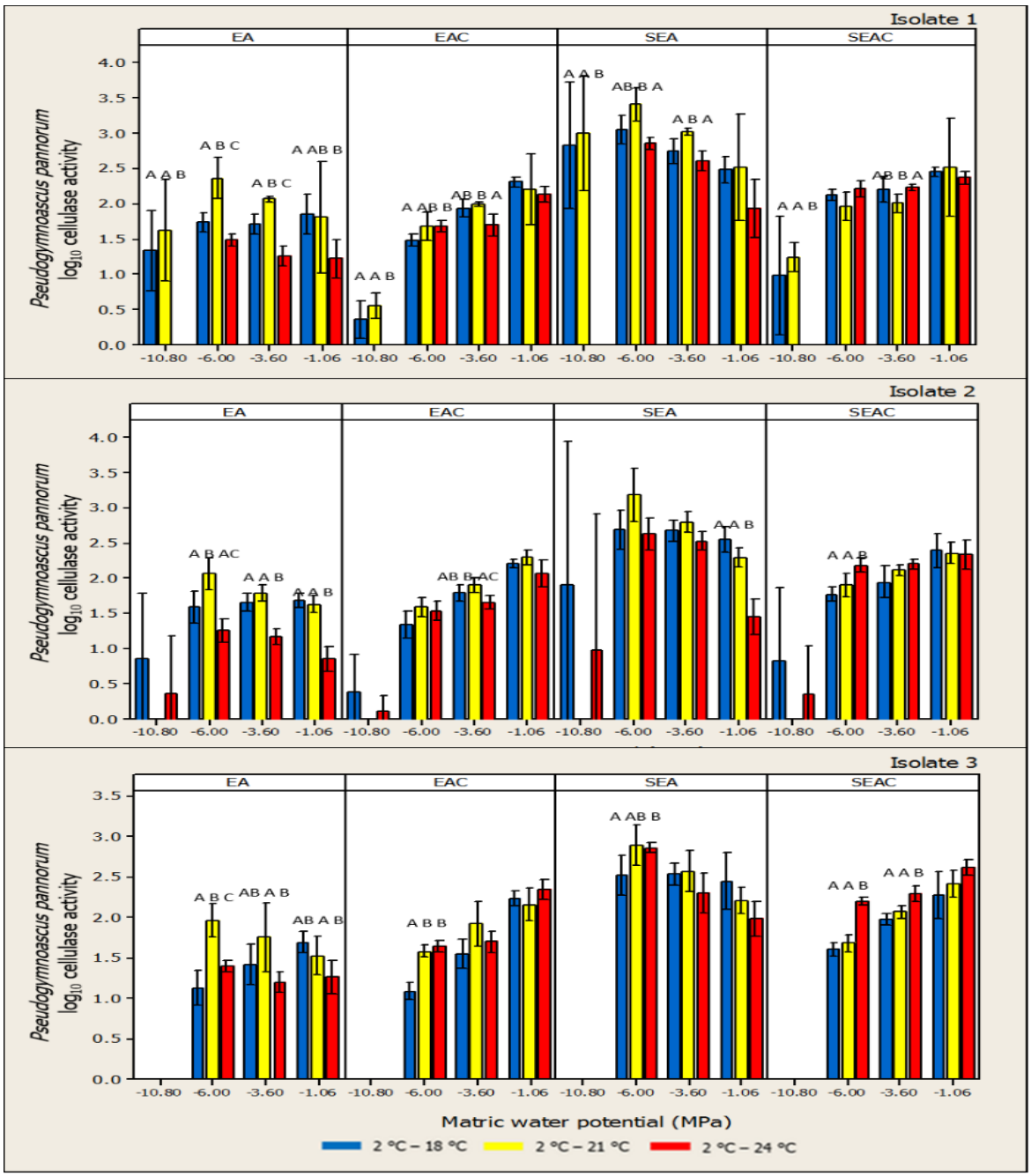



*Figure 4.6 Cellulase activities of three* P. pannorum *isolates at 2–18 °C, 2–21 °C and 2–24 °C temperature cycles and at matric water potentials of -10.80, -6.00, -3.60 and -1.06 MPa. Values are means of four replicates ± SEM. EAC; enzyme activity per colony, SEAC; specific enzyme activity per colony, EA; enzyme activity per biomass, SEA; specific enzyme activity per biomass. Differen letters indicate significant (P < 0.005) differences between treatments based on one way ANOVA. Data were log$_{10}$ transformed prior to statistical analyses.*

interactive effect of temperature cycle and $\Psi_m$ on cellulase EAC ($F_{6, 47}$ = 157.32–459.94, all $P < 0.001$; Fig. 4.6). When it was measurable, cellulase EAC was reduced by 20 % and 90 % at -10.8 MPa under the 2–21 °C and 2–24 °C temperature cycles, compared with the 2–18 °C cycle (Figure 4.6). The 2–21 °C cycle increased cellulase EAC by 1.5–2 times at -6.0 MPa and -3.6 MPa, compared with the 2–18 °C cycle, but this effect was not observed for SEAC. There was no effect of the 2–21 °C temperature cycle on cellulase EAC, nor SEAC, at -1.06 MPa (Figure 4.6). A temperature increase from 2–18 °C to 2– 24 °C typically doubled cellulase EAC and SEAC at -6.0 MPa. Combined increases in $\Psi_m$ and temperature reduced cellulase EAC and SEAC by *ca*. 10–20 % and *ca*. 20–50 %, respectively, compared with the 2–18 °C temperature cycle. Similair trends were observed for EA as for EAC, and for SEA as for SEAC (Figure 4.6).

Analyses of response ratios for cellulase activity indicated that EAC, EA and SEA were typically higher under the 2–21 °C temperature cycle, compared with the 2–18 °C cycle, at -6.0 MPa and -3.6 MPa (Figure 4.7). There were negligible effects on cellulase SEAC of increasing temperature from 2–18 °C to 2–21 °C (Figure 4.6). At -1.06 MPa, most

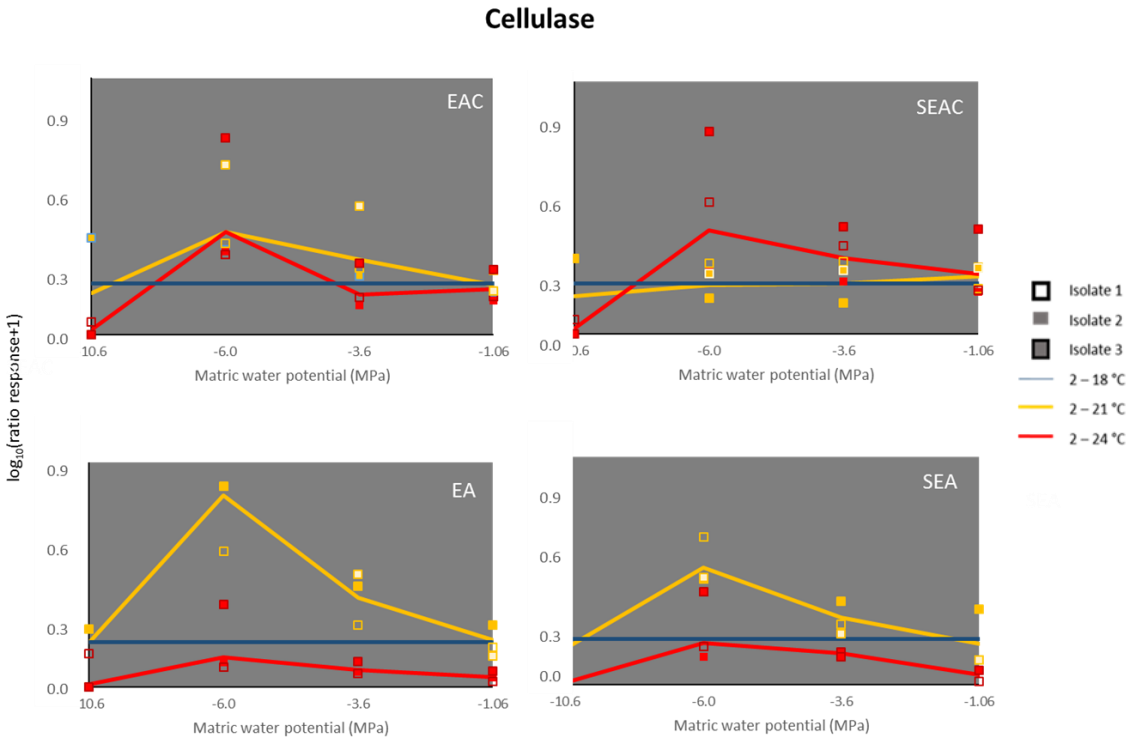



*Figure 4.7 $Log_{10}$ response ratios (relative to the 2 – 18 °C temperature cycle, denoted by blue line) of the cellulase activities of three* P. pannorum *isolates at 2–21 °C (yellow line) and 2–24 °C (red line) temperature cycles and at water potentials of -10.80, -6.00, -3.60 and -1.06 MPa. EAC; enzyme activity per colony, SEAC; specific enzyme activity per colony, EA; enzyme activity per biomass, SEA; specific enzyme activity per biomass.*

measures of cellulase activity declined under the 2–21 °C cycle, compared with the 2– 18 °C cycle (Figure 4.7). In contrast to the responses for chitinase, increasing temperature from 2–18 °C to 2–24 °C increased cellulase EAC and SEAC at -6.0 MPa and -3.6 MPa, with the magnitude of this effect declining as more water became available (Figure 4.7). As for chitinase activity, cellulase EA and SEA were consistently lower at the 2–24 °C temperature cycle, relative to the 2–18 °C cycle (Figure 4.7).

#### *4.3.3.3 Leucine aminopeptidase*

Leucine aminopeptidase activity (expressed as EA, EAC, SEA and SEAC) of all isolates was affected by $\Psi_m$ ($F_{3, 46}$ = 58.00–93.04, all $P < 0.001$; Appendix 11), with the EA highest values at -6.0 MPa (Figure 4.8). The activities were also affected by temperature ($F_{2, 46}$ = 4.82–146.63, all $P < 0.001$; Appendix 11), with lowest values at 2–24 °C. There were significant interactive effects of temperature cycle and water potential on leucine aminopeptidase EA, EAC, SEA and SEAC of each isolate ($F_{6, 46}$ = 8.52–28.07, all $P < 0.001$; Appendix 11). Response ratios showed that the 2–21 °C temperature cycle usually increased leucine aminopeptidase activity, relative to the 2–18 °C cycle at -6.0 MPa and -3.6 MPa (Figure 4.9). At the highest water availability (-1.06 MPa), the 2–21 °C cycle decreased all measures of leucine aminopeptidase activity of the isolates, relative to the 2–18 °C cycle (Figure 4.9). All measures of leucine aminopeptidase activity were consistently decreased in all isolates by the increase in temperature from 2–18 °C to 2– 24 °C (Figure 4.9). No leucine aminopeptidase activity was recorded for isolates 2 and 3 at -10.8 MPa, with a small amount of activity being measured for isolate 1 (Figure 4.9).

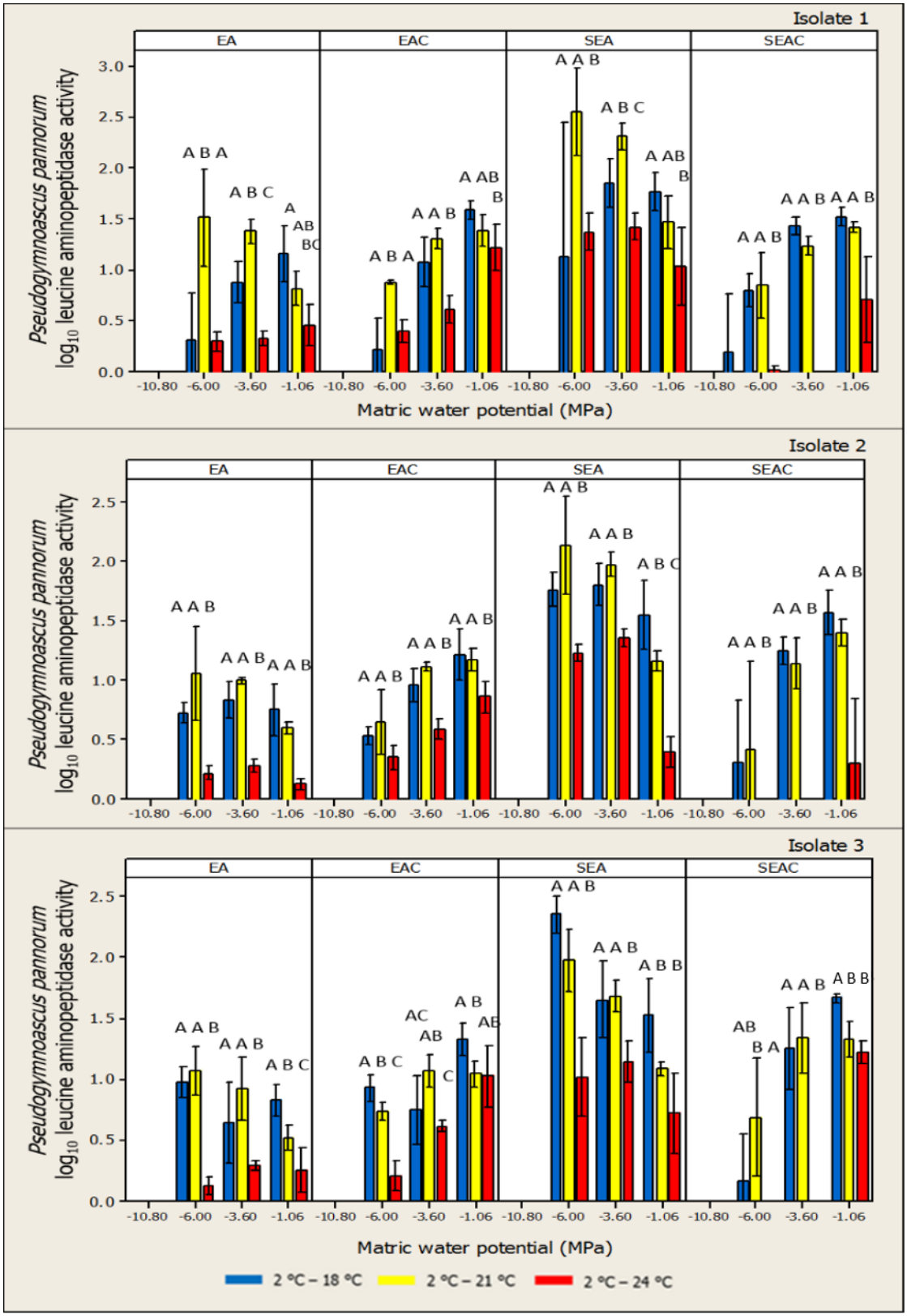



*Figure 4.8 Leucine aminopeptidase activities of three* P. pannorum *isolates at 2–18 °C, 2–21 °C and 2–24 °C temperature cycles and at matric water potentials of -10.80, -6.00, -3.60 and -1.06 MPa. Values are means of four replicates ± SEM. EAC; enzyme activity per colony, SEAC; specific enzyme activity per colony, EA; enzyme activity per biomass, SEA; specific enzyme activity per biomass. Different letters indicate significant (*P *< 0.05) differences between treatments, based on one way ANOVA. Data were* $log_{10}$ *transformed prior to statistical analyses. Lack of bars means there was no leucine aminopeptidase activity recorded for that treatment.*

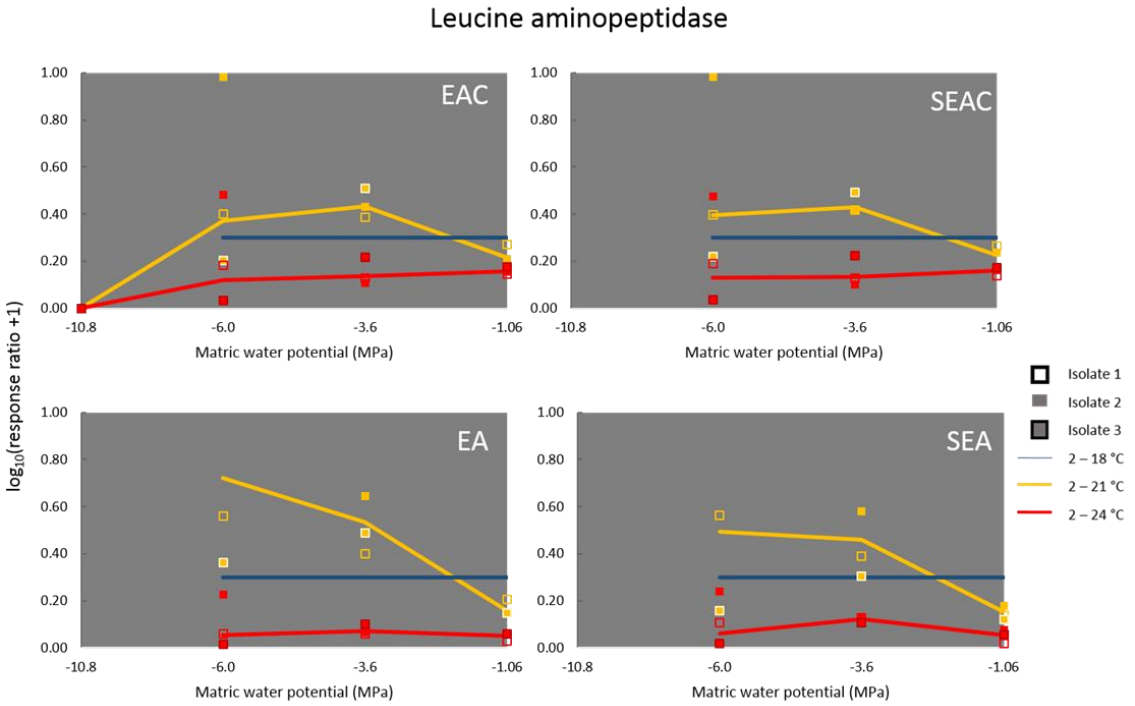



*Figure 4.9 $Log_{10}$ response ratios (relative to the 2 – 18 °C temperature cycle, denoted by blue line) of the leucine aminopeptidase activities of three* P. pannorum *isolates at 2–21 °C (yellow line) and 2–24 °C (red line) temperature cycles and at water potentials of -10.80, -6.00, -3.60 and -1.06 MPa. EAC; enzyme activity per colony, SEAC; specific enzyme activity per colony, EA; enzyme activity per biomass, SEA; specific enzyme activity per biomass. Missing values means there was no leucine aminopeptidase recorded for that treatment.*

#### *4.3.3.4 Alkaline phosphatase*

The water potential treatments significantly affected alkaline phosphatase EAC across all three isolates ($F_{2, 46}$ = 13.85–31.41, all $P$ < 0.001). Alkaline phosphatase EAC was highest at -1.06 MPa, and decreased gradually with decreasing $\Psi_m$ to -6.00 MPa, with no activity at -10.80 MPa except for isolate 1 at 2–24 °C (Figure 4.10). The 2–24 °C treatment decreased alkaline phosphatase EAC by 95–98 %, compared to the 2–18 °C treatment ($F_{2, 46}$ = 7.61–9.77, $P$ < 0.001). For all isolates, temperature cycle interacted with water potential to influence EA, EAC, SEA and SEAC ($F_{6, 46}$ = 5.36–31.2, all $P$ < 0.05, Appendix 11).

As with the other enzymes that were assayed for, the 2–21 °C temperature cycle increased all measures of alkaline phosphatase activity at -6.0 MPa (by two fold when expressed as EAC, SEAC and SEA, and by four fold for EA), compared with the 2–18 °C cycle. These effects diminished in magnitude at -3.60 and -1.06 MPa. Exposure to the

2– 24 °C treatment consistently inhibited alkaline phosphatase activity at -6.0 MPa, - 3.6 MPa and -1.06 MPa, relative to the 2-18 °C temperature cycle (Figure 4.11).

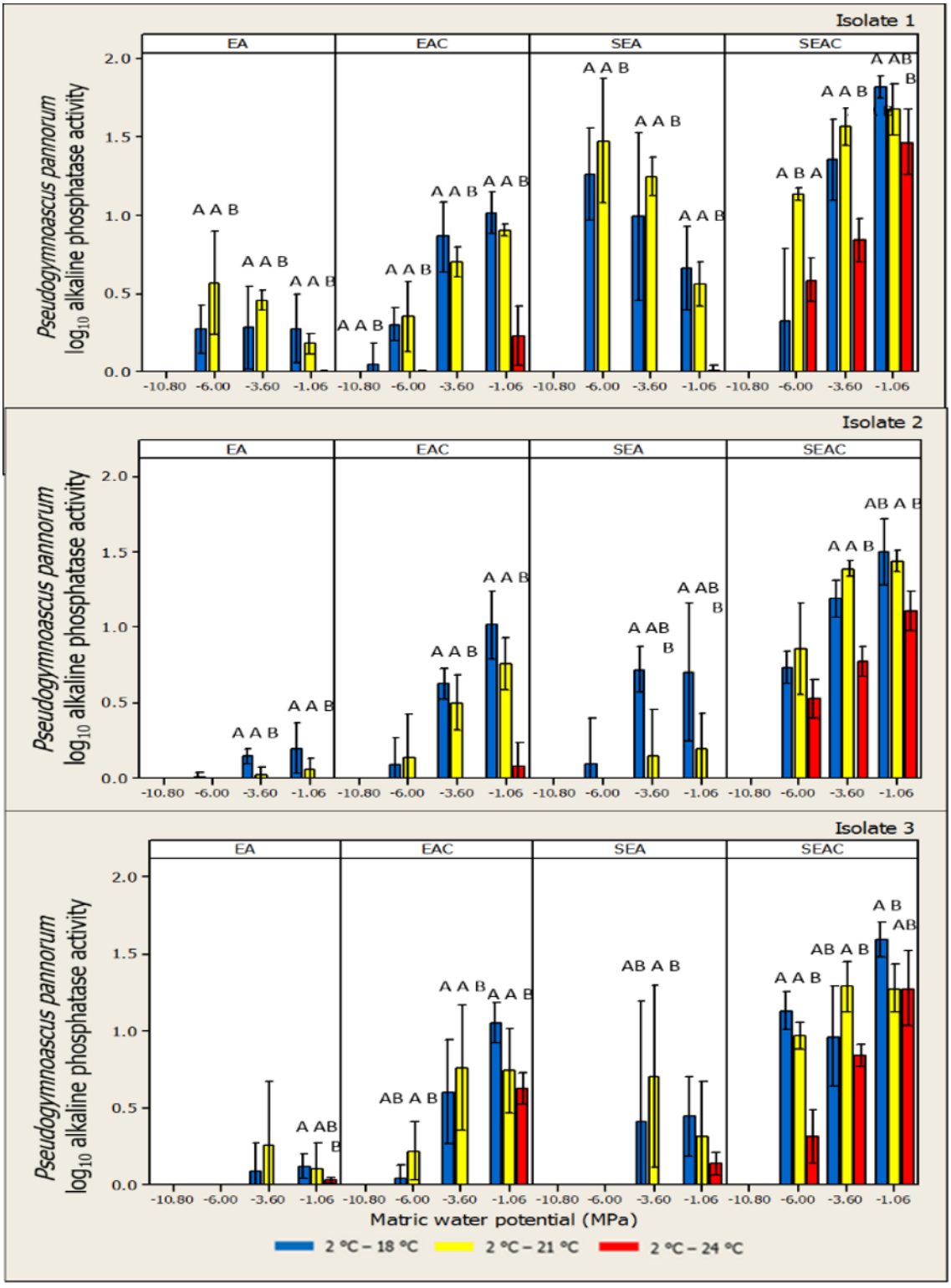



*Figure 4.10 Alkaline phosphatase activities of three* P. pannorum *isolates at 2–18 °C, 2–21 °C and 2–24 °C temperature cycles and at matric water potentials of -10.80, -6.00, -3.60 and -1.06 MPA. Values are means of four replicates ± SEM. EAC; enzyme activity per colony, SEAC; specific enzyme activity per colony, EA; enzyme activity per biomass, SEA; specific enzyme activity per biomass. Differently subscripted letters indicate significant (*P < 0.05*) differences between treatments, based on one way ANOVA. Data were log10 transformed prior to statistical analyses. Lack of bars means there was no alkaline phosphatase activity recorded for the specific treatment.*

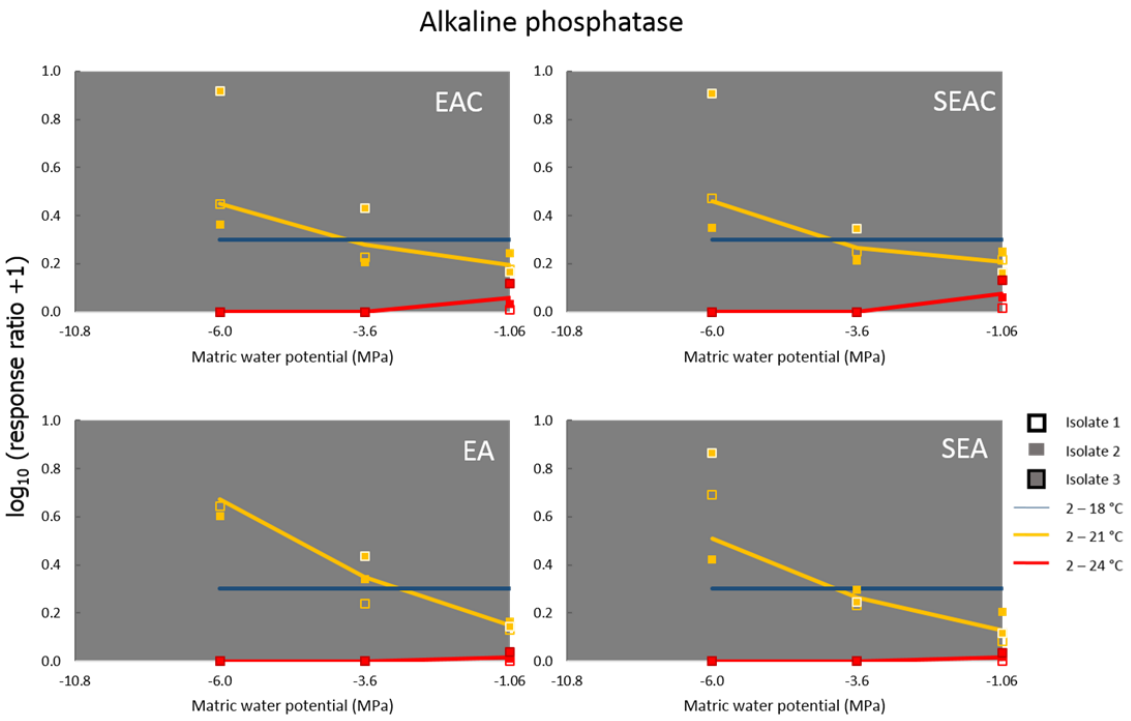



*Figure 4.11 $Log_{10}$ response ratios (relative to the 2 – 18 °C temperature cycle, denoted by blue line) of the alkaline phosphatase activities of three* P. pannorum *isolates at 2–21 °C (yellow line) and 2–24 °C (red line) temperature cycles and at water potentials of -10.80, -6.00, -3.60 and -1.06 MPA. EAC; enzyme activity per colony, SEAC; specific enzyme activity per colony, EA; enzyme activity per biomass, SEA; specific enzyme activity per biomass. Missing values means there was no alkaline phosphatase recorded for that treatment.*

#### *4.3.3.5 Acid phosphatase*

Acid phosphatase EAC was significantly affected by $\Psi_m$ across all three isolates ($F_{2, 46}$ = 8.43–15.29, all $P < 0.001$; Fig. 4.12). EAC was highest at -1.06 MPa, and decreased to 0.13 U $hr^{-1}$ $colony^{-1}$ at -6.00 MPa, with little or no activity being recorded at -10.8 MPa. Compared with the 2–18 °C temperature cycle, the 2–24 °C cycle decreased acid phosphatase EAC in isolate 1 by 98 % at the three highest water potentials, and completely inhibited EAC for isolate 2 ($F_{2, 32}$ = 5.76–11.70, both $P < 0.006$). There were significant interactions between temperature cycles and $\Psi_m$ for EA, EAC, SEA and SEAC ($F_{6,46}$ = 3.02–23.05, all $P < 0.05$, except for EA and EAC for *P. pannorum* 3; Appendix 11).

Analyses of response ratios indicated that, in common with the other enzymes, the 2–21 °C temperature cycle tended to increase acid phosphatase activity at -6.0 MPa and -3.6 MPa (by 1.2–3.2 times), with a decline in this effect at -1.06 MPa (Figure 4.13).

At 2–24 °C, however, all measures of acid phosphatase activity were consistently decreased at -6.0 MPa, -3.6 MPa and -1.06 MPa, relative to the 2–18 °C treatment (Figure 4.13).

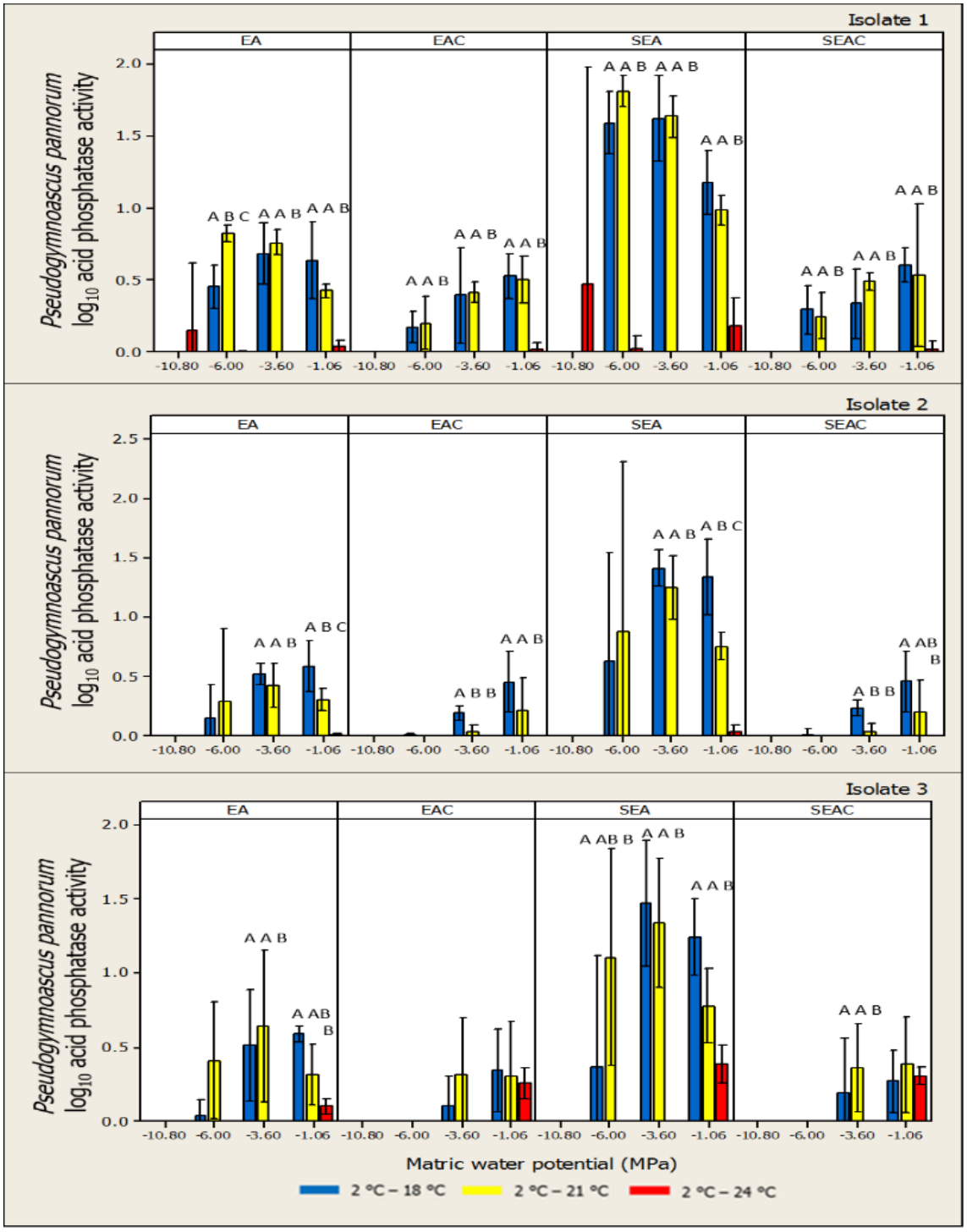



*Figure 4.12 Acid phosphatase activities of three* P. pannorum *isolates at 2–18 °C, 2–21 °C and 2–24 °C temperature cycles and at water potentials of -10.80, -6.00, -3.60 and -1.06 MPA. Values are means of four replicates ± SEM. EAC; enzyme activity per colony, SEAC; specific enzyme activity per colony, EA; enzyme activity per biomass, SEA; specific enzyme activity per biomass. Different letters indicate significant (P < 0.05) differences between treatments based on one way ANOVA. Data were log$_{10}$ transformed prior to statistical analyses. Lack of bars means there was no alkaline phosphatase activity recorded for the specific treatment.*

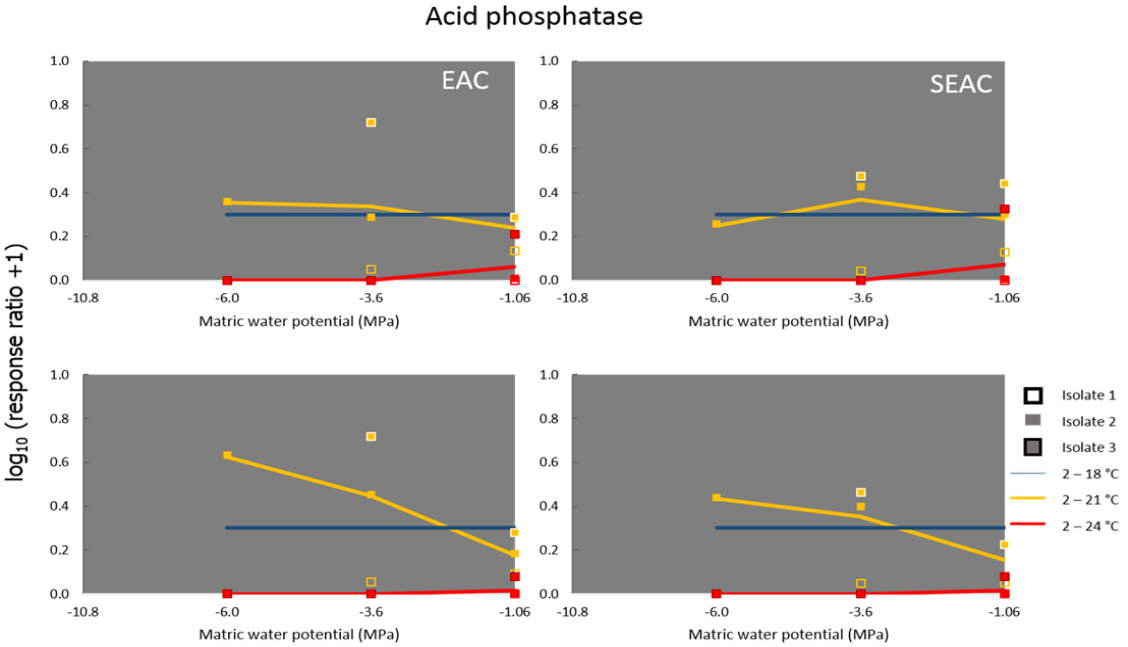



*Figure 4.13 $Log_{10}$ response ratios (relative to the 2 – 18 °C temperature cycle, denoted by blue line) of the acid phosphatase activities of three* P. pannorum *isolates at 2–21 °C (yellow line) and 2–24 °C (red line) temperature cycles and at water potentials of -10.80, -6.00, -3.60 and -1.06 MPA. EAC; enzyme activity per colony, SEAC; specific enzyme activity per colony, EA; enzyme activity per biomass, SEA; specific enzyme activity per biomass. No acid phosphatase activity was recorded at -10.8 MPa. Missing values means there was no acid phosphatase recorded for that treatment.*

## 4.4 Discussion

The observations reported here support Hypothesis 1 that warming to > 21 °C inhibits the hyphal extension rate of *Pseudogymnoascus pannorum*, a widespread psychrophilic fungus of Antarctic soils (Newsham *et al*. 2016). They corroborate the finding that the concentration of *Pseudogymnoascus* spp. DNA is lower in a maritime Antarctic soil that is warmed for five years (Chapter 3). Furthermore, they are in broad agreement with previous studies showing that optimum growth of Antarctic *P. pannorum* isolates occurs at 15–20 °C or below (Azmi and Seppelt 1997, Hughes *et al*. 2002, Edgington *et al.* 2014, Krishnan *et al.* 2016, Krishnan *et al.* 2018), and that temperature increases to > 20 °C inhibit the growth of the taxon (Duncan *et al.* 2008, Krishnan *et al.* 2016). The observations reported here do, however, differ from those reported by previous studies in that they were derived from experiments in which the isolates were exposed to diurnally oscillating daily temperature cycles of 2–18 °C,

2– 21 °C and 2–24 °C. The mean temperatures in each of these treatments were 10.1 °C, 12.0 °C and 12.9 °C, respectively (Section 2.6), all of which are considerably below the temperatures at which the growth of Antarctic *Pseudogymnoascus* sp. isolates is inhibited (Hughes et al. 2013, Krishnan *et al*. 2018). The current observations suggest that exposing polar soil microbes to constant temperatures in the laboratory may not accurately predict their responses to warming in the maritime Antarctic natural environment, where temperatures at surfaces can vary diurnally by up to 30 °C (Longton and Holdgate 1967). It may hence be important for future *in vitro* experiments to mimic the oscillating temperatures encountered in the field more closely to predict biological responses to projected climate change more accurately (Dang *et al*. 2009, Barnes *et al.* 2010).

Also in support of Hypothesis 1, warming in the present study also had main effects on enzyme activities. In contrast to the effects of warming on hyphal extension rates, increases in temperature from 2–18 °C to 2–21 °C increased enzyme activities by *ca*. 5–250 %, with further warming to 2–24 °C decreasing the activities of enzymes by *ca*. 20–90 %, relative to the 2–18 °C temperature cycle. These observations are in contrast to those from previous studies, with experiments at constant temperatures indicating that the enzyme activity of *P. pannorum*, including cellulase activity (derived from halo area measurements and expressed as mean relative cellulase activity) increases at higher temperatures and is highest at ≥ 25 °C, despite optimal growth being recorded at 15 °C (Krishnan *et al*. 2018). Similarly, Duncan *et al.* (2008) showed that temperature increases from 4 °C to 15 °C had no consistent effect on the specific cellulase activity of *P. pannorum*, and that the effects of warming were isolate dependent. The disparity between these studies could arise from differences in experimental design, such as employing constant temperature treatments, the use of different media and variation in sampling times. For example, Krishnan *et al*. (2018) used a bacteriological agar medium that was rich in nutrients and sampled the cultures for enzyme analyses after 10 days of treatment. In the present study, a soil extract medium that was poor in nutrients was used, and colonies were sampled in the log phase of growth after 11 weeks. Krishnan *et al*. (2018) explained the negative correlation between colony extension and relative enzyme activity at higher temperatures as

a stress response that resulted in a reduction of resource allocation to growth. The current study does however suggest that at 10 days, colonies would still have been in the lag phase of growth (Appendix 6), suggesting that hyphae may still have been acquiring resources for growth.

Although warming affected the extension rate and enzyme activity of *P. pannorum* in the experiments reported in this chapter, the main factor influencing the growth and enzyme activities of *P. pannorum* was determined to be water potential. This supported Hypothesis 2, that lower water availability would influence the physiology of *P. pannorum*, and corroborates numerous studies showing strong inhibitory effects of declining water availability on the growth and enzyme activities of crop pathogenic, wood decomposer and food spoilage fungi (e.g., Magan and Lynch 1986, Jurado *et al.* 2008, Alam *et al.* 2009, A'Bear *et al*. 2014). Declining water availability had a strong inhibitory effect on the growth of *P. pannorum* in the present study, with its extension rate being reduced by up to 93 % between -1.06 MPa and -10.80 MPa. The growth of the fungus was consistently fastest at -1.06 MPa, which is the approximate water potential measured in soil at Mars Oasis when snow cover had recently melted. Given that snowmelt is the most significant input of water to the soil at Mars Oasis, with summertime precipitation being exceedingly rare, this suggests that growth of the fungus mainly occurs in the period just prior to snowmelt when the soil is thawed, and in the days following snowmelt, when the soil is sufficiently moist to allow prolific extension of the fungus. In agreement with this, it has previously been reported that soil fungal activity is highest during winter and spring, compared with summertime, when the ratio of fungal to bacteria biomass decreases in cold regions (Lipson *et al*. 2002). It is notable that the isolates of *P. pannorum* in the present study all showed measurable extension down to a water potential of -10.8 MPa, suggesting that hyphae of the fungus may ramify through soil during the Antarctic summer well after snowmelt has occurred.

Studies into the effects of water availability on other Antarctic fungi are limited, but Gonçalves *et al.* (2013) examined the effects of salinity on *Penicillium solitum* isolated from marine sediments from Southern Ocean marine core sediments sampled near King George Island. The authors showed that culturing the fungus at 15 °C on malt extract agar containing 1, 5, 10 and 15 % NaCl (which progressively reduced osmotic

water availability) had a negative effect on its extension rate. Similarly, Andrews and Pitt (1987) showed that altering water availability in media with salt (NaCl), sugar (glucose/fructose) or glycerol had a negative effect on the growth of *Pseudogymnoascus* species. The extension rate of the fungus at 25 °C was optimal at water activities ($a_w$) of 0.98–0.93 (*ca*. -2.7 MPa to -8 MPa) but decreased at lower water availabilities. Similarly, Gunde-Cimerman *et al.* (2003) found that adding salt (NaCl) or sugar (glucose) to growth media had a negative effect on fungal counts isolated from Arctic seawater, melted sea ice and glacial ice water. The counts were highest on 20 % glucose media ($a_w$ = 0.941), and were lowest on 50 % glucose media ($a_w$ = 0.890), irrespective of where the samples were collected from. As for glucose, increased salt concentration in the media decreased fungal counts (Gunde-Cimerman *et al.* 2003). However, $Na^+$ and $Cl^-$ ions are toxic and glucose can be metabolised, so the use of salt in media to alter $a_w$ can confound water potential effect (Rath *et al*. 2016).

The present study confirmed Hypothesis 3 that warming and water potential would interact to influence the growth rate of *P. pannorum*. These interactions mainly arose from the inhibitory effects of warming on the hyphal extension rate of *P. pannorum* at the highest water potential (-1.06 MPa), and an absence of effects of warming at the two lowest potentials (-6.0 MPa and -10.8 MPa). At the moderate water potential of -3.6 MPa, the effect of warming on extension rate and enzyme activities was inconsistent and was also isolate dependent. The joint effects of the two factors on the growth rate of *P. pannorum* have not been examined before. In partial support of the observations here, Duncan *et al*. (2008) showed that the effect of increased temperature on the extension rate of *P. pannorum* cultured on media enriched with different sources of carbon varied between the types of growth media used. The extension of the fungus increased at 25 °C compared with 15 °C on carboxymethyl cellulose enriched media, but decreased on cellulose carbon substrates, presumably because of altered osmotic potentials.

In further confirmation of Hypothesis 3, significant interactive effects of warming and water potential on the enzyme activities of the three *P. pannorum* isolates were found here, with temperature increases from 2–18 °C to 2–21 °C usually resulting in increases in enzyme activity at -6.0 MPa and -3.6 MPa. At -1.06 MPa, however the

activities of the enzymes consistently decreased with temperature increases from 2– 18 °C to 2–24 °C or 2–21 °C to 2–24 °C. A reduction in enzyme activity under wetter conditions could be associated with anaerobic conditions limiting enzyme activity. Field studies support this view, with higher soil moisture in maritime Antarctic tundra resulting in lower ecosystem respiration compared with drier tundra, even though respiration at the seasonal scale is affected by temperature (Bao *et al*. 2018). Higher enzyme activity at increased temperature also agrees with the findings of Krishnan *et al*. (2018), who found that warming resulted in *P. pannorum* exhibiting a stress response characterised by an investment in enzyme production and a reduction in growth. In this study, however, an increase in temperature from 2–18 °C to 2–24 °C almost always reduced enzyme activities at all water potentials, indicating that the three strains of *P. pannorum* studied here exibited a stress threshold beyond which both the growth and activity of the isolates were inhibited.

## 4.5 Conclusion

Linking community composition to ecosystem function is a challenge in complex microbial communities (Oliverio *et al.* 2017). The quantification of specific functional groups of fungi in soil allows the activities of enzymes that drive key biogeochemical cycles to be predicted (Talbot *et al.* 2015), but phylogeny is a poor predictor for enzyme activity if the physiologies of the microbes of interest are not well understood. Using an autecological approach, the study illustrates that responses of Antarctic isolates of *P. pannorum* to climate warming will depend on water availability. Increasing temperatures and reducing water potential inhibited the colony extension rates of three *P. pannorum* isolates in the laboratory, with similar reductions in the enzyme activities of the isolates under warmer and drier conditions. Given that the experiments reported here were conducted on soil extract medium at diurnally oscillating temperatures and water potentials known to be encountered in the natural environment, they suggest that similar inhibitory effects of warming may occur in the field.

# 5 The effects of warming and water addition on fungal abundances in a High Arctic soil

## 5.1 Introduction

Arctic ecosystems are undergoing rapid change, forced by anthropogenic greenhouse gas emissions (Blaud *et al.* 2015), with current air temperature rises of 0.06– 0.10 °C year$^{-1}$ (Comiso and Hall 2014, IPCC 2014) and anticipated increases in air temperature of 3–6 °C by 2080 (Comiso and Hall 2014, Blaud *et al*. 2015). These rises in air temperature will lead to reductions in sea ice and snow cover, glacial retreat, thawing of permafrost and probable changes in plant cover (ACIA 2005, Stroeve *et al*. 2012b, Kivlin *et al*. 2013, Blaud *et al*. 2015). Because of the vast stocks of organic carbon present in Arctic permafrost (*ca*. 1.7 × $10^{15}$ kg; Tarnocai *et al*. 2009), changes to soil microbial activities associated with climate warming also have the potential to influence the flux of $CO_2$ to the Earth's atmosphere and to impact global carbon storage.

In Arctic soils, microbial growth and activity are limited by frequent freeze–thaw cycles and low temperatures, precipitation and nutrient availability (Margesin and Miteva 2011, Timling and Taylor 2012). Increasing temperature and changes to precipitation patterns in the High Arctic are hence thought likely to affect the microbes present in these soils (Buckeridge *et al*. 2013, Blaud *et al*. 2015), with potential changes to soil microbial community composition and biomass being predicted as climate change occurs across the region (Schimel *et al*. 2004, Timling and Taylor 2012). Amongst the predominant groups of soil microbes, fungi are favoured in the cold, arid environments characteristic of the Arctic (Russell 1990, Buckeridge *et al*. 2015) owing to their ability of remaining physiologically active at water potentials below -1.5 MPa, beyond which the majority of bacterial and protozoan growth is halted (Whitford 1989). They contribute to a wide range of ecosystem processes, including the decomposition of organic carbon, deposition of recalcitrant carbon, and recycling of soil nitrogen and phosphorus (Tedersoo *et al*. 2014, Treseder and Lennon 2015). Despite their crucial ecological role in rapidly-changing Arctic ecosystems, the responses of most Arctic soil fungi to climate change is not fully understood, with responses varying between specific taxa, and soil

fungal community composition being dependent on site characteristics, such as precipitation, soil moisture, pH or vegetation type (Wallenstein *et al*. 2007, Deslippe *et al*. 2011, Timling and Taylor 2012, Siciliano *et al*. 2014, Tedersoo *et al*. 2014, Timling *et al*. 2014, Geml *et al*. 2015, Christiansen *et al*. 2017).

Most of the studies into the effects of climate change on fungi in Arctic soils have focused on ectomycorrhizal fungi associated with the roots of woody plants, typically *Betula nana,* and *Salix* spp. and *Dryas* spp. (Clemmensen and Michelsen 2006, Deslippe *et al*. 2011, Timling *et al*. 2012). Other research has focused on the identification of soil fungal communities in High Arctic soils using molecular methods, principally Sanger sequencing (Bergero *et al*. 1999, Kurek *et al*. 2007, Ozerskaya *et al.* 2009, Singh *et al*. 2012, Singh and Singh 2012). More recently, next generation sequencing (NGS) methods have led to an increased number of studies reporting the composition of Arctic soil fungal communities and how they might respond to warming (e.g. Tedersoo *et al*. 014, Timling *et al*. 2014, Geml *et al*. 2015, Morgado *et al.* 2016). Despite these studies, current knowledge regarding the quantitative responses of Arctic saprotrophic fungal communities to projected climate change remains limited (Deslippe *et al*. 2011, Blaud *et al*. 2015). Although providing huge amounts of data, with a single lane of a typical Illumina MiSeq run generating up to 15 Gb of data, NGS studies do not estimate changes to the biomasses of specific fungal groups, which is vital information for understanding whether or how soil functioning might alter in a warmer and wetter Arctic (Taylor *et al.* 2016). To circumvent this problem, Q-PCR has been used in recent years to quantify fungal abundances in Arctic soils using primers for total fungi, targeting 18S and ITS regions of rRNA genes (Buckeridge *et al*. 2012, Haugwitz *et al*. 2014). Qantitative-PCR, a widely applied technique in microbial ecology studies (Smith and Osborn 2009, Brankatsch *et al.* 2012), has been used to investigate specific fungal genes involved in nitrogen cycling (Henry *et al*. 2006) and biodegradation (Lee *et al*. 2008, McKew *et al.* 2007). The method has also been used for quantifying fungal DNA in a range of soils, and for the detection and differentiation of *P. destructans* and other *Pseudogymnoascus* species responsible for white nose syndrome disease in bats (Haughland *et al*. 2002).

Few studies have addressed the question of how interactions between abiotic factors, such as soil moisture (Blaud *et al*. 2015), temperature (Semenova *et al*. 2015) or fertility (Schuur *et al*. 2008), might affect the abundances of Arctic soil fungi, and our understanding of such effects at a quantitative level is still limited. *In situ* multifactorial experiments mimicking realistic environmental changes are, therefore, crucial for determining the effects of climate change on soil microbial diversity and function (Blaud *et al*. 2015). As demonstrated for a maritime Antarctic soil in Chapter 4, it is possible that the combined effects of warming and water availability may lead to the reduced growth and enzyme activities of psychrophilic fungi such as *Pseudogymnoascus* spp. in polar soils, with possible effects on the cycling of soil organic compounds.

In this study Q-PCR is used to investigate how warming, applied with open top chambers (OTCs), and watering, simulating increased summer precipitation, will affect the abundances of seven fungal taxa that are frequent in High Arctic soil. It is hypothesised that:

1) warming with OTCs will increase the concentrations of fungal gDNA in soil,
2) water application will alter the concentrations of fungal gDNA in soil, and
3) warming with OTCs and water application will interact to influence the concentrations of fungal gDNA in soil.

## 5.2 Methods

### 5.2.1 Site location, field experiment and soil sample collection

Full details of the field experiment are given in Section 2.2. Briefly, the fieldsite was located at Kongsfjordneset on the Brøggerhalvøya Peninsula, on Svalbard in the High Arctic. The site is a typical High Arctic polar desert habitat with limited plant cover (CAVM Team, 2013). It is a flat coastal terrace, with a mean July air temperature of 5 °C, an annual precipitation of *ca*. 400 mm, and a mean summer precipitation of *ca*. 80 mm (Førland *et al.* 1997, Brossard *et al.* 2002).

The field experiment was established in September 2014 to examine the *in situ* effects of warming and water addition on the soil at the site (Figure 5.1). Four treatments (control plots, warmed plots, watered plots, and warmed and watered

plots), each replicated 12 times, were applied to 48 plots (Appendix 1). Open top chambers, installed all year round, were used to increase the mean temperature of surface soils by *ca*. 2 °C during summer (Section 2.2.1). The water treatment was effected by applying 1 L of deionised water twice every summer, usually in late June and late August–early September, and approximated to an increase in summer precipitation of 40 mm, equating to a *ca*. 50 % increase in mean summer precipitation and a *ca*. 10 % increase in mean annual precipitation. The relatively high moisture concentration of the soil at Kongsfjordneset precluded the application of dry substrates, as were applied to soil at Mars Oasis in the Antarctic (see Chapter 3).

The soil samples used in the analyses described below were collected on 12 September 2014, prior to any treatment applications, and on 21 August 2017, after almost three years of treatment. After collection, samples were frozen at -20 °C, transported to the UK under cool conditions and refrozen at -20 °C prior to soil physicochemical determinations (described in detail in Section 2.3) and Q-PCR analyses.

*Figure 5.1 The field experiment established on Svalbard in 2014.*

### 5.2.2 Q-PCR

Full details of Q-PCR analyses are given in Section 2.4.4. Briefly, Q-PCR was used to determine the concentrations in soil of genomic (g)DNA of *Pseudogymnoascus* spp., *Mortierella* spp., *Tetracladium* spp., *Leptosphaeria* spp., and

*Cadophora* and *Phialocephala* spp. (as a single group), and members of the class Dothideomycetes. A combination of cloning, denaturing gradient gel electrophoresis and isolations onto solid media identified these taxa as being the most frequent fungi in the soil at Kongsfjordneset. Concentrations of gDNA of each taxon derived from these analyses were expressed in units of ng or pg of DNA $g^{-1}$ dwt soil. Preliminary analyses on the soil from the site used the ITS86F/ITS4 primer set described by De Beeck *et al.* (2014) to quantify total fungal DNA. Despite the success of this primer set in amplifying total fungal DNA from Antarctic soil (Section 3.6.2), high background readings, which introduced bias associated with the amplification of targets from mixed community template DNA (Polz and Cavanaugh 1998, Smith and Osborn 2008), precluded the measurement of the total fungal gDNA in the soil. Only 2017 samples were used for Q-PCR statistical analyses, as 2014 samples had gDNA concentrations that were below the limit of detection.

### 5.2.3 Statistical analyses

All statistical analyses were performed either in the R statistical package or MINITAB 17 (Version 17.2.1). Analysis of Variance was used to test whether warming or water application affected soil properties (surface temperature, pH, carbon:nitrogen ratio, and moisture, organic carbon and total carbon and nitrogen concentrations). General Linear Models (GLMs) were applied to test the effects of warming, water application, fungal taxon and block, including all interaction terms, on summed gDNA concentrations in soil. The effects of the treatments and blocks on each fungal taxon were also analysed separately using GLMs. Data were $\log_{10}(n+1)$-transformed prior to analysis to improve data distribution. Pearson's correlations were also used to investigate associations between soil properties and the concentrations of gDNA of each taxon.

## 5.3 Results

### 5.3.1 Soil properties

Warming of soil with OTCs in summer (July–August) 2017 increased the minimum soil surface temperature by 0.50 °C, the maximum soil surface temperature by 2.94 °C

and the mean soil surface temperature by 0.86 °C, compared with unchambered plots ($P < 0.05$). Annual (2016–2017) minimum and mean soil surface temperatures were not affected by OTCs. The maximum annual 2017 soil surface temperature was marginally significantly higher (3.08 °C; $F_{1, 8} = 5.85$, $P = 0.052$) in chambered plots compared with control plots (Fig. 5.2).

No main or interactive effects of OTCs and water application were found on soil pH and moisture, organic matter or total carbon and nitrogen concentrations (Appendix 12). Soil moisture, organic matter and carbon concentrations differed between the blocks, with soil from Block 1 having the highest soil moisture concentration (11.9 %), which was *ca*. 2– 3 % higher than that from the other two blocks ($F_{2, 43} = 9.21$, $P < 0.001$). Soil from Block 1 also had a higher organic matter concentration (1.64 %) than soils from Blocks 2 and 3 (1.35 % and 1.25 %, respectively; $F_{2, 47} = 8.75$, $P = 0.001$). Total carbon concentration was also highest in Block 1 (8.5 %), compared with Blocks 2 and 3 (8.4 % and 7.9 %, respectively; $F_{2, 47} = 6.25$, $P = 0.004$). Soil nitrogen concentration was generally low at 0.14–0.18 % and did not differ significantly between blocks (Appendix 12).

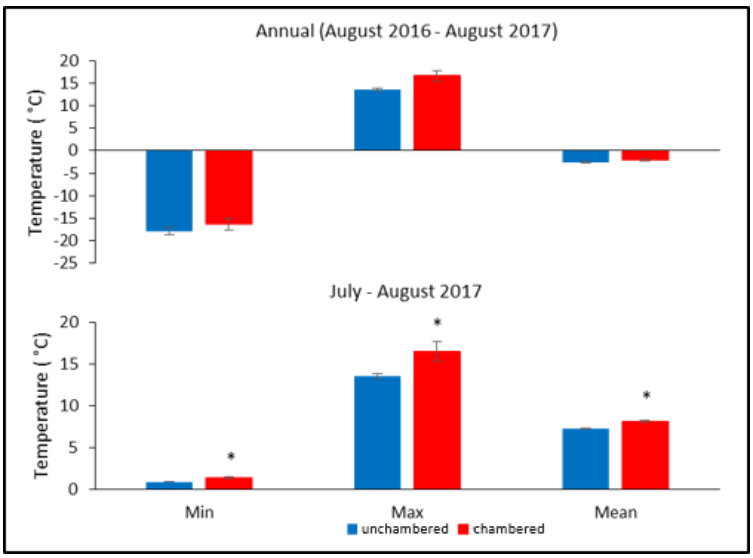



*Figure 5.2 The effects of warming, applied with open top chambers (OTCs) on annual (top) and summer (July–August, bottom) soil surface temperatures. Asterisks indicate significantly different temperatures (P < 0.05) based on one way ANOVA test. Bars are standard deviations, n=4.*

### 5.3.2 Concentrations of gDNA of individual fungal taxa

The concentrations of gDNA in soil varied between individual taxa ($F_{5, 258} = 5.37$, $P < 0.001$). The highest concentration of gDNA in soil was recorded for *Pseudogymnoascus* spp. (787.30 fg $g^{-1}$ dwt soil), followed by *Cadophora* spp. (539.36 fg $g^{-1}$dwt soil), *Leptosphaeria* spp. (221.97 fg $g^{-1}$dwt soil), Dothideomycetes

(94.49 fg $g^{-1}$dwt soil) and *Mortierella* spp. (76.67 fg $g^{-1}$dwt soil). *Tetracladium* spp. had the lowest gDNA abundance (0.02 fg $g^{-1}$; Figure 5.3).

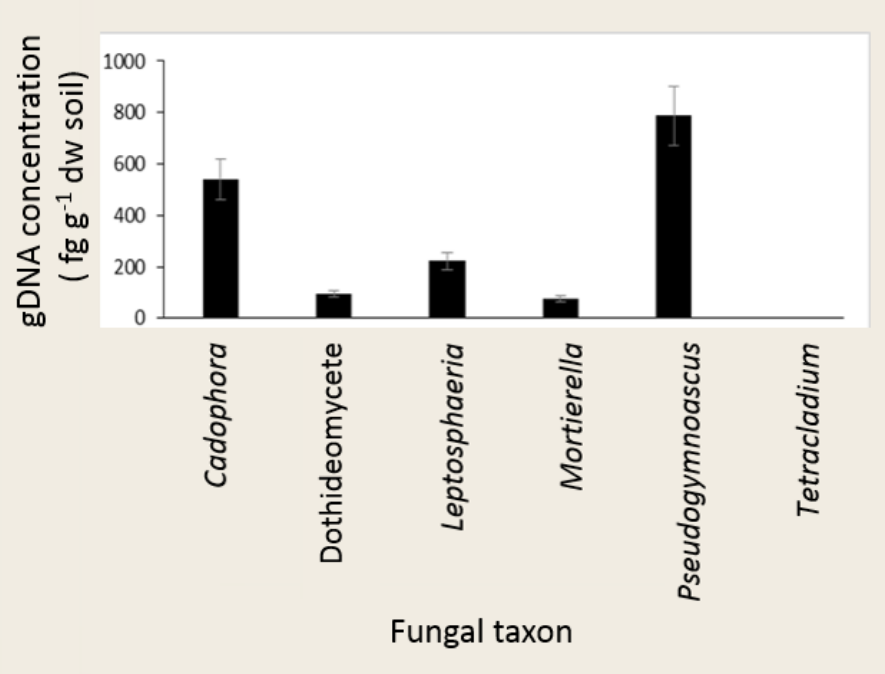



*Figure 5.3 gDNA concentrations of fungal taxa in High Arctic soil collected from the field experiment in August 2017. Bars are SEM,* n=*48. Italics – spp. level, non-italics – genus level.*

### 5.3.3 Responses of summed fungal gDNA concentrations to treatments

Block affected summed fungal gDNA concentrations in soil ($F_{2, 48}$ = 3.82, $P$ < 0.001), with gDNA concentrations being lower in Block 1 than in Blocks 2 and 3. OTCs had no effect on fungal gDNA concentrations ($P$ > 0.05; Table 5.1). General Linear Models, however, suggested a significant effect of watering on the concentration of fungal gDNA in soil, with the summed fungal gDNA concentrations of all six taxa being 8 % lower in soil to which water had been added, compared with unwatered soil ($F_{1, 48}$ = 4.05, $P$ < 0.045). One way Analysis of Variance test indicated that this effect was marginally significant ($F_{1, 64}$ = 3.82, $P$ = 0.052; Table 5.1).

*Table 5.1 Outputs from GLM (four way ANOVA) testing for the effects of taxon, block, OTCs and water application on the gDNA concentrations of seven fungal taxa in soil collected from the field experiment after three years of treatment. Data were log10 (n+1) transformed prior to analysis. Bold – treatments that were statistically significant at P < 0.05 based on ANOVA test, $F_{degrees\ of\ freedom}$.*

| Factor | $F_{258}$ | P |
|---|---|---|
| Taxon | **5.37 (5.51)** | **< 0.001 (<0.001)** |
| Block | **8.31 (8.57)** | **< 0.001 (<0.001)** |
| OTC | 0.38 | 0.538 |
| Water | **4.05** (3.82) | **0.045** (0.052) |
| Taxon x block | 1.08 | 0.377 |
| Taxon x OTC | 0.45 | 0.81 |
| Taxon x water | 0.2 | 0.963 |
| Block x OTC | 0.05 | 0.948 |
| Block x water | 0.78 | 0.459 |
| OTC x water | 0.07 | 0.788 |
| Taxon x block x OTC | 0.28 | 0.986 |
| Taxon x block x water | 0.83 | 0.601 |
| Taxon x OTC x water | 0.43 | 0.825 |
| Block x OTC x water | 0.82 | 0.444 |
| Taxon x block x OTC x water | 0.59 | 0.821 |

*Abbreviation: OTC, open top chamber, (One Way ANOVA results)*

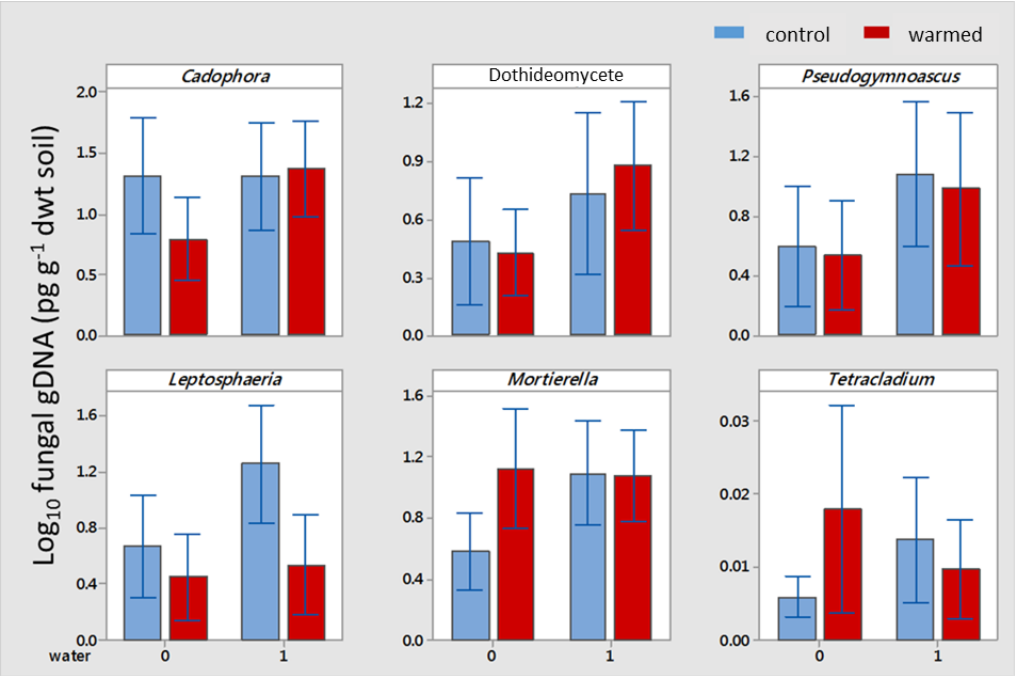



*Figure 5.4 The effects of warming with OTCs and water application on the abundance of gDNA of fungal taxa in soil collected from the High Arctic field experiment after three years of treatment (mean values, n=10–12). Bars are SEM.*

### 5.3.4 Responses of gDNA concentrations of individual taxa to treatments

As fungal taxon had an effect on the concentrations of gDNA (Table 5.1), the responses of each taxon to the treatments were analysed separately. General Linear Models using Analysis of Variance indicated no significant main effects of warming with OTCs or water application on the concentrations of gDNA of any taxon (Table 5.2, Figure 5.5). The concentration of *Cadophora* spp. gDNA in soil, however, was marginally significantly affected by the warming treatment, with 36 % lower concentrations of the gDNA of this genus in chambered soil, compared with unchambered plots ($F_{1, 46} = 3.28$, $P = 0.087$; Table 5.2).

Further investigation of the effects of block on different fungal taxonomic groups showed that the concentration of *Pseudogymnoascus* spp. gDNA was 84 % and 99 %

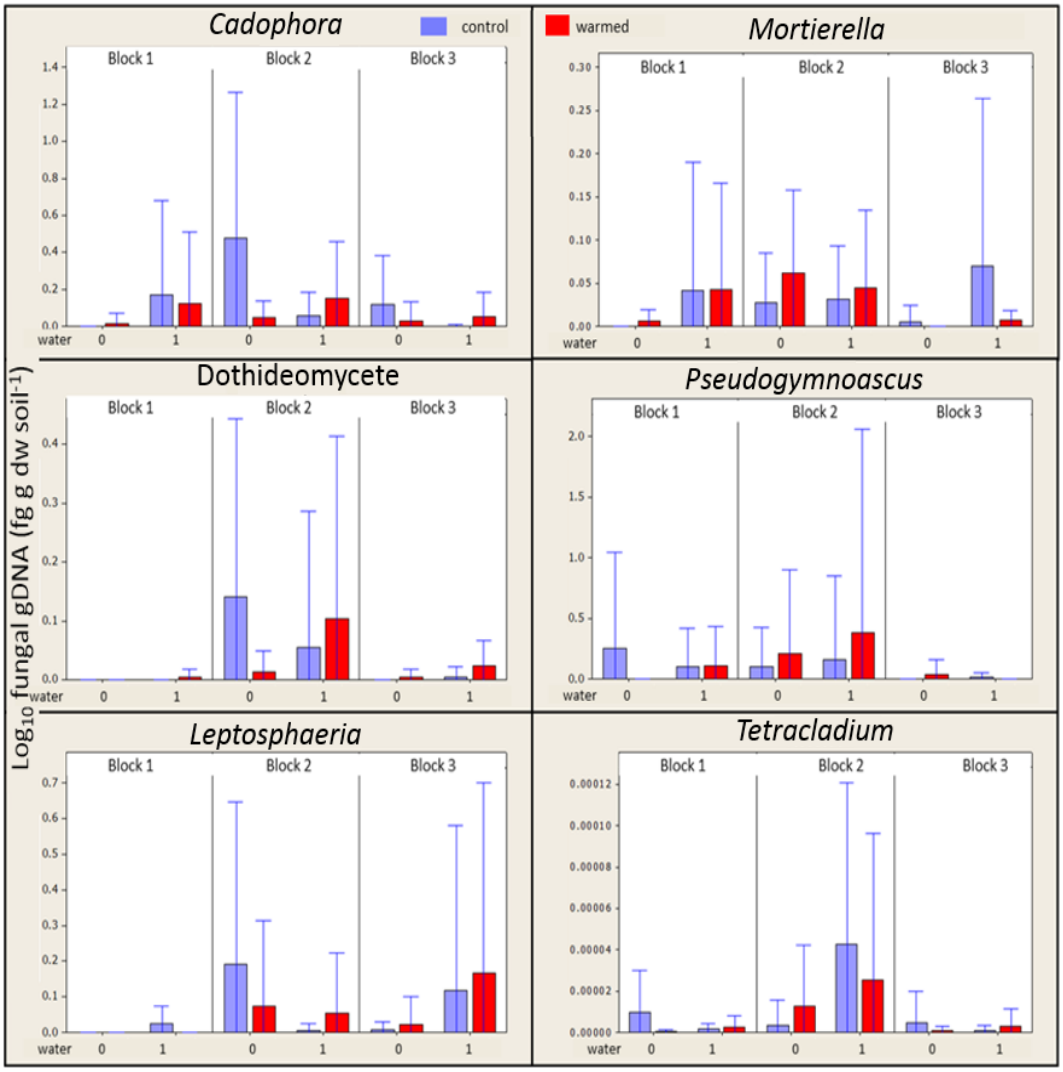



*Figure 5.5 Concentrations of gDNA of fungal taxa in High Arctic soil after three years of treatment. Values are means (n=3-4) ± SEM.*

*Table 5.2 Summary of treatment effects (three-way ANOVA for all block and two-way ANOVA for individual blocks) of warming (OTC), water application and block (if applicable), and interactions on concentrations of gDNA of six fungal taxa in soil from the field experiment. Data were log10(n+1) transformed prior to statistical analysis. Values in grayed cells indicate statistically significant effects (P<0.05 .F degrees of freedom for three-way ANOVA/degrees of freedom for two-way ANOVA.*

| | | *Cadophora* spp. | | Dothideomycete | | *Leptosphaeria* spp. | | *Mortierella* spp. | | *Pseudogymnoascus* spp. | | *Tetracladium* spp. | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Block | Factor | $F_{1, 46/15}$ | *P* | $F_{1, 48/16}$ | *P* | $F_{1, 45/15}$ | *P* | $F_{1, 45/15}$ | *P* | $F_{1, 46/15}$ | *P* | $F_{1, 45/15}$ | *P* |
| All | OTC | 3.28 | 0.087 | 1.53 | 0.232 | 0.56 | 0.466 | 1.08 | 0.312 | 0.12 | 0.731 | 0.28 | 0.600 |
| | Water | 0.00 | 0.964 | 0.81 | 0.380 | 0.68 | 0.421 | 2.00 | 0.175 | 0.45 | 0.509 | 0.01 | 0.926 |
| | Block | 0.38 | 0.686 | 1.25 | 0.300 | 1.29 | 0.288 | 0.88 | 0.423 | 3.39 | **0.045** | 4.05 | **0.026** |
| | OTC x water | 1.09 | 0.365 | 1.04 | 0.382 | 0.87 | 0.466 | 0.93 | 0.434 | 0.69 | 0.564 | 0.95 | 0.336 |
| | OTC x block | 2.61 | 0.101 | 1.64 | 0.222 | 0.70 | 0.512 | 0.94 | 0.408 | 1.20 | 0.324 | 0.60 | 0.553 |
| | Water x block | 2.07 | 0.156 | 0.83 | 0.453 | 1.37 | 0.282 | 0.55 | 0.585 | 2.71 | 0.080 | 0.01 | 0.989 |
| | OTC x water x block | 2.25 | 0.120 | 0.78 | 0.465 | 0.84 | 0.441 | 1.07 | 0.353 | 0.05 | 0.950 | 1.93 | 0.160 |
| *1* | OTC | 0.00 | 0.988 | 1.00 | 0.337 | 3.00 | 0.109 | 0.42 | 0.528 | 0.43 | 0.524 | 1.44 | 0.253 |
| | Water | 1.56 | 0.236 | 1.00 | 0.337 | 3.00 | 0.109 | 3.12 | 0.103 | 0.16 | 0.693 | 0.67 | 0.427 |
| | Water x OTC | 0.68 | 0.425 | 1.00 | 0.337 | 3.00 | 0.109 | 2.02 | 0.181 | 0.44 | 0.521 | 2.40 | 0.147 |
| *2* | OTC | 0.26 | 0.622 | 0.49 | 0.496 | 0.13 | 0.720 | 0.15 | 0.708 | 0.03 | 0.867 | 0.44 | 0.519 |
| | Water | 0.20 | 0.662 | 0.16 | 0.697 | 0.62 | 0.445 | 0.35 | 0.567 | 1.23 | 0.289 | 0.01 | 0.909 |
| | Water x OTC | 1.49 | 0.246 | 0.00 | 0.991 | 0.52 | 0.483 | 0.07 | 0.796 | 0.00 | 0.979 | 1.63 | 0.226 |
| *3* | OTC | 0.17 | 0.692 | 1.45 | 0.252 | 0.70 | 0.421 | 0.09 | 0.767 | 0.03 | 0.869 | 0.09 | 0.769 |
| | Water | 0.24 | 0.631 | 1.58 | 0.233 | 2.01 | 0.182 | 0.35 | 0.566 | 0.03 | 0.869 | 0.09 | 0.772 |
| | Water x OTC | 0.39 | 0.545 | 0.06 | 0.817 | 1.04 | 0.328 | 0.28 | 0.609 | 1.97 | 0.186 | 1.27 | 0.281 |

higher in Block 2 (0.00462 pg $g^{-1}$ dwt soil) than it was in Blocks 1 and 3, respectively ($F_{2, 46}$ = 3.39, $P$ = 0.045; Table 5.2). There was also a marginally significant interactive effect of water and block on the concentration of *Pseudogymnoascus* spp. gDNA ($F_{2, 46}$ = 2.71, $P$ = 0.080). In Blocks 1 and 3, water addition reduced the concentration of *Pseudogymnoascus* spp. gDNA by 66 % and 73 %, respectively, but increased the concentration of gDNA of the fungus by 3.7 times in Block 2. The highest concentration of *Tetracladium* spp. gDNA was recorded in Block 2 (0.077 fg $g^{-1}$ dwt soil), with 99.9 % and 92.7 % higher concentrations recorded in this block than in Blocks 1 and 3, respectively ($F_{1, 45}$ = 4.05, $P$ = 0.026; Table 5.2). The block effect was not significant for the other fungal taxa, but soil from Block 2 typically had consistently higher concentrations of gDNA than that from Blocks 1 and 3. Variation between replicate plots within the same block was typically high, with SEM values often exceeding those of means (Figure 5.5).

### 5.3.5 Correlations between fungal gDNA concentrations and soil physicochemical properties

Pearson's correlation tests showed significant associations between soil physicochemical properties and the concentrations of gDNA of the analysed fungal taxa, values > 0 showed positive correlation between analysed parameters, and values < 0 represented negative correlation between the parameters (Table 5.3). Soil carbon:nitrogen ratio was positively correlated with the concentrations of *Tetracladiu* spp. and *Cadophora* spp. gDNA, and those of the summed gDNA of the analysed taxa (Table 5.3). The concentration of *Leptosphaeria* spp. gDNA was marginally significantly correlated with carbon:nitrogen ratio, as was the summed concentration of gDNA of all seven taxa with soil carbon concentration (Table 5.3). Total carbon concentration and soil moisture showed a strong negative relationship, as did total nitrogen concentration and carbon:nitrogen ratio (Table 5.3).

*Table 5.3 Pearson's coefficients and* P *values (in brackets) for correlations between soil properties and the abundance of gDNA of fungal taxa present in High Arctic soil from the field experiment. Significant probability values (*P*<0.05) are marked in bold.*

| **Pearson correlation** | **C** | **N** | **C:N ratio** | **Moisture** |
|---|---|---|---|---|
| N | 0.263 (0.071) | | | |
| C:N ratio | -0.134 (0.362) | -0.884 (**<0. 001**) | | |
| Moisture | -0.448 (**0.001**) | -0.070 (0.634) | -0.140 (0.342) | |
| *Pseudogymnoascus* spp. | -0.245 (0.101) | 0.040 (0.794) | 0.109 (0.472) | -0.140 (0.354) |
| *Mortierella* spp. | -0.30 (0.842) | 0.010 (0.949) | 0.090 (0.554) | -0.094 (0.534) |
| *Tetracladium* spp. | -0.119 (0.425) | -0.215 (0.146) | 0.299 (**0.041**) | -0.113 (0.451) |
| Dothidiomycete | 0.049 (0.745) | -0.146 (0.328) | 0.187 (0.207) | -0.118 (0.429) |
| *Leptosphaeria* spp. | -0.091 (0.543) | -0.205 (0.167) | 0.283 (0.054) | -0.081 (0.590) |
| *Cadophora* spp. | -0.099 (0.507) | -0.203 (0.171) | 0.325 (**0.026**) | -0.016 (0.916) |
| Summed fungal gDNA | -0.249 (0.089) | -0.114 (0.440) | 0.302 (**0.037**) | -0.139 (0.347) |

*Abbreviations: C, total carbon concentration; N, total nitrogen concentration*

## 5.4 Discussion

Empirical observations indicate that the Arctic is warming at a rate of approximately 0.6–1.0 °C per decade (Comiso and Hall 2014, IPCC 2014), with increased precipitation in winter (as snowfall) and summer (as rainfall and snowmelt), and climate models predicting further increases in precipitation across the region towards end of this century (Bintanja and Andry 2017, Song and Liu 2017). These changes in climate are hypothesised to have an impact on the abiotic and biotic properties of Arctic soils (Blaud *et al*. 2015, Hursh *et al*. 2017). Although the OTCs used here elevated mean summer and annual surface soil temperatures by 0.9 °C and 0.4 °C, and maximum summer and annual surface soil temperatures by 2.9°C and 3.1 °C, respectively, the findings indicate no effects of OTCs on soil physicochemical properties. Furthermore, water application to soil failed to result in subsequent measurable changes to any soil properties analysed here, including soil moisture concentration. Given that *ca*. 40 mm of water was applied to each frost boil, it is, however, inevitable that water availability would have been increased at the soil surface, but for how long these effects persisted is currently not known.

The analyses here indicated no significant effects of OTCs after three years of treatment on either the summed or individual concentrations of soil fungal gDNAs, offering no support for Hypothesis 1. These observations are consistent with previous studies showing that artificial warming only exerts measurable effects on Arctic saprotrophic and mycorrhizal fungal communities after 10–20 years of treatment (Deslippe *et al*. 2011, Sistla and Schimel 2013, Peltoniemi *et al*. 2015, Semenova *et al*. 2015). These previous long-term changes to soil fungal abundances arising from warming have been attributed to secondary effects caused by altered cover and community composition of higher plants (Rinnan *et al*. 2007). The lack of significant effects of warming with OTCs on the abundances of fungal taxa at Kongsfjordneset might also have been due to high natural spatial variability of fungal gDNA in soil, with significant differences being recorded between blocks in DNA concentrations, and large variation between replicates in blocks, suggesting that the four replicate plots of each treatment per block were insufficient to detect within-block treatment effects. Nevertheless, a marginally significant ($P < 0.09$) negative effect of warming with OTCs was found on the amount of *Cadophora* spp. gDNA, which is in agreement with previous studies reporting that this genus is frequent in cold and arid Antarctic soils (Blanchette *et al*. 2004). Given that most of the reported changes to soil fungal biomass and community composition in Arctic soils have been caused by nutrient additions (Rinnan *et al*. 2007, Rinnan *et al*. 2013), it is possible that if growth substrates had been applied to the High Arctic soil, as they were to the Antarctic soil (Chapter 3), then fungal gDNA concentrations would have risen to levels at which significant main effects of OTCs might have been detected.

The bioavailability of liquid water is one of the most important factors limiting the distribution, abundance and activities of terrestrial organisms in polar ecosystems (Kennedy 1993, Onofri *et al*. 2004, Arenz and Blanchette 2011). Changes to soil moisture thus have the potential to alter fungal taxonomic and functional groups in the soil (Tedersoo *et al*. 2014, Morgado *et al*. 2015). It was hence surprising that the watering treatment, simulating a 50 % increase in summer precipitation at the study site, did not have consistent effects on the abundances of gDNA of any of the fungal taxa studied here. Nevertheless, an 8 % decrease was observed in the gDNA concentrations of the

analysed fungal taxa in watered soil, providing support for Hypothesis 2. The reduction in fungal gDNA in watered plots may have been owing to a lower oxygen availability in waterlogged soil, which would have affected fungal growth (Flanagan & Vaum 1974, Rosswall & Hall 1974), or may have been owing to competition from bacteria in soils with higher water availability (Boer *et al.* 2005, Zhang *et al*. 2016). The finding here of lower soil fungal gDNA in watered soil corroborates previous observations showing that moisture is an important factor in determining soil fungal community composition and richness (Tedersoo *et al*. 2014, Geml *et al*. 2015), but counters previous observations that soil moisture could have positive effects on fungal abundance in Antarctic soil (Arenz and Blanchette 2011), and that increases in soil moisture stimulate microbial biomass in Arctic soil during springtime thaw (Sistla and Schimel 2013).

The analyses here showed no interactive effects of OTCs and water application on the abundances of fungal gDNA in an Arctic soil, providing no support for Hypothesis 3. This agrees with the findings of Zhang *et al*. (2016), who showed that warming and water treatments had no effect on the soil fungal community in alpine grassland on the Tibetan Plateau applied for one year, while the bacterial community was positively affected by soil moisture. Previous studies have, however, suggested that such interactions may occur. In a study in dry shrub heath and wet sedge tundra at Disko Island, Greenland, Christiansen *et al*. (2017) found that the abundance and diversity of fungi in *Betula glandulosa* litter was reduced by warming with OTCs in dry, but not in wet, soils. The authors suggested that OTCs only affected fungal abundance (assessed using Q-PCR analyses) in dry soils because increased evapotranspiration in OTCs lowered litter moisture to the point at which fungal physiology was inhibited. In contrast, Geml *et al.* (2016) showed that warming with OTCs in the Low Arctic significantly altered soil fungal community composition (assessed using NGS analyses), with decreased ectomycorrhizal and lichenized fungal diversity, and increased saprotrophic and pathogenic fungal richness in response to warming in moist tussock tundra, but not in dry heath (Geml *et al*. 2016). These constrasting responses are likely due to different fungal functional groups dominating soil communities in distinct habitats (Geml *et al.* 2015, Geml *et al*. 2016, Semenova *et al*. 2016).

Soil chemistry is an important factor driving fungal community composition in polar ecosystems (Wallenstein *et al*. 2007, Deslippe *et al*. 2011). In Arctic and Antarctic soils, the frequencies of different fungal taxonomic groups are linked to vegetation cover and soil fertility, specifically organic matter, $Na^+$ and $Cl^-$ concentrations and pH (Siciliano *et al*. 2014, Timling *et al*. 2014). In the present study, soil carbon concentration was negatively correlated with soil moisture concentration, suggesting higher utilization of carbon when water is available, and corroborating studies showing increased decomposition in wetter Arctic soils (Christiansen *et al.* 2012). Significant positive associations were also found between soil carbon:nitrogen ratio and the concentrations of *Cadophora* spp. and *Tetracladium* spp. gDNA. Soil carbon:nitrogen ratio has similarly been found to correlate positively with the abundances of other saprotrophic fungi in Antarctic soils (*Cladosporium* spp., *Pseudeurotium* spp. and *Penicillium* spp.; Newsham *et al.* 2016) and to be a determinant of soil fungal community composition on other continents (Tedersoo *et al*. 2014). In Canadian mesic low Arctic tundra, Buckeridge *et al*. (2013) also showed a decline in soil fungal biomass in summer, when compared to winter, corresponding with decreases in soil carbon and nitrogen concentrations.

## 5.5 Conclusion

Current knowledge of the influence of climate change on the biomasses of different fungal taxa in polar soils is scarce and needs to be integrated with taxonomic and functional data (Makhalanyane *et al.* 2016), using *in situ* multifactorial experiments to determine soil fungal responses to climate change scenarios (Blaud *et al*. 2015). In this study, there is no evidence that warming with OTCs and water application, simulating a 50 % increase in summertime rainfall, affected the gDNA concentrations of individual fungal taxa in a High Arctic soil. Water application did however lead to an 8 % reduction in the summed concentration of gDNA of the taxa, suggesting lower abundances of saprotrophic fungi in Arctic soils receiving increased precipitation, and possible negative effects on soil decomposition processes.

# 6 The *in vitro* effects of elevated temperature and altered water availability on the growth and extracellular enzyme activities of Arctic soil fungi

## 6.1 Introduction

Arctic regions, characterized by sub-zero temperatures for most of the year, frequent freeze-thaw cycles during summer, low water and nutrient availability and large seasonal variations in solar radiation, are currently subjected to rapid climate change (Anisimov 2007, Prach *et al*. 2010, Zalar and Gunde-Cimerman 2014, Blaud *et al*. 2015). As in all other regions, terrestrial productivity in the Arctic is directly linked to the activity of the soil microbial community (Sinsabaugh *et al.* 1991, Wallenstein 2008, Tedersoo *et al*. 2014). Hence, abiotic changes associated with climate warming, such as increased temperatures or altered water availability, have the potential to affect the growth and activity of soil micro-organisms (Lennon *et al.* 2012, Arnosti *et al*. 2014), which might influence the release of $CO_2$ to the atmosphere from the huge stocks of organic carbon present in Arctic permafrost (Tarnocai *et al*. 2009, Scharlemann *et al*. 2014).

Soil microbial communities in cold regions are dominated by fungi (Schmidt and Bölter 2002). These microbes play a key role in decomposition processes, in which complex organic molecules are broken down into forms that can be re-assimilated by roots (Sistla and Schimel 2012, Buckeridge *et al.* 2013, Krishnan *et al.* 2018). They are able to survive and remain physiologically active under conditions of low temperatures, reduced nutrient availability and restricted access to water (Robinson 2001, Margesin and Miteva 2011, Cararasco *et al*. 2012, Krishnan *et al*. 2016). Previous studies have reported that climate change, and its associated effects, such as rising temperatures and increased snow depth, affects fungal community composition and enzyme activities in Arctic soils (Waldrop *et al.* 2010, Arnosti *et al.* 2014, Peltoniemi *et al*. 2015, Christiansen *et al*. 2016, Geml *et al*. 2016, Mundra *et al*. 2016). Global warming, and increased temperature in particular, is likely to boost the growth of soil micro-organisms

in cold Arctic soils. Increased temperatures will also result in improved access to liquid water, one of the most important factors limiting the distribution and abundance of terrestrial organisms in polar regions (Onofri *et al*. 2004, Arenz and Blanchette 2011), and such changes are hence likely to lead to increased soil microbial growth (Bokhorst *et al.* 2007, Singh *et al*. 2010).

Several studies into the responses of Arctic soil fungi to warming have used next generation sequencing (NGS) methods to characterise soil fungal community composition (Semenova *et al*. 2015, Geml *et al*. 2016, Morgado *et al*. 2016). Investigating total microbial community structure using NGS methods may, however, overlook changes in the functional roles of soil micro-organisms (Yergeau and Kowalchuk 2008), and without knowing the functions of each taxon in biogeochemical processes (Oliverio *et al.* 2017), it is difficult to predict from NGS analyses how soil processes will be affected by climate change based simply on taxonomic descriptions of soil microbial communities. Such information can be derived from enzymological studies. Arctic fungi produce a range of extracellular enzymes (ECE) that decompose complex organic molecules and contribute towards carbon, nitrogen and phophorus turnover in soil (Sinsabaugh *et al*. 1993, Allison 2008, Krishnan *et al*. 2016). These enzymes include amylase, cellulase, chitinase, lipase, phosphatases and pectinase (Kurek *et al.* 2007, Singh *et al.* 2012, Singh and Singh 2012). An enzyme's activity depends on its stability and the immediate environment into which it is secreted, and is affected by external environmental factors, such as temperature and water availability (Hankin and Anagnostakis 1975, Allison 2008, Sinsabaugh *et al*. 2013). Despite clear links between enzyme activities and temperature and water availability, the influence of these abiotic factors on the enzymatic capacities of Arctic soil fungi are poorly defined.

Here, in controlled laboratory experiments, the effects of simulated climate change, consisting of warming (applied using environmentally-realistic oscillating temperatures) and altered water availability, on the growth rates and enzyme activities of 13 Arctic soil fungi growing on soil extract medium are studied. In previous studies into the effects of temperature on the growth and activities of Arctic soil fungi, constant temperatures, which do not mimic natural temperature cycles, have been used (e.g., Singh *et al.* 2012, Krishnan *et al.* 2011, Krishnan *et al.* 2016, Krishnan *et al.* 2018).

Furthermore, previous ecophysiological studies on Arctic fungi have not controlled water availability by altering matric water potential, which strongly influences microbial growth in soil (Wynn-Williams 1990, Robinson 2001). Based on previous research (Allison 2008, Jurado *et al*. 2008, Sinsabaugh *et al*. 2013, Chen *et al*. 2015, Damialis *et al*. 2015, Krishnan *et al*. 2018), it is hypothesised that:

1) warming will increase the growth rates and enzyme activities of Arctic soil fungi,
2) water availability will affect the growth rates and enzyme activities of the isolates, and
3) water availability will interact with warming to determine the growth rates and enzyme activities of the isolates.

## 6.2 Materials and methods

### 6.2.1 Fungal isolates and experimental design

Full details of the experiment to determine the effects of elevated temperature and changes in water availability on the growth and enzyme activities of 13 Arctic soil fungal isolates (Figure 6.1) are given in Section 2.6. Briefly, soil collected in September 2014 from the High Arctic field warming experiment (Section 2.2.1) was used for fungal isolations. Identification of the isolates was determined by sequencing of ITS regions of DNA (Section 2.4.2). The isolates were maintained on half strength potato dextrose agar


*Figure 6.1 Arctic fungal isolates used in the laboratory experiment. 1)* Acremonium *sp., 2)* Cadophora *sp., 3) Dothideomycete, 4)* Isaria farinosa*, 5)* Leptosphaeria *sp., 6)* Mortierella alpina*, 7)* M. polygonia*, 8)* M. verticilliata*, 9)* Mortierella *sp., 10)* Phialocephala lagerbergii*, 11)* Pseudogymnoascus pannorum*, 12) and 13)* Pseudogymnoascus *sp.*

medium at < 15°C. For the experiment, they were exposed, on cellophane film overlaying soil extract medium (see Section 2.6), to three diurnally oscillating temperature cycles (5–18 °C, 5–21 °C and 5–24 °C), representing daily temperature changes occurring at soil surfaces in the High Arctic during the summer, and potential future temperature increases arising from climate warming (see Figure 2.8). The isolates were also exposed to three water potentials (-0.2 MPa, -0.6 MPa and -1.6 MPa), representing water availabilities in wet (20 % moisture) through to intermediate (8– 10 %) and dry (3 % moisture) conditions, respectively (Appendix 2), which were typical of the soil at the warming experiment during summer. The water availability of the medium was altered by manipulating matric water potential using polyethylene glycol 8000 (see Section 2.6 for details). Temperature cycles and water potential treatments were applied factorially, resulting in nine treatment combinations. Each treatment was replicated five times for each of the fungal isolates.

### 6.2.2 Extension rate analyses

Colonies of Arctic fungi inoculated onto cellophane film were exposed to the treatments for 3–12 weeks, depending on the growth rate of each isolate. Once the growth of the fungal colony was visible beyond the inoculation point, an image of the colony was taken once a week with a digital camera (Canon SX 700HS) from a height of 20 cm, with exceptions to *Mortierella* spp. The surface area of each colony was measured with ImageJ software (Version 1.49v, National Institute of Health, Bethesda, Maryland, USA) and the growth rate of each colony was determined using the slope value from linear regressions between time and colony surface area (Section 2.6.1).

### 6.2.3 Extracellular enzyme assays

Colorimetric enzyme assays were used to determine the concentrations of enzymes (acid and alkaline phosphatase, chitinase, cellulase and leucine aminopeptidase) in extracts from the colonies at the end of the experiment. The cellophane film on which the fungal colony was growing was submerged upside down in 4 ml of 10 mM phosphate buffer in a 90 mm diameter Petri dish and was gently shaken for 2 hours at 7 °C. Concentrations of the enzymes were measured using p-nitrophenol

substrates (Appendix 7) as described by Alam *et al*. (2009). Further details of these analyses are given in Section 2.6.2. Total protein concentration was also measured, using a BCA protein kit following the manufacturer's protocol (Section 2.6.2) and biomass was measured by washing colonies onto pre-weighed filter paper and drying overnight at 105 °C prior to reweighing. Enzyme activities, calculated as mmol p-nitrophenol released (U), were expressed as enzyme activity per biomass (EA; U $hr^{-1}$ mg $hyphae^{-1}$) and specific enzyme activity per biomass (SEA; U $hr^{-1}$ mg $hyphae^{-1}$ mg $protein^{-1}$).

### 6.2.4 Statistical analyses

All statistical analyses were performed either in the R statistical package or MINITAB 17 (version 17.2.1). General Linear Models were used to test the main and interactive effects of isolate, temperature cycles and water potentials on extension rate, protein concentration and enzyme activities. For enzyme data, negative values were treated as zero, indicating no enzyme production. Data were $\log_{10}(n+1)$-transformed prior to statistical analyses to improve data distribution. One way ANOVA and Tukey's multiple comparisons tests were used to determine significant differences between treatments. EA and SEA were analysed separately for each enzyme and fungal taxon.

## 6.3 Results

### 6.3.1 Growth rate analyses

The growth rates of the Arctic soil fungi were significantly affected by isolate ($F_{12, 528}$ = 292.35, $P$ < 0.001), matric water potentials ($\Psi_m$; $F_{2, 528}$ = 518.6, $P$ < 0.001) and temperature cycles ($F_{2, 528}$ = 34.03, $P$ < 0.001). Isolate × temperature, isolate × $\Psi_m$ and temperature × $\Psi_m$ × isolate interaction terms were all significant ($P \leq 0.001$, Table 6.1). The $\Psi_m$ × temperature interaction term was marginally significant ($P$ = 0.053). *Mortierella* isolates, along with *Acremonium* sp., *Isaria farinosa* and *Leptosphaeria* sp., were the fastest growing isolates (46–78 $mm^2$ $day^{-1}$). The three *Pseudogymnoascus pannorum* isolates and the *Cadophora* sp. isolate grew at *ca*. 30 $mm^2$ $day^{-1}$.

*Phialocephala lagerbergii* and the Dothideomycete were the slowest growing fungi (*ca*. 9 mm$^2$ day$^{-1}$; $F_{12,\ 528}$ = 50.71, $P$ < 0.001). With the notable exception of *P. pannorum* 1 and 2, increases in temperature usually had positive effects on growth, with main effects of temperature cycles on the extension rates of *Acremonium* sp., *I. farinosa*, *Leptosphaeria* sp., *Mortierella* spp. and *P. lagerbergii* (Table 6.2) and 2–122 % increases in the extension rates of these taxa at 5–21 °C and 5– 24 °C, relative to 5–18 °C, respectively (Table 6.3). At 5– 21 °C, the extension rates of *P. pannorum* 1 and 2 were increased by 15 % and 2 % respectively, relative to 5–18 °C, but at 5–24 °C, were reduced by 11 % and 34 % relative to the 5– 18 °C temperature cycle (Table 6.3). The growth of all isolates was affected by $\Psi_m$ (all $P \leq 0.016$; Table 6.2). The growth of the fungi typically decreased at lower water potentials, with extension rates being fastest at -0.2 MPa (*ca*. 65 mm$^2$ day$^{-1}$) and reducing, on average, by 19 % and 58 % at - 0.6 MPa and -1.6 MPa, with this effect being most pronounced for *Cadophora* sp., *Leptosphaeria* sp., *P. pannorum* 3 and all *Mortierella* isolates except for *M. polygonia* (Table 6.3, Figure 6.2). Reductions in $\Psi_m$ from -0.2 MPa to -0.6 MPa decreased the extension rates of seven isolates by 9–63 %, with reductions from -0.2 MPa to -1.6 MPa inhibiting those of 10 isolates by 8–89 % (Table 6.3). The growth of *Acremonium* sp., the Dothideomycete, *Mortierella polygonia*, *P. lagerbergii* and *P. pannorum* 1 and 2, however, was increased at lower $\Psi_m$, with 2–81 % increases in the extension rates of these taxa at -0.6 MPa and -1.6 MPa, relative to -0.2 MPa (Table 6.3). The interactive effect of $\Psi_m$ and temperature cycles was significant for *Acremonium* sp., the Dothideomycete and *Leptosphaeria* sp. and was marginally significant ($P$ = 0.052–0.083) for the three *P. pannorum* isolates (Table. 6.2). At -1.6 MPa, an increase in temperature to 5–21 °C and 5–24 °C increased the growth of *Acremonium* sp. by 57 % and 87 %

*Table 6.1 General Linear Model (three-way ANOVA) outputs of main and interactive effects of isolate, temperature cycles (5–18 °C, 5–21 °C and 5–24 °C) and water potential (- 1.6 MPa, -0.6 MPa and -0.2 MPa) on the hyphal extension rates of 13 Arctic soil fungal isolates. Bold* P *values indicate statistically significant effects (*P < 0.05*); $F_{degress\ of\ freedom}$.*

| | Statistics | |
|---|---|---|
| **Factor** | $F_{2,\ 528}$ | $P$ |
| Isolate | 309.53 | **< 0.001** |
| T | 38.17 | **< 0.001** |
| $\Psi_m$ | 567.37 | **< 0.001** |
| Isolate x T | 5.13 | **< 0.001** |
| Isolate x $\Psi_m$ | 57.76 | **< 0.001** |
| T x $\Psi_m$ | 2.35 | 0.053 |
| Isolate x T x $\Psi_m$ | 1.85 | **0.001** |

*Abbreviations: $\Psi_m$, water potential, T, temperature cycle*

compared with the 5–18 °C temperature cycle, but had no effect at other $\Psi_m$. The interactive effect on the growth of the Dothideomycete was caused by the significant reductions in extension rate under the 5–21 °C cycle, relative to the 5– 18 °C cycle, that occurred at -0.6 MPa, but not at other $\Psi_m$ (Figure 6.2).

*Table 6.2 General Linear Model (two-way ANOVA) outputs of main and interactive effects of temperature cycles and water potentials on the hyphal extension rates ($mm^2\ day^{-1}$) of the Arctic fungal isolates. Bold* P *values indicate statistically significant effects (*P *< 0.05);* $F_{degress\ of\ freedom}$.

| | | Treatment | | |
|---|---|---|---|---|
| **Fungal isolate** | **Statistics** | **T** | **$\Psi_m$** | **T x $\Psi_m$** |
| *Acremonium* sp. | $F_{2, 38}$ | 24.87 | 24.53 | 24.15 |
| | *P* | **<0.001** | **<0.001** | **<0.001** |
| *Cadophora* sp. | $F_{2, 45}$ | 2.17 | 95.63 | 1.55 |
| | *P* | 0.129 | **<0.001** | 0.21 |
| Dothideomycete | $F_{2, 41}$ | 1.28 | 5.17 | 3.94 |
| | *P* | 0.293 | **0.011** | **0.01** |
| *Isaria farinosa* | $F_{2, 38}$ | 62.08 | 4.76 | 0.39 |
| | *P* | **<0.001** | **0.016** | 0.816 |
| *Leptosphaeria* sp. | $F_{2, 45}$ | 35.82 | 181.89 | 3.92 |
| | *P* | **<0.001** | **<0.001** | **0.01** |
| *M. alpina* | $F_{2, 40}$ | 2.07 | 94.93 | 0.35 |
| | *P* | 0.143 | **<0.001** | 0.844 |
| *M. polygonia* | $F_{2, 42}$ | 0.91 | 6.3 | 0.44 |
| | *P* | 0.412 | **0.005** | 0.78 |
| *M. verticilliata* | $F_{2, 42}$ | 10.28 | 268.91 | 0.3 |
| | *P* | <0.001 | **<0.001** | 0.875 |
| *Mortierella* sp. | $F_{2, 43}$ | 6.22 | 177.42 | 1.47 |
| | *P* | **0.005** | **<0.001** | 0.234 |
| *P. lagerbergii* | $F_{2, 39}$ | 6.31 | 14.24 | 1.61 |
| | *P* | **0.005** | **<0.001** | 0.198 |
| *P. pannorum* 1 | $F_{2, 36}$ | 12.14 | 57.58 | 2.32 |
| | *P* | **<0.001** | **<0.001** | 0.083 |
| *P. pannorum* 2 | $F_{2, 39}$ | 36.73 | 7.08 | 2.65 |
| | *P* | **<0.001** | **0.003** | 0.052 |
| *P. pannorum* 3 | $F_{2, 41}$ | 2.14 | 439.46 | 2.50 |
| | *P* | 0.135 | **<0.001** | 0.062 |

*Abbreviations: $\Psi_m$, water potential; T, temperature cycle*

*Table 6.3 The effects of temperature cycles and water potentials on the hyphal extension rates of 13 Arctic soil fungal isolates. For the effects of $\Psi_m$, the values shown are percentage increases or decreases relative to the responses at – 0.2 MPa. For the effects of temperature cycles, the values shown are percentage increases or decreases relative to responses at 5–18 °C. Only values that were statistically significant (P < 0.05) are shown, based on one way ANOVA test.*

| | T (°C) | | $\Psi_m$ (MPa) | |
|---|---|---|---|---|
| **Fungal isolate** | **5–21** | **5–24** | **-1.6** | **-0.6** |
| *Acremonium* sp. | 34 | 22 | 49 | 28 |
| *Cadophora* sp. | | | -89 | -60 |
| Dothideomycete | | | -25 | 9 |
| *I. farinosa* | 40 | 63 | -14 | -9 |
| *Leptosphaeria* sp. | 67 | 122 | -77 | -63 |
| *M. alpina* | | | -80 | -28 |
| *M. polygonia* | | | -8 | 26 |
| *M. verticilliata* | | | -89 | -11 |
| *Mortierella* sp. | 43 | 65 | -86 | -19 |
| *P. lagerbergii* | 23 | 51 | 39 | 81 |
| *P. pannorum* 1 | 15 | -11 | -18 | 36 |
| *P. pannorum* 2 | 2 | -34 | 2 | 26 |
| *P. pannorum* 3 | | | -78 | -32 |

*Abbreviations: $\Psi_m$, water potential; T, temperature cycle*

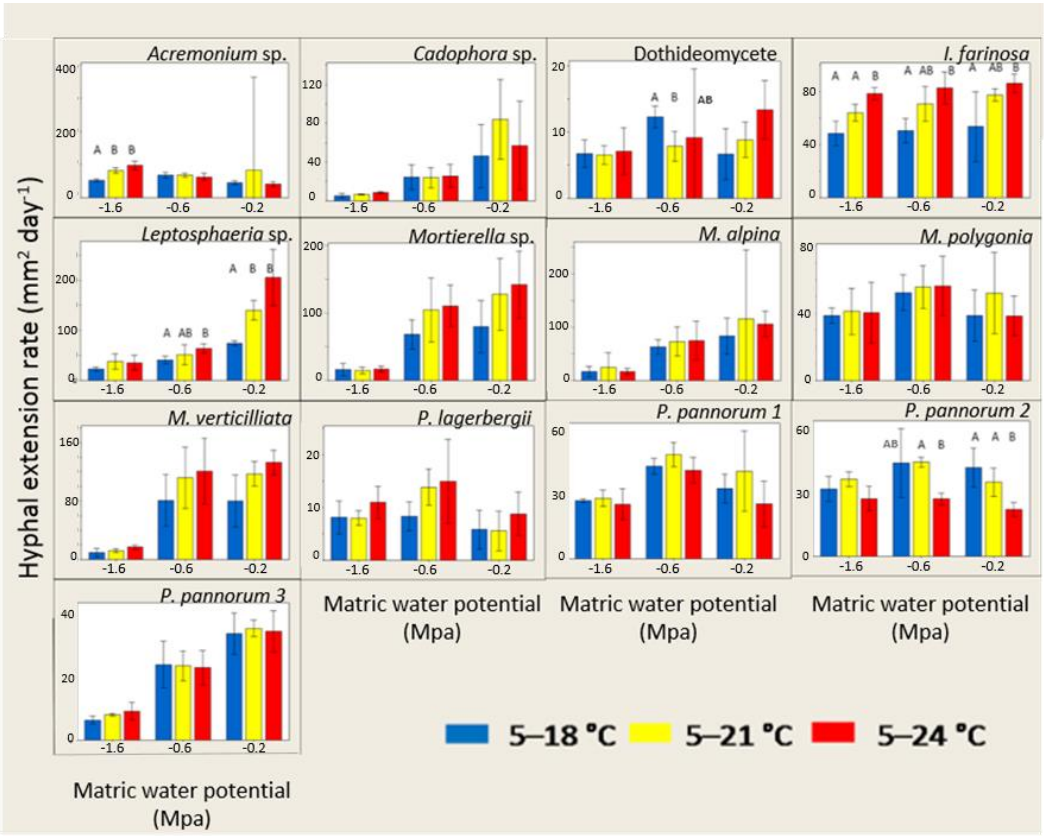



*Figure 6.2 Extension rates of Arctic fungal isolates at different temperature cycles and water potentials in the laboratory experiment. Values are means of 3–5 replicates ± SEM. Different letters indicate significant (P < 0.05) differences between treatments, based on one-way ANOVA.*

That on the growth of *Leptosphaeria* sp. was caused by increases in growth under the 5–21°C and 5–24°C cycles, compared with the 5–18°C cycle, that were recorded at -0.6 MPa and - 0.2 MPa, but not at -1.6 MPa (Figure 6.2). *Pseudogymnoascus pannorum* isolates were the only fungi negatively affected by temperature increases to 5–24 °C, but the effect was dependent on isolate and $\Psi_m$, and was only significant for *P. pannorum* 2 (Figure 6.2). Compared with the 5–18 °C temperature cycle, the growth of this isolate at 5–24 °C was reduced by 43 % and 38 % at -0.6 MPa and -0.2 MPa, respectively, but was not affected at -1.6 MPa (Figure 6.2).

### 6.3.2 Protein concentration

Protein concentration varied between isolates and was significantly affected by $\Psi_m$, but not by temperature cycle (Table 6.4). The isolate × $\Psi_m$ and isolate × $\Psi_m$ × temperature interaction terms also affected protein concentration ($P < 0.05$, Table 6.4). The three *P. pannorum* isolates, *I. farinosa*, *Acremonium* sp. and *Leptosphaeria* sp. had the highest concentrations of proteins (59–100 µg $ml^{-1}$), whereas *Mortierella* isolates, along with *P. lagerbergii* and the Dothideomycete, had the lowest amounts of protein (16–31 µg $ml^{-1}$). Compared with a $\Psi_m$ of -0.2 MPa, the concentration of protein for all isolates was reduced by 12 % and 14 % at -0.6 MPa and -1.6 MPa, respectively.

*Table 6.4 General Linear Models (three-way ANOVA) outputs of the main and interactive effects of isolates, temperature cycles and water potentials on protein concentration in extracts from Arctic soil fungal isolates. Bold text indicates significant (P < 0.05) effects of treatments, $F_{degrees\ of\ freedom}$.*

| | Statistics | |
|---|---|---|
| **Factor** | $F_{2, 571}$ | *P* |
| Isolate | 90.96 | **< 0.001** |
| T | 0.00 | 0.998 |
| $\Psi_m$ | 6.76 | **0.001** |
| Isolate x T | 1.22 | 0.222 |
| Isolate x $\Psi_m$ | 14.36 | **<0.001** |
| T x $\Psi_m$ | 1.67 | 0.155 |
| Isolate x T x $\Psi_m$ | 1.63 | **0.007** |

*Abbreviations: $\Psi_m$, matric water potential, T, temperature cycle*

The effects of decreases in $\Psi_m$ on protein concentrations varied between the isolates, and, as for growth rate, had either positive, negative or neutral effects (Figure 6.3). Concentrations of proteins extracted from colonies of the Dothideomycete, *P. lagerbergii* and *M. verticilliata* were not affected by changes in $\Psi_m$. Those from colonies of *Acremonium* sp., *P. pannorum* 1 and *P. pannorum* 2 decreased with increases in $\Psi_m$, whereas *Cadophora* sp., *Leptosphaeria* sp., *Mortierella alpina*, *Mortierella* sp. and *P. pannorum* 3

*Table 6.5 Genereal Linear Models (two-way ANOVA) outputs of main and interactive effects of temperature cycles and water potentials on protein concentration in extracts from Arctic fungal isolates. Bold text indicates significant (P < 0.05) value, based on ANOVA test, $F_{degress\ of\ freedom}$.*

| | | Treatment | | |
|---|---|---|---|---|
| **Fungal isolate** | **Statistics** | **$\Psi_m$** | **T** | **T x $\Psi_m$** |
| *Acremonium* sp. | $F_{2, 45}$ | 5.61 | 0.50 | 2.01 |
| | *P* | **0.008** | 0.609 | 0.114 |
| *Cadophora* sp. | $F_{2, 45}$ | 26.80 | 0.46 | 0.72 |
| | *P* | **<0.001** | 0.633 | 0.582 |
| Dothideomycete | $F_{2, 45}$ | 3.83 | 0.91 | 1.26 |
| | *P* | **0.031** | 0.410 | 0.302 |
| *Isaria farinosa* | $F_{2, 45}$ | 6.66 | 0.04 | 0.46 |
| | *P* | **0.003** | 0.959 | 0.763 |
| *Leptosphaeria* sp. | $F_{2, 45}$ | 26.62 | 3.31 | 4.78 |
| | *P* | **<0.001** | **0.048** | **0.003** |
| *M. alpina* | $F_{2, 45}$ | 5.05 | 0.21 | 2.74 |
| | *P* | **0.012** | 0.813 | **0.044** |
| *M. polygonia* | $F_{2, 45}$ | 1.24 | 1.50 | 1.43 |
| | *P* | 0.302 | 0.236 | 0.245 |
| *M. verticilliata* | $F_{2, 45}$ | 4.14 | 2.17 | 0.50 |
| | *P* | **0.024** | 0.128 | 0.735 |
| *Mortierella* sp. | $F_{2, 45}$ | 6.97 | 0.10 | 1.47 |
| | *P* | **0.003** | 0.907 | 0.233 |
| *P. lagerbergii* | $F_{2, 45}$ | 0.65 | 1.43 | 1.14 |
| | *P* | 0.530 | 0.252 | 0.352 |
| *P. pannorum* 1 | $F_{2, 45}$ | 19.45 | 0.36 | 0.36 |
| | *P* | **<0.001** | 0.701 | 0.837 |
| *P. pannorum* 2 | $F_{2, 45}$ | 20.16 | 2.15 | 2.90 |
| | *P* | **<0.001** | 0.131 | **0.035** |
| *P. pannorum* 3 | $F_{2, 45}$ | 40.88 | 2.97 | 2.17 |
| | *P* | **<0.001** | 0.064 | 0.092 |

*Abbreviations: T, temperature cycles, $\Psi_m$, matric water potential*

secreted more protein at higher $\Psi_m$ (Figure 6.4). Temperature cycle only had a marginally significant (*P* = 0.048) main effect on protein concentration for one isolate, *Leptosphaeria* sp. (Table 6.5).

The application of $\Psi_m$ × temperature cycle together only affected protein concentration in *Leptosphaeria* sp., *M. alpina* and *P. pannorum* 2 (Table 6.5). At - 0.2 MPa, temperature increases had positive effects on protein concentration in the former isolate (2.1 and 2.5 times increases at 5–21 °C and 5–24 °C, compared with 5– 18°C), but had no effects at other $\Psi_m$. At - 1.6 MPa, an increase in temperature had a negative effect on protein concentration in *M. alpina* (a 60 % reduction at 5–24 °C, compared with 5–18°C), but had no effect at other $\Psi_m$. Also at -1.06 MPa, the concentration of proteins extracted from *P. pannorum* 2 increased by 1.6 times with an increase in temperature from 5–18°C to 5– 21 °C, but protein concentration in extracts from *P. pannorum* 2 was unaffected by an increase in temperature at other $\Psi_m$.

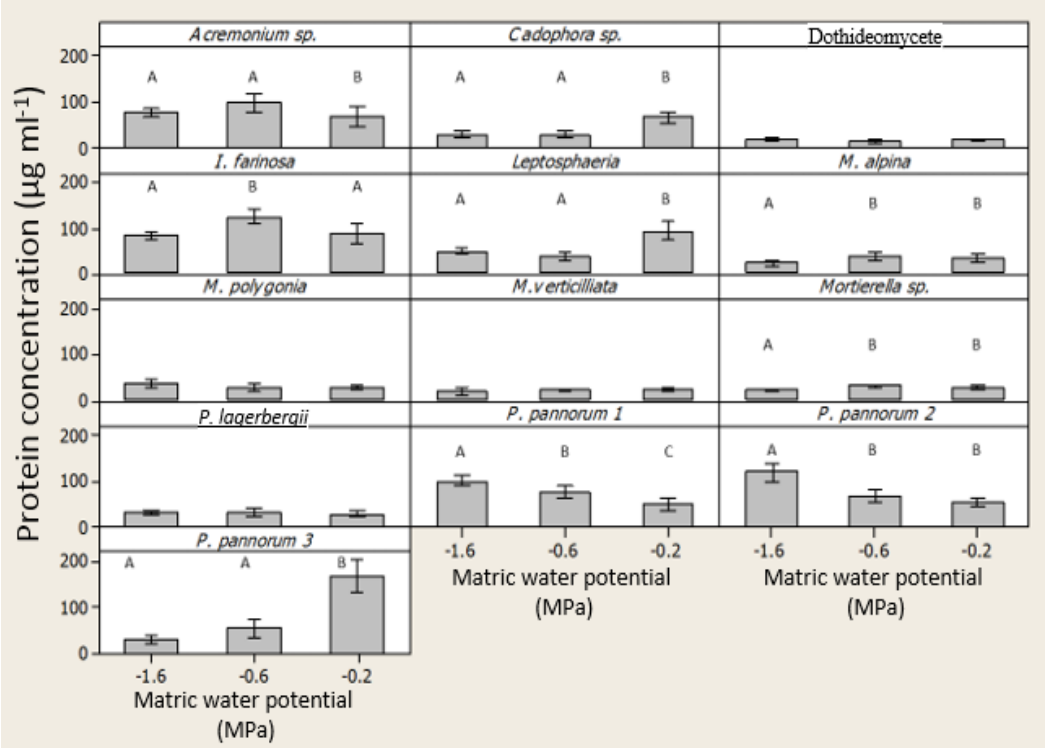



*Figure 6.3 Protein concentrations (µg ml$^{-1}$) in extracts from Arctic soil fungal isolates cultured at different water potentials, mean ± SEM, n = 12. Different letters indicate significant (P < 0.05) differences between treatments based on one-Way ANOVA test.*

### 6.3.3 Extracellular enzyme activities

Total enzyme activity (EA) differed significantly between isolates, enzymes, temperature cycles and water potentials (all *P* < 0.001, Table 6.6). All interaction terms (2- and 3-level) were significant, except for enzyme × temperature cycle, isolate × enzyme x temperature cycle and enzyme × temperature × $\Psi_m$ (Table 6.6).

*Table 6.6 General Linear Models (four way ANOVA) of main and interactive effects of isolate, enzyme, temperature cycles and water potentials on enzyme activity and specific enzyme activity of 13 Arctic fungal isolates. Bold text indicates significant (P < 0.05), $F_{degrees\ of\ freedom}$.*

| | Enzyme activity | | Specific enzyme activity | |
|---|---|---|---|---|
| **Factor** | $F_{3,\ 2709}$ | *P* | $F_{3,\ 2709}$ | *P* |
| Isolate | 698.83 | **<0.001** | 76.36 | **<0.001** |
| Enzyme | 319.44 | **<0.001** | 84.71 | **<0.001** |
| T | 0.40 | 0.672 | 0.91 | 0.404 |
| $\Psi_m$ | 203.24 | **<0.001** | 79.66 | **<0.001** |
| isolate x enzyme | 46.9 | **<0.001** | 16.77 | **<0.001** |
| isolate x T | 14.7 | **<0.001** | 8.56 | **<0.001** |
| isolate x $\Psi_m$ | 28.6 | **<0.001** | 13.65 | **<0.001** |
| enzyme x T | 0.66 | 0.727 | 0.94 | 0.483 |
| enzyme x $\Psi_m$ | 4.49 | **<0.001** | 4.24 | **<0.001** |
| T x $\Psi_m$ | 4.29 | **0.002** | 5.82 | **<0.001** |
| Isolate x enzyme x T | 1.05 | 0.358 | 0.89 | 0.779 |
| Isolate x enzyme x $\Psi_m$ | 7.83 | **<0.001** | 6.16 | **<0.001** |
| Enzyme x T x $\Psi_m$ | 1.56 | 0.070 | 0.71 | 0.791 |

*Abbreviations: T, temperature cycles, $\Psi_m$, matric water potential*

*Acremonium* sp. and *P. pannorum* isolates exhibited the highest EA values (*ca.* 30 and 15 U $hr^{-1}$ mg $hyphae^{-1}$, respectively) and *Cadophora* sp., *Mortierella spp.* and *P. lagerbergii* had the lowest EAs (< 1 U $hr^{-1}$ mg $hyphae^{-1}$; Table 6.7).

*Table 6.7 Summary of the effects of fungal isolate, enzyme, water potentials and temperature cycles on enzyme activity and specific enzyme activity of 13 Arctic soil fungal isolates. Values are means of 27–45 replicates ± SEM.*

| Factor | Level | Enzyme Activity (EA) (U $hr^{-1}$ mg $hyphae^{-1}$) | Specific Enzyme Activity (SEA) (U $hr^{-1}$ mg $hyphae^{-1}$ mg $protein^{-1}$) |
|---|---|---|---|
| | *Acremonium* sp. | 29.14 ±4.22 | 0.430 ± 0.056 |
| | *Cadophora* sp. | 0.62 ± 0.07 | 0.020 ± 0.002 |
| | Dothideomycete | 1.08 ± 0.15 | 0.0732 ± 0.01 |
| | *P. pannorum* 1 | 16.34 ± 1.91 | 0.203 ± 0.020 |
| | *P. pannorum* 2 | 16.84 ± 4.31 | 0.187 ± 0.035 |
| | *P. pannorum* 3 | 10.47 ± 1.35 | 0.316 ± 0.071 |
| Isolate | *I. farinosa* 1 | 9.1 ± 1.05 | 0.103 ± 0.011 |
| | *Leptosphaeria* sp. | 1.84 ± 0.24 | 0.045 ± 0.006 |
| | *M. alpina* | 0.35 ± 0.06 | 0.019 ± 0.006 |
| | *M. polygonia* | 0.25 ± 0.04 | 0.011 ± 0.02 |
| | *M. verticilliata* | 1.41 ± 0.19 | 0.101 ± 0.023 |
| | *Mortierella* sp. | 0.27 ± 0.02 | 0.014 ± 0.001 |
| | *P. lagerbergii* | 0.902 ± 0.24 | 0.029 ± 0.007 |
| | | | |
| | Acid phosphatase | 6.44 ± 1.47 | 0.095 ± 0.017 |
| | Alkaline phosphatase | 3.68 ± 1.18 | 0.055 ± 0.010 |
| Enzyme | Cellulase | 11.29 ±1.00 | 0.259 ± 0.031 |
| | Chitinase | 8.34 ± 1.34 | 0.117 ± 0.015 |
| | Leucine aminopeptidase | 2.96 ± 0.40 | 0.058 ± 0.006 |
| | | | |
| | -1.6 MPa | 12.07 ± 1.40 | 0.205 ± 0.021 |
| $\Psi_m$ | -0.6 MPa | 3.73 ± 0.29 | 0.064 ± 0.004 |
| | -0.2 MPa | 3.56 ± 0.37 | 0.076 ± 0.009 |
| | | | |
| | 5–18 °C | 5.98 ± 1.06 | 0.124 ± 0.019 |
| T | 5–21 °C | 6.34 ± 0.73 | 0.102 ± 0.010 |
| | 5–24 °C | 7.28 ± 0.85 | 0.124 ± 0.013 |

*Abbreviations: T, temperature cycles, $\Psi_m$, matric water potential*

Cellulase and chitinase were the most active enzymes (Table 6.7). Enzyme activity values increased at lower $\Psi_m$ (Table 6.6 and 6.7). Compared with -0.2 MPa, EA was 3.4 times higher at -1.6 MPa (Table 6.7). Temperature cycles had no main effect on overall EA. Specific enzyme activity (SEA) showed similar trends to EA, varying

significantly between enzymes, isolates and $\Psi_m$ (all $P < 0.001$), but not between temperature cycles (Table 6.6). All interaction terms (2- and 3-level) were significant, except for enzyme × temperature cycle, isolate × enzyme × temperature cycle and enzyme × temperature cycle x $\Psi_m$ (Table 6.6).

As for EA, SEA was highest for *Acremonium* sp. and *P. pannorum* isolates, and was lowest for *Mortierella* spp., *P. lagerbergii* and *Cadophora* sp. (Table 6.7). The SEA increased at lower $\Psi_m$, by 2.7 and 3.2 times at -1.6 MPa, compared to -0.2 MPa and - 0.6 MPa, respectively (Table 6.7).

#### *6.3.3.1 Chitinase*

Chitinase EA varied between isolates ($F_{12, 541}$ = 2525.7, $P < 0.001$) and was significantly affected by changes in $\Psi_m$ ($F_{2, 541}$ = 10.94, $P < 0.001$). Overall, decreases in $\Psi_m$ from -0.2 MPa to -1.6 MPa were found to double chitinase EA (Figure 6.4). Chitinase EA, however, was not affected by temperature cycle (Appendix 13, Figure 6.4) nor by the temperature cycles x $\Psi_m$ interaction (Appendix 13). The highest values of chitinase EA were recorded for *Acremonium* sp. (*ca.* 41 U $hr^{-1}$ mg $hyphae^{-1}$), followed by *I. farinosa* (*ca*. 18 U $hr^{-1}$ mg $hyphae^{-1}$) and *P. pannorum* isolates (*ca*. 17 U $hr^{-1}$ mg $hyphae^{-1}$). The EA values were lowest for *Cadophora* sp. and *Mortierella* spp. isolates.

When expressed as SEA, chitinase activity was affected by $\Psi_m$ and isolate ($P < 0.05$, Appendix 13, Figure 6.5) and was marginally significantly influenced by temperature cycle ($F_{2, 541}$ = 2.99, $P$ = 0.051). Interaction terms (isolate x temperature, isolate × $\Psi_m$, temperature × and isolate × temperature × $\Psi_m$) were all significant (all $P < 0.05$, Appendix 13), with further analyses showing that the changes were driven by isolate, and not by temperature or $\Psi_m$. The temperature cycle × $\Psi_m$ interaction had an effect on chitinase SEA only in *P. pannorum* isolates (Appendix 13, Figure 6.5). At - 0.2 MPa an increase in temperature from 5– 18 °C to 5–24 °C had a significant negative effect on SEA for *P. pannorum* 1. At - 0.6 MPa SEA for *P. pannorum* 3 decreased by 61 % with a temperature increase to 5– 21 °C, compared to 5–18 °C, but had no effect at other $\Psi_m$. None of the *P. pannorum* isolates were affected by temperature changes at - 1.6 MPa (Figure 6.5).

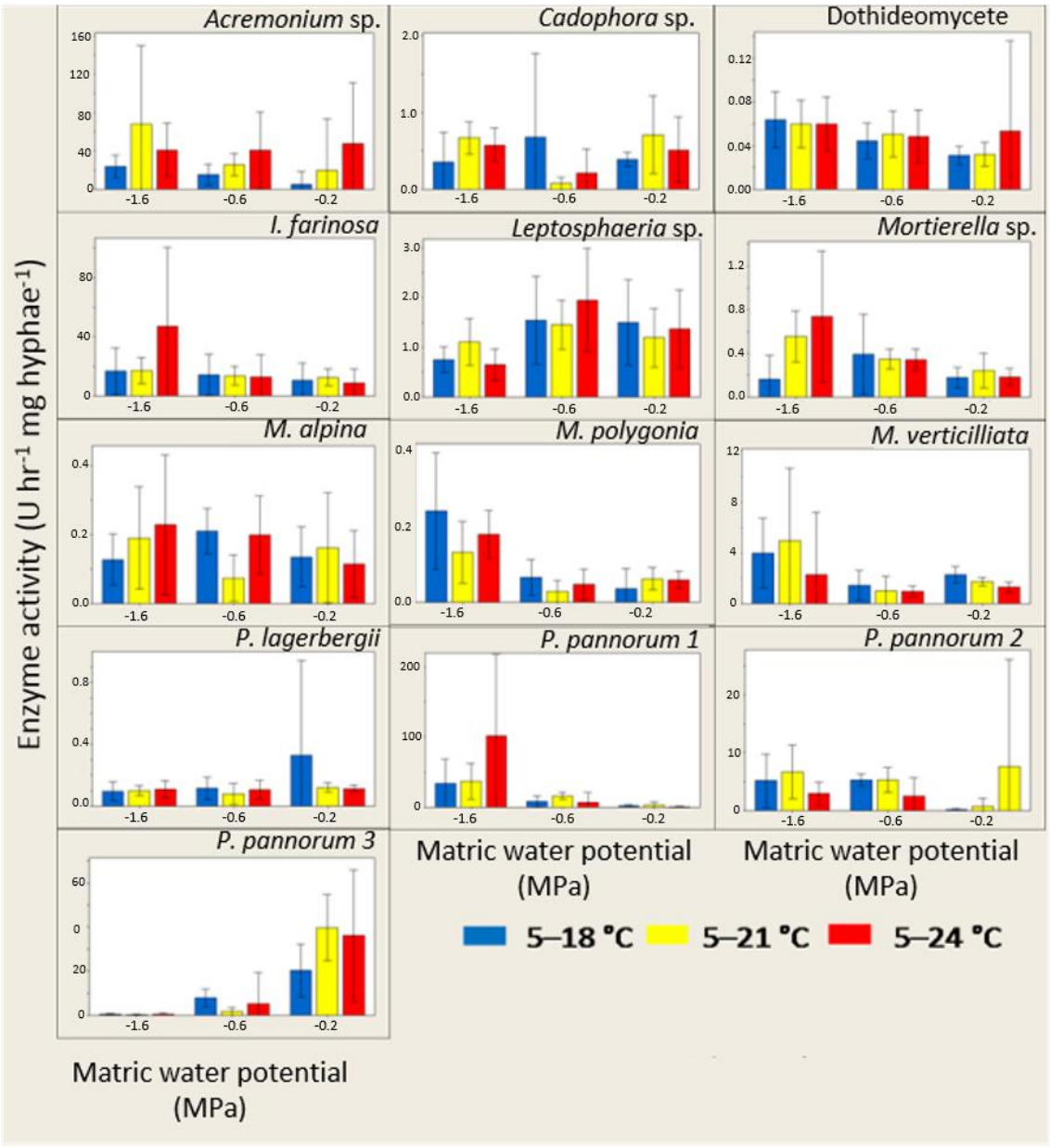



*Figure 6.4 Chitinase activities of 13 Arctic soil fungal isolates at the 5–18 °C, 5–21 °C and 5–24 °C temperature cycles and at water potentials of -1.6 MPa, -0.6 MPa and -0.2 MPa. Values are means of 3–5 replicates ± SEM. Note that y-axes for each isolate are scaled differently.*

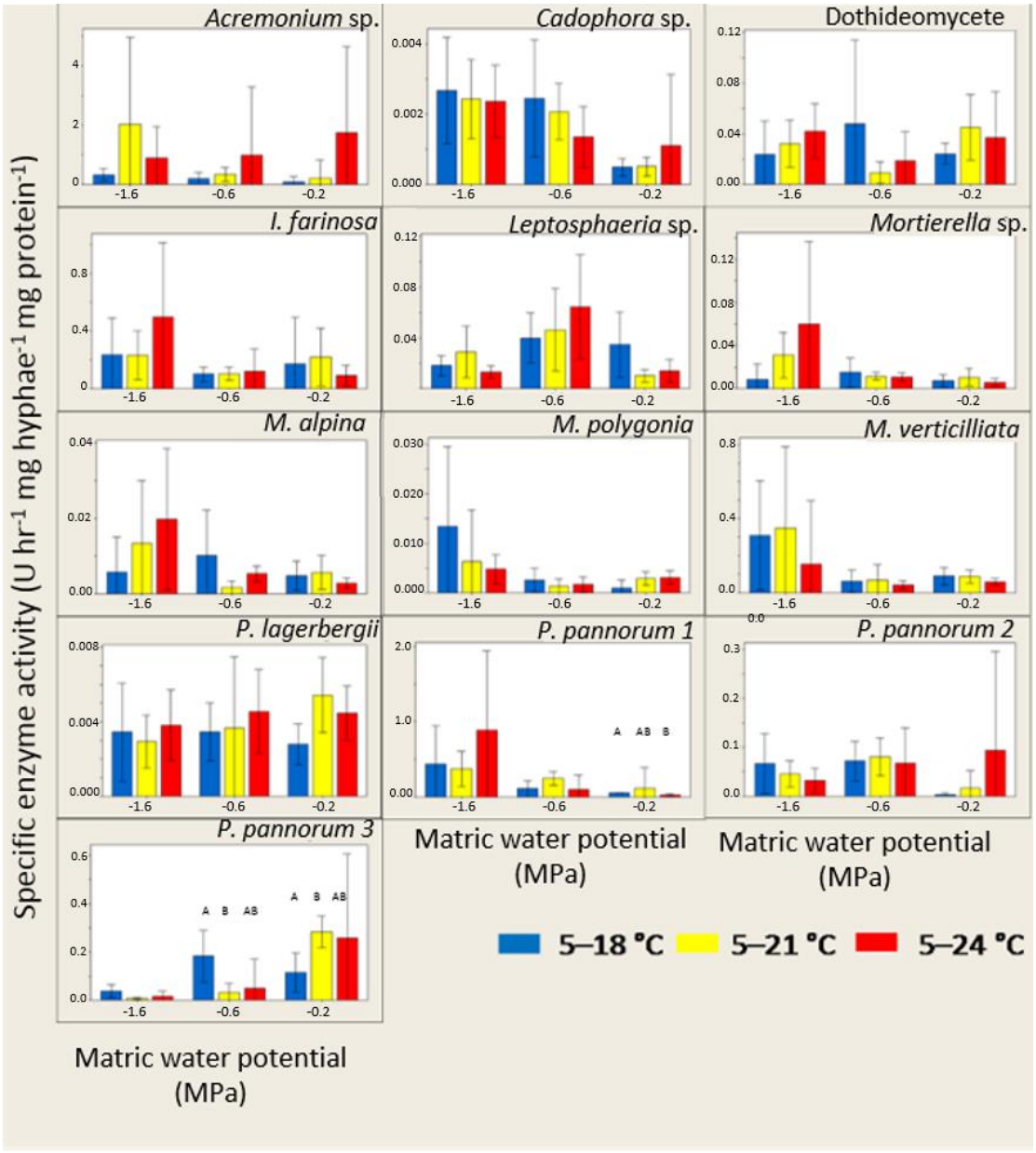



*Figure 6.5 Specific chitinase activities of 13 Arctic soil fungal isolates at the 5–18 °C, 5–21 °C and 5–24 °C temperature cycles and at water potentials of -1.6 MPa, -0.6 MPa and -0.2 MPa. Values are means of 3–5 replicates ± SEM. Note that y-axes for each isolate are scaled differently. Different letters indicate significant (P < 0.05) differences between treatments, based on one-way ANOVA.*

### 6.3.3.2 Cellulase

Cellulase EA varied between different soil fungal isolates ($F_{12,\ 542}$ = 2525.7, $P$ < 0.001) and was significantly affected by $\Psi_m$ ($F_{2,\ 542}$ = 55.27, $P$<0.001) but not by temperature cycles (Appendix 13, Figure 6.6). Temperature cycle × $\Psi_m$ changes applied together had a marginally significant effect on cellulase EA ($F_{4,\ 542}$ = 2.32, $P$ = 0.056). *Pseudogymnoacus pannorum* isolates had the highest cellulase EA (*ca*. 34 U $hr^{-1}$

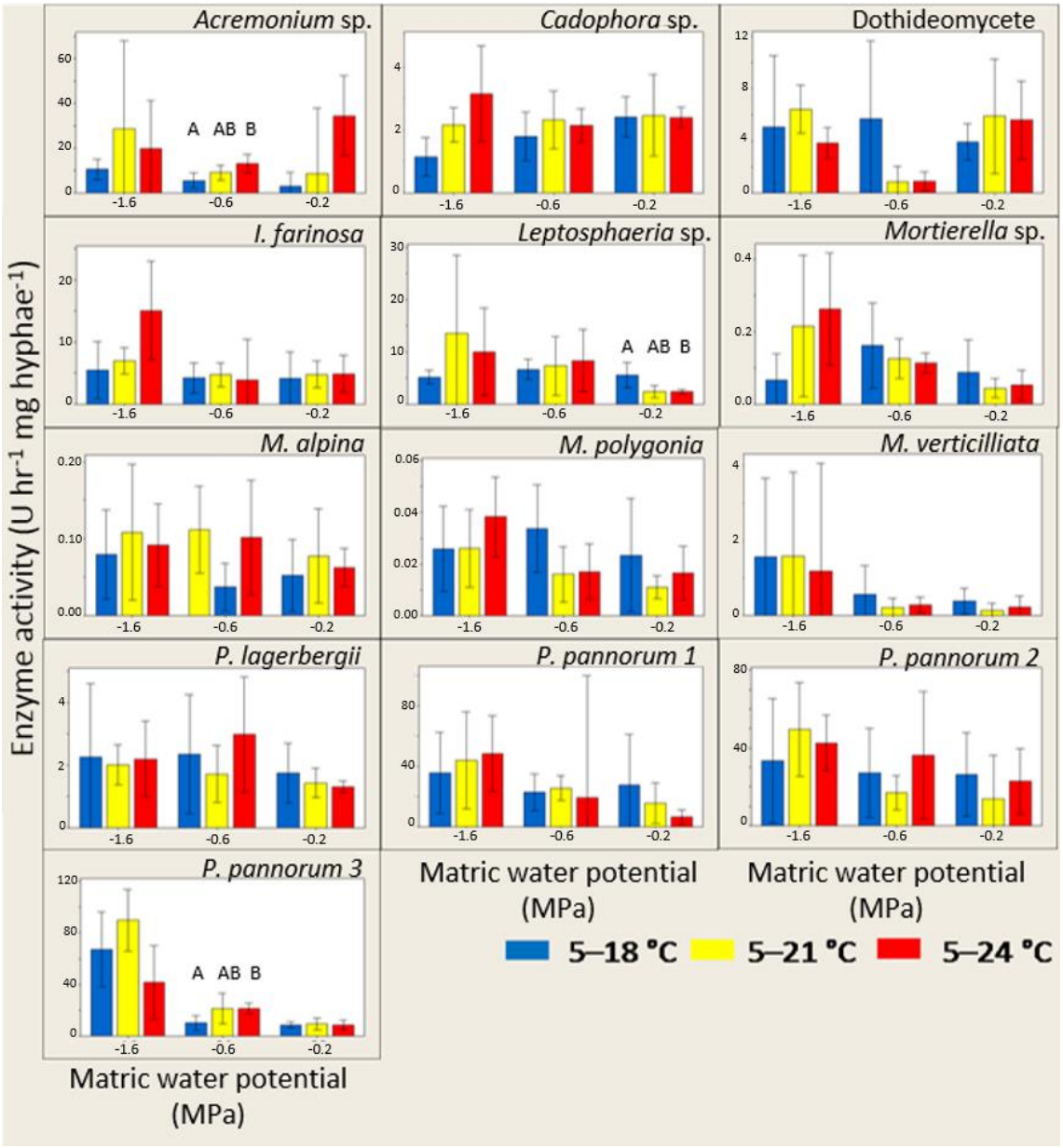



*Figure 6.6 Cellulase activities of 13 Arctic soil fungal isolates at the 5–18 °C, 5–21 °C and 5–24 °C temperature cycles and at water potentials of -1.6 MPa, -0.6 MPa and -0.2 MPa. Values are means of 3–5 replicates ± SEM. Note that y- axes for each isolate are scaled differently. Different letters indicate significant (P < 0.05) differences between treatments, based on one-way ANOVA.*

mg hyphae$^{-1}$), followed by *Acremonium* sp. (*ca.* 23 U hr$^{-1}$ mg hyphae$^{-1}$), and with *Mortierella* sp. having the lowest values of cellulase EA amongst the studied fungi (< 1 U hr$^{-1}$ mg hyphae$^{-1}$). Cellulase EA for the remaining fungal taxa ranged from 2– 7 U hr$^{-1}$ mg hyphae$^{-1}$. Overall, a decrease in $\Psi_m$ increased cellulase activity by 22 % and 2.7 times at -0.6 MPa and -1.6 MPa, respectively, compared with -0.2 MPa. The interactive effects of temperature cycles and $\Psi_m$ affected cellulase EA in *Acremonium* sp., *Leptosphaeria* sp. and *P. pannorum* 3 ($P < 0.05$; Figure 6.8). At -0.6 MPa,

a temperature increase from 5– 18 °C to 5–24 °C increased cellulase EA by 2.4 times in *Acremonium* sp., and by 2.1 times in *P. pannorum* 3, but had no effect at other $\Psi_m$. An increase in temperature from 5–18 °C to 5–24 °C reduced cellulase EA in *Leptosphaeria* sp. by 57 % at -0.2 MPa, but had no effect at other $\Psi_m$ (Figure 6.6).

Similar trends were recorded for cellulase SEA (Figure 6.7, Appendix 13). The exception was the temperature cycle x $\Psi_m$ interaction, which was only significant for

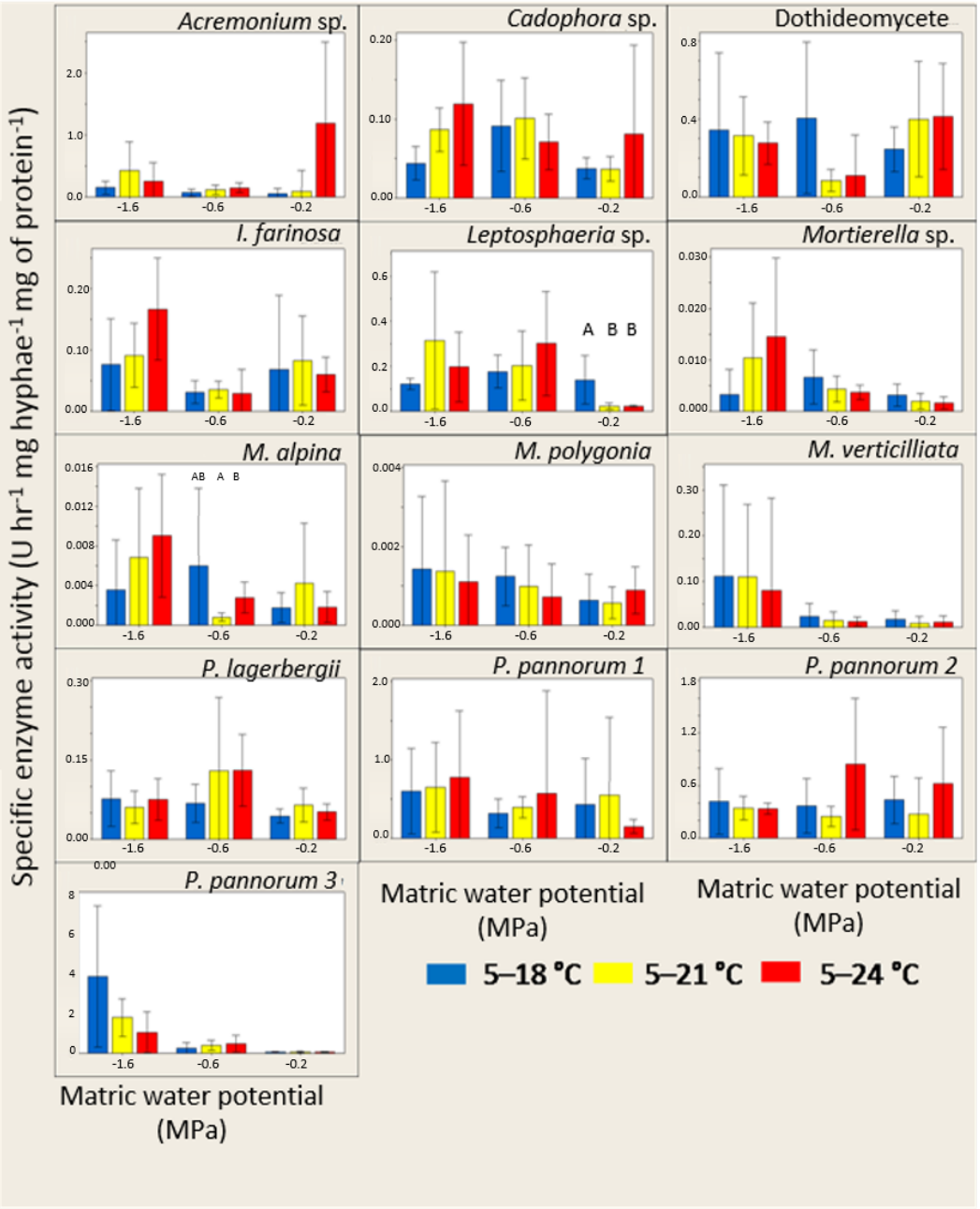



*Figure 6.7 Specific cellulase activities of 13 Arctic soil fungal isolates at the 5–18 °C, 5–21 °C and 5–24 °C temperature cycles and at water potentials of -1.6 MPa, -0.6 MPa and -0.2 MPa. Values are means of 3–5 replicates ± SEM. Note that y-axes for each isolate are scaled differently. Different letters indicate significant (P < 0.05) differences between treatments, based on one-way ANOVA.*

*Leptosphaeria* sp., but not for *Acremonium* sp., nor for *P. pannorum* isolates, mainly due to large variation between replicates (Figure 6.7). At -0.2 MPa, temperature increases above 5–18 °C decreased cellulase SEA in *Leptosphaeria* by 85 % and 83 %, but had no effect at other $\Psi_m$. At this $\Psi_m$, an increase in temperature from 5–21 °C to 5–24 °C was also found to increase SEA in cellulase in *M. alpina* by 53 % (Figure 6.7).

### 6.3.3.3 Leucine aminopeptidase

Leucine aminopeptidase EA varied between isolates ($F_{12, 543}$ = 138.43, $P$ < 0.001) and was significantly affected by $\Psi_m$ ($F_{2, 543}$ = 95.61, $P$ < 0.001, Figure 6.8) but not by

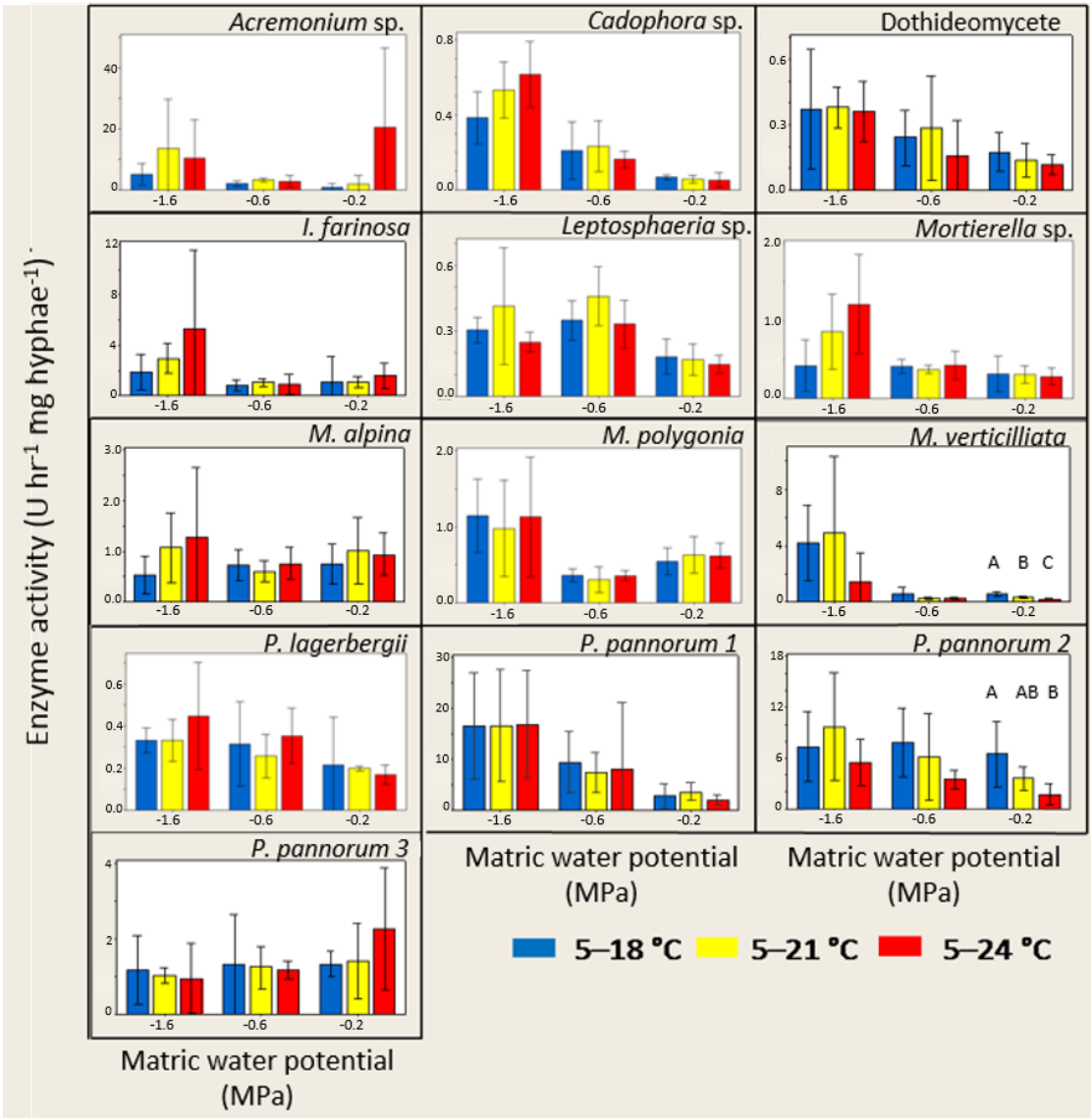



*Figure 6.8 Leucine aminopaptidase activities of 13 Arctic soil fungal isolates at the 5–18 °C, 5–21 °C and 5–24 °C temperature cycles and at water potentials of -1.6 MPa, -0.6 MPa and -0.2 MPa. Values are means of 3–5 replicates ± SEM. Note that y-axes for each isolate are scaled differently. Different letters indicate significant (P < 0.05) differences between treatments, based on one-way ANOVA.*

temperature cycle. Temperature cycle × $\Psi_m$ had no effect on leucine aminopeptidase EA. *Pseudogymnoascus pannorum* isolates 1 and 2 and *Acremonium* sp. had the highest leucine aminopeptidase EA values (9–12 U $hr^{-1}$ mg $hyphae^{-1}$). Leucine aminopeptidase EA values for the remaining fungal isolates ranged from 0.3–3.0 U $hr^{-1}$ mg $hyphae^{-1}$. Over all isolates, at -1.6 MPa, leucine aminopeptidase EA was increased by 3.9 and 3.2 times, compared with -0.6 MPa and -0.2 MPa, with these effects being particularly pronounced for *Cadophora* sp., the Dothideomycete, *Mortierella* spp. isolates and *P. pannorum* 1 and 2 (Figure 6.8).

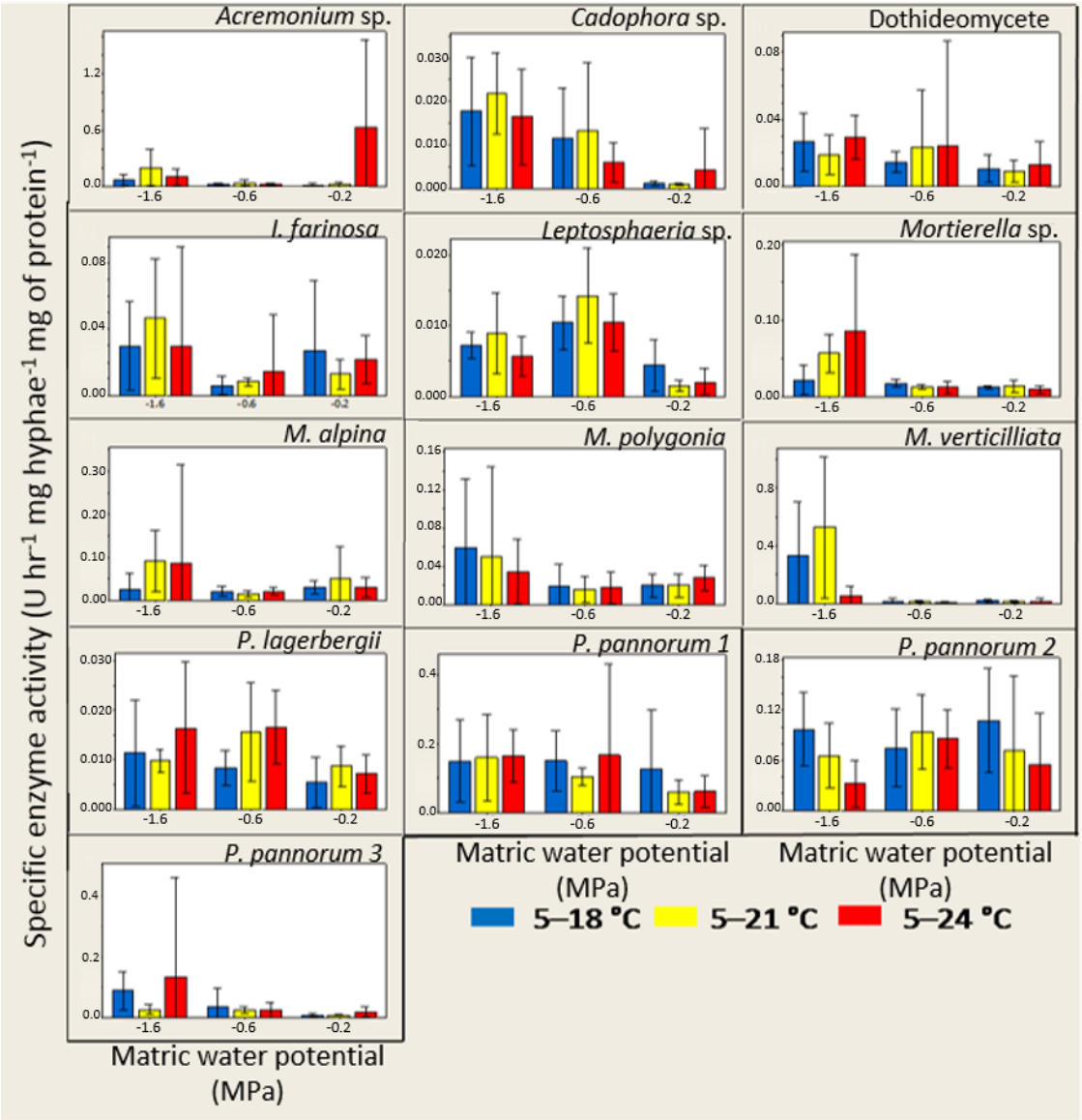



*Figure 6.9 Specific leucine aminopeptidase activities of 13 Arctic soil fungal isolates at the 5–18 °C, 5–21 °C and 5– 24 °C temperature cycles and at water potentials of -1.6 MPa, -0.6 MPa and -0.2 MPa. Values are means of 3–5 replicates ± SEM. Note that y-axes for each isolate are scaled differently.*

Changes in temperature x $\Psi_m$ affected leucine aminopeptidase EA only in

*M. verticilliata* and *P. pannorum* 2, and only at -0.2 MPa (Figure 6.8). At this $\Psi_m$, an increase in temperature to 5–21 °C and 5–24 °C decreased leucine aminopeptidase EA in *M. verticilliata* by 37 % and 58 %, compared to 5–18 °C. For *P. pannorum* 2, an increase in temperature to 5–24 °C decreased EA by 73 %, compared with 5–18 °C. Similar trends and significance of factors were found for leucine aminopeptidase SEA (Figure 6.9, Appendix 13). There were no significant temperature × $\Psi_m$ interactions for any of the isolates for leucine aminopeptidase SEA.

#### *6.3.3.4 Alkaline phosphatase*

Alkaline phosphatase EA varied between isolates ($F_{12, 544}$ = 137.3, $P$ < 0.001) and was significantly affected by $\Psi_m$ ($F_{2, 544}$ = 51.97, $P$<0.001) but not by temperature cycle (Figure 6.10, Appendix 13). *Acremonium* sp. had the highest alkaline phosphatase EA (*ca*. 13 U $hr^{-1}$ mg $hyphae^{-1}$), followed by *Pseudogymnoascus* sp. (4–18 U $hr^{-1}$ mg $hyphae^{-1}$) and *I. farinosa* (5 U $hr^{-1}$mg $hyphae^{-1}$). The remaining isolates produced alkaline phosphatase at < 0.5 U $hr^{-1}$ mg $hyphae^{-1}$. At -1.60 MPa, alkaline phosphatase activity was increased by 6.6 and 4.6 times, compared with -0.2 MPa and -0.6 MPa, respectively, with this effect being particularly pronounced for *I. farinosa*, *M. polygonia*, and *P. pannorum* 1 and 3 (Figure 6.10). Temperature cycles and $\Psi_m$ applied together had only a marginally significant effect on alkaline phosphatase EA ($F_{4, 544}$ = 2.2, $P$ = 0.068). An increase in temperature from 5–18 °C to 5–24 °C increased alkaline phosphatase EA in *I. farinosa* by four times at -0.2 MPa, but did not affect EA at other $\Psi_m$. Alkaline phosphatase enzyme activity in *Mortierella* sp. was also positively affected by an increase in temperature, with values being 14 times higher at 5–24 °C, compared to 5–18 °C, but only at -1.6 MPa (Figure 6.10). Alkaline phosphatase EA for *P. pannorum* was negatively affected by temperature increases. At -0.6 MPa, EA for *P. pannorum* 2 decreased by 79 % at 5–24 °C, relative to 5–18 °C. That for *P. pannorum* 3 was also reduced by 89 % by an increase in temperature from 5–18 °C to 5–24 °C, but only at -1.6 MPa.

Similar trends and significance of factors were recorded for alkaline phosphatase SEA (Figure 6.11, Appendix 13). *Pseudogymnoascus pannorum* 2 and 3 SEA was negatively affected at -1.6 MPa by temperature increases from 5–18 °C to 5–24 °C (97 % and 95 % decreases at -1.6 MPa, respectively; Figure 6.11). *I. farinosa* and *Mortierella* sp.

were not affected by temperature × $\Psi_m$ interactions, but SEA for *Leptosphaeria* sp. at - 0.6 MPa increased by 1.9 times at 5–21 °C, and by 3 times at 5–24 °C, compared to 5– 18 °C, with temperature increases having no effects at other $\Psi_m$ (Figure 6.11).

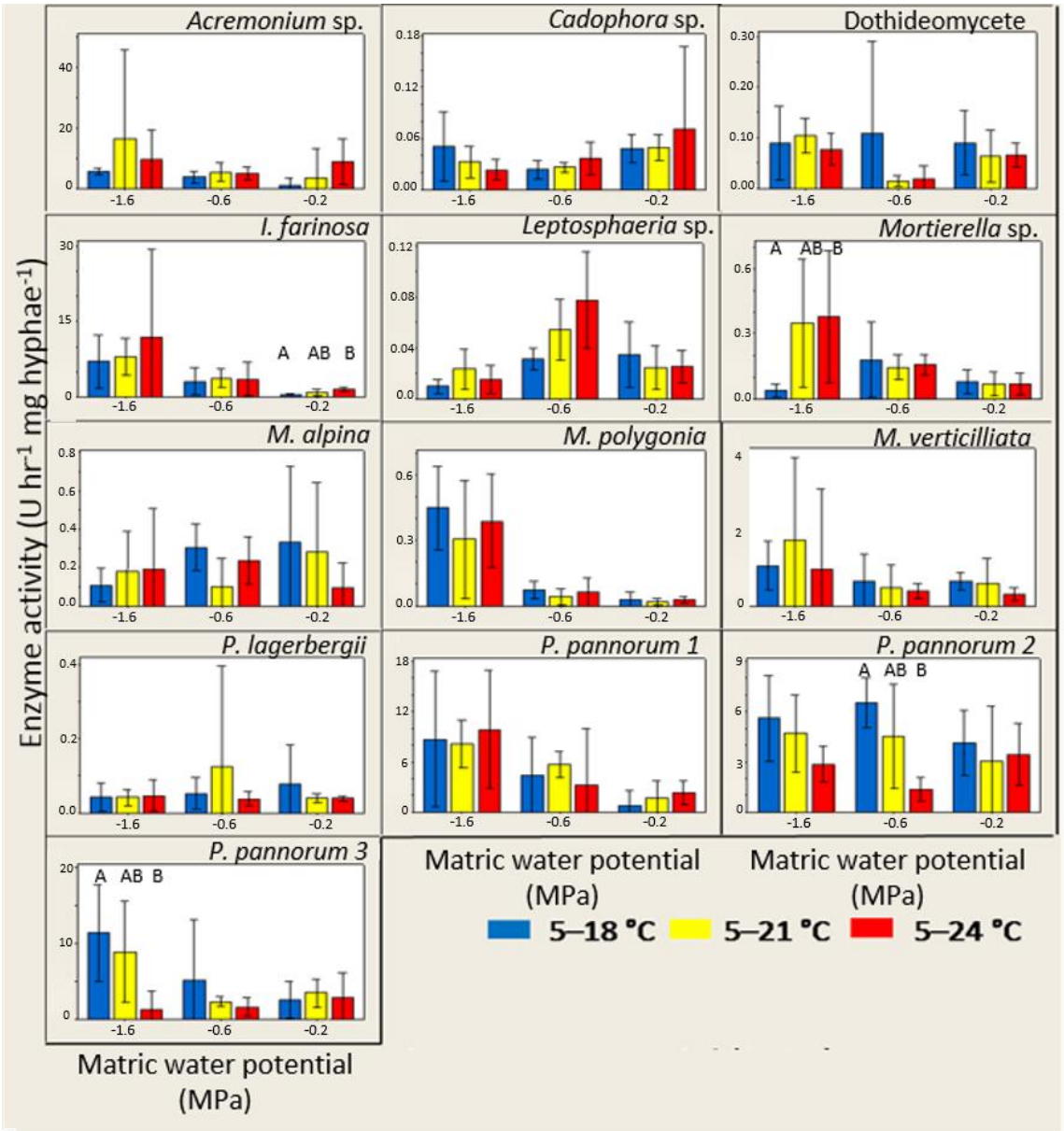



*Figure 6.10 Alkaline phosphatase activities of 13 Arctic soil fungal isolates at the 5–18 °C, 5–21 °C and 5–24 °C temperature cycles and at water potentials of -1.6 MPa, -0.6 MPa and -0.2 MPa. Values are means of 3–5 replicates ± SEM. Note that y-axes for each isolate are scaled differently. Different letters indicate significant (P < 0.05) differences between treatments, based on one-way ANOVA.*

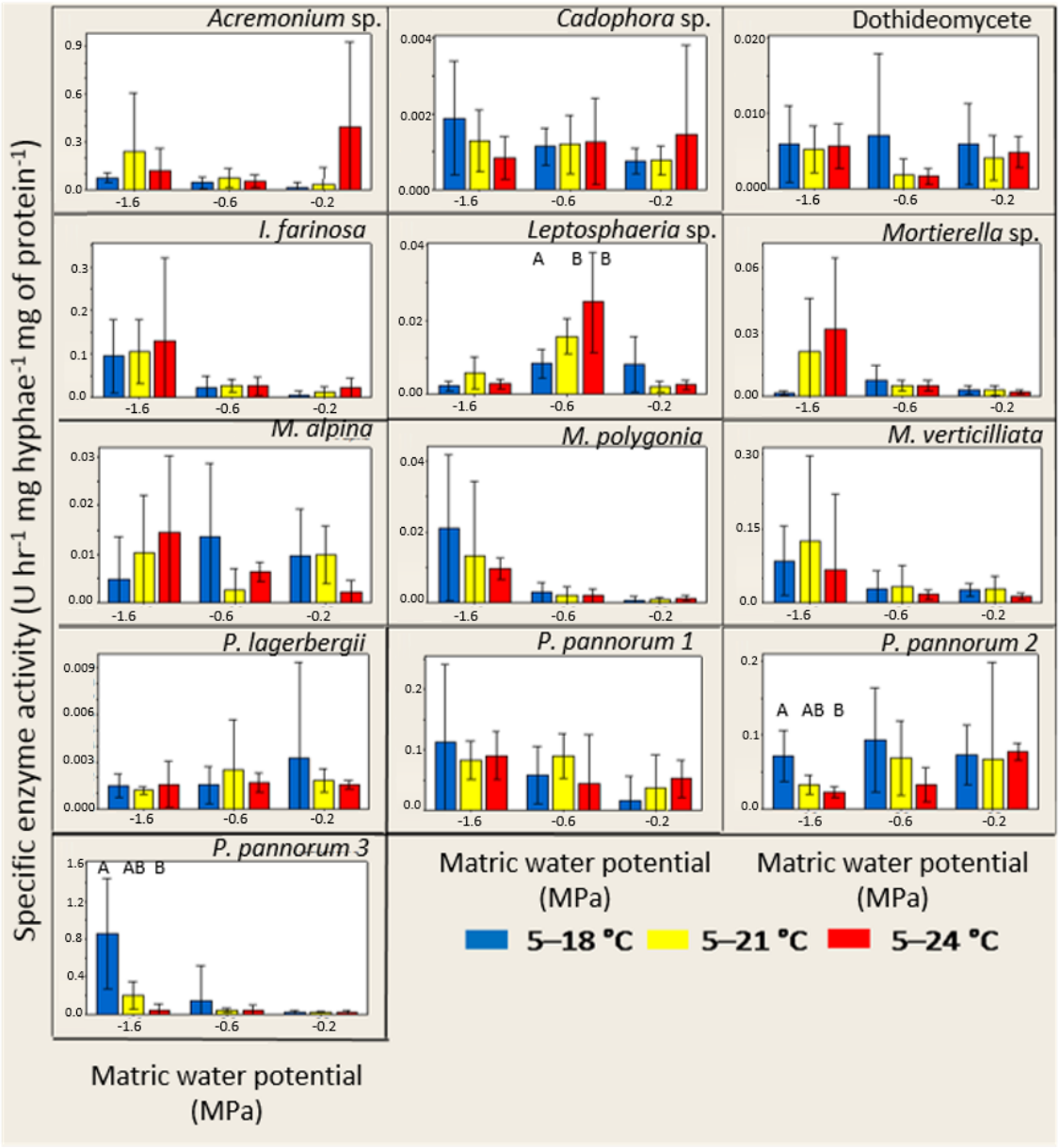



*Figure 6.11 Specific alkaline phosphatase activities of 13 Arctic soil fungal isolates at the 5–18 °C, 5–21 °C and 5– 24 °C temperature cycles and at water potentials of -1.6 MPa, -0.6 MPa and -0.2 MPa. Values are means of 3–5 replicates ± SEM. Note that y-axes for each isolate are scaled differently. Different letters indicate significant (P < 0.05) differences between treatments, based on one-way ANOVA.*

### 6.3.3.5 Acid phosphatase

Acid phosphatase EA varied between soil fungal isolates ($F_{12, 542}$ = 173.94, $P$ < 0.001) and was significantly affected by $\Psi_m$ ($F_{2, 542}$ = 54.87, $P$ < 0.001) but not by temperature cycle (Appendix 13, Figure 6.12). In contrast with all other measures of enzyme activity, however there was a highly significant interactive effect of temperature and $\Psi_m$ on acid phosphatase EA ($F_{4, 542}$ = 4.55, $P$ = 0.001, Appendix 13). At - 0.2 MPa, an

increase in temperature from 5–18 °C to 5–24 °C increased acid phosphatase EA by *ca.* 9 times, but had no effect at -0.6 MPa, nor at -1.6 MPa. As for the other enzymes tested, *I. farinosa, Acremonium* sp. and the three *P. pannorum* isolates had the highest acid phosphatase EA values (0.072–0.86 U $hr^{-1}$ mg $hyphae^{-1}$). Acid phosphatase EA values for the remaining fungal taxa were all ≤ 0.01 U $hr^{-1}$ mg $hyphae^{-1}$, with *Mortierella* spp. being one of the lowest producers of this enzyme. An increase in temperature from 5– 18 °C to 5–24 °C at -0.2 MPa increased acid phosphatase EA by 28 times in

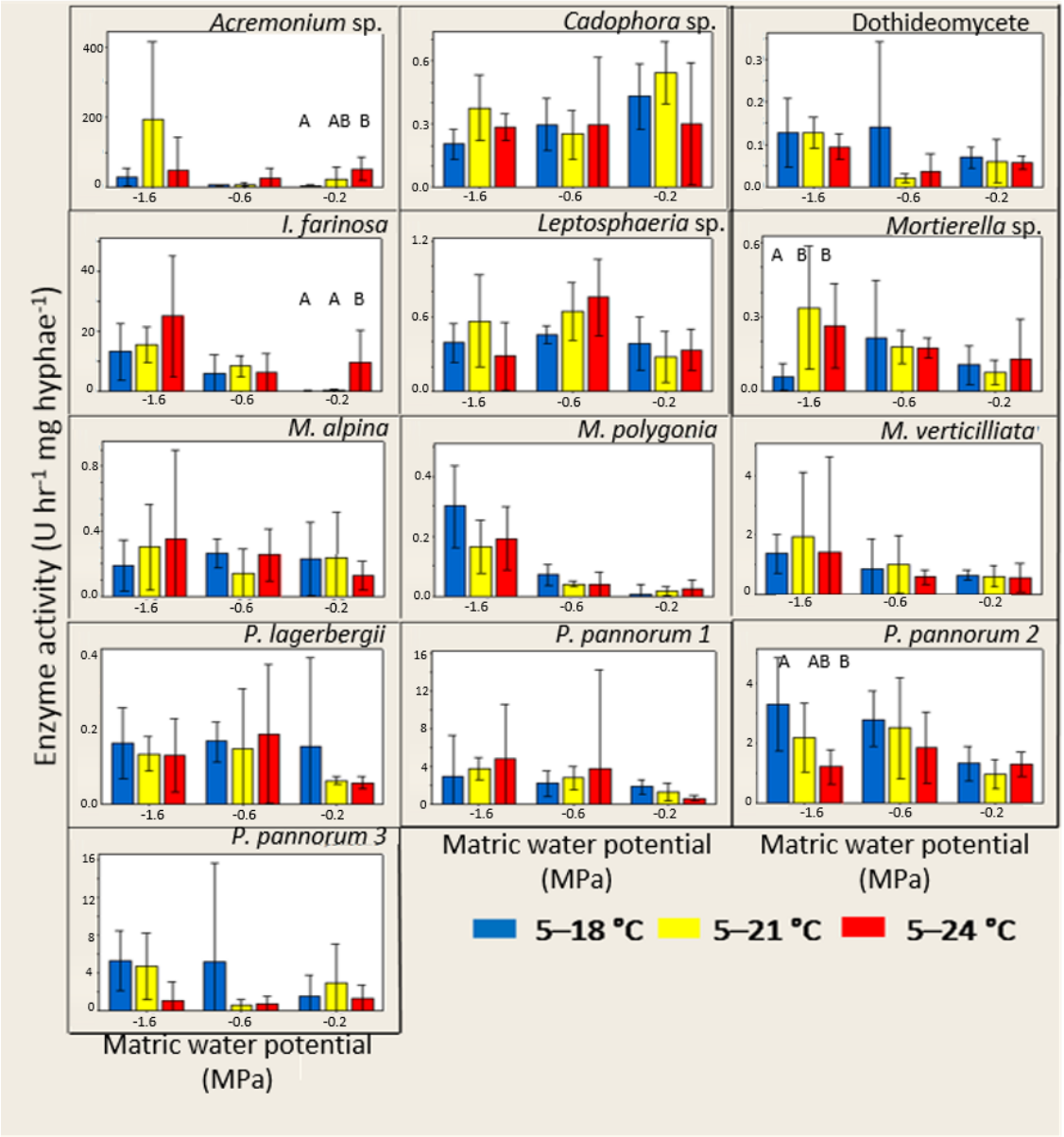



*Figure 6.12 Acid phosphatase activities of 13 Arctic soil fungal isolates at the 5–18 °C, 5–21 °C and 5–24 °C temperature cycles and at water potentials of -1.6 MPa, -0.6 MPa and -0.2 MPa. Values are means of 3–5 replicates ± SEM. Note that y-axes for each isolate are scaled differently. Different letters indicate significant (P < 0.05) differences between treatments, based on one-way ANOVA.*

*Acremonium* sp., and 44 times in *I. farinosa*, but did not affect the EA of these isolates at other $\Psi_m$. *Mortierella* sp. EA was increased by 7.8 times at 5–21 °C, and by 6 times at 5–24 °C, compared to the 5–18 °C cycle, but only at -1.6 MPa (Figure.6.12). An increase in temperature from 5–18 °C to 5–24 °C did decrease acid phosphatase EA of *P. pannorum* 2 by 62 % at -1.6 MPa, with the effect, however, not being significant at - 0.6 MPa or -0.2 MPa (Figure 6.12). Similar trends and significance of factors were recorded for acid phosphatase SEA, but with no effects on *Acremonium* sp. (Appendix 13, Figure 6.13).

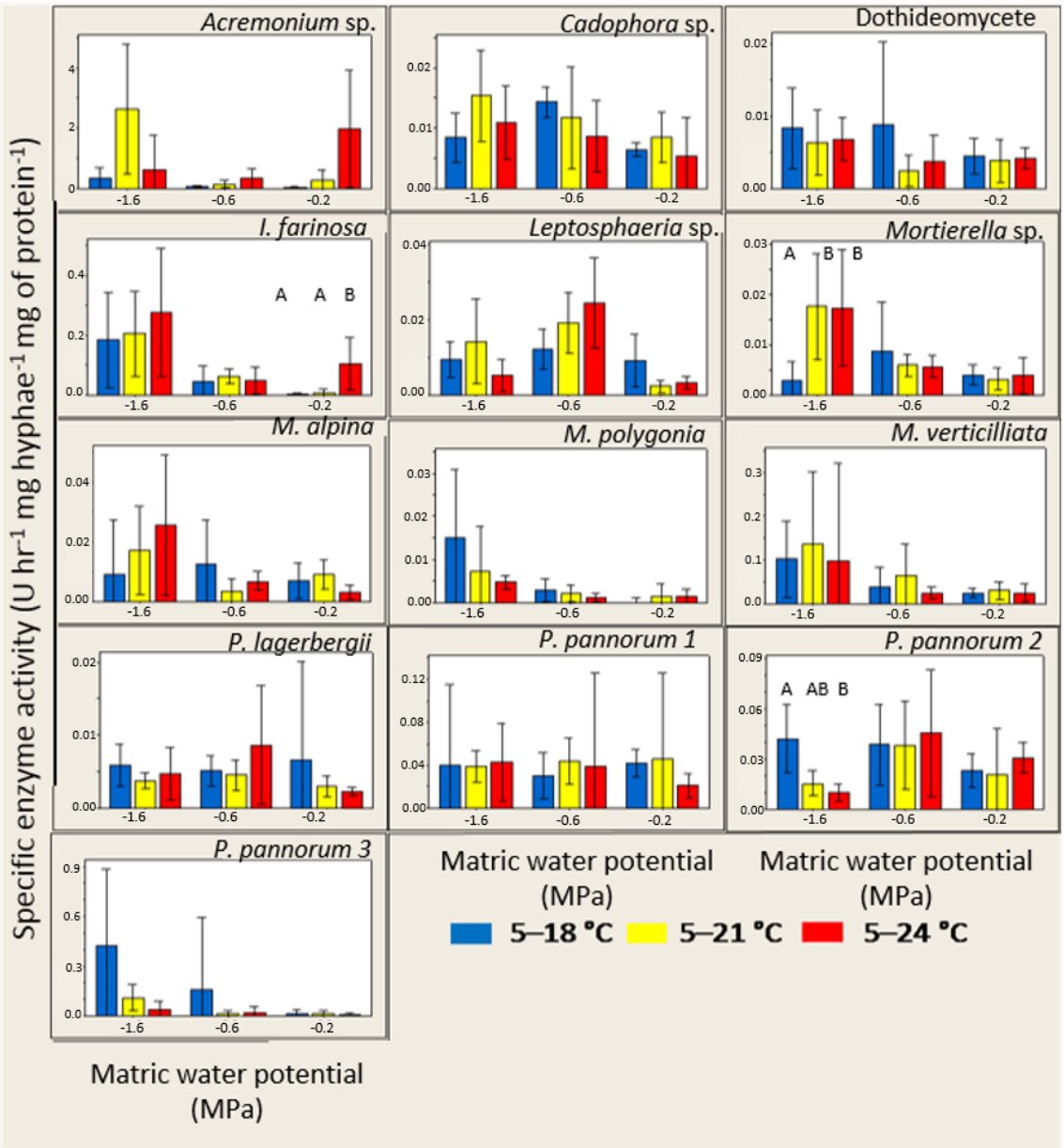



*Figure 6.13 Specific acid phosphatase activities of 13 Arctic soil fungal isolates at the 5–18 °C, 5–21 °C and 5–24 °C temperature cycles and at water potentials of -1.6 MPa, -0.6 MPa and -0.2 MPa. Values are means of 3–5 replicates ± SEM. Note that y-axes for each isolate are scaled differently. Different letters indicate significant (P < 0.05) differences between treatments, based on one-way ANOVA.*

## 6.4 Discussion

This study, investigating the effects of warming and altered matric water potentials on the growth and enzyme activities of Arctic soil fungi, showed that increased temperature had a consistently positive effect on the mycelial extension rates of five out of the 13 fungal isolates (*Acremonium* sp., *I. farinosa*, *Leptosphaeria* sp., *Mortierella* spp. and *P. lagerbergii*), partially supporting Hypothesis 1. This finding in part corroborates the study of Geml *et al.* (2015), who reported that warming led to increases in the number of operational taxonomic units of *Acremonium sp.*, *Phialocephala sp.* and *Pseudogymnoascus* spp. in an Alaskan Low Arctic tundra soil when OTCs were used to warm soil by *ca*. 1.5–2.0 °C for 18 years. In contrast to the findings of Geml *et al*. (2015), the extension rate of *P. pannorum* was affected negatively by the 5–24 °C warming treatment in the present study. This observation agrees with other studies that have found the optimal growth temperature for *P. pannorum* isolates from cold regions to be < 20 °C (Krishnan *et al*. 2016, Krishnan *et al.* 2018). These studies did however use constant temperature treatments (Krishnan *et al*. 2016, Krishnan *et al.* 2018), whilst here cycling temperatures with average daily means of 11.4 °C, 13.1 °C and 14.2 °C for the 5–18 °C, 5–21 °C and 5–24 °C temperature cycles, respectively, were used. These mean temperatures are below the previously reported maximum growth temperatures reported for *P. pannorum* isolates from cold soils (Bergero *et al*. 1999, Hughes *et al*. 2003, Krishnan *et al*. 2018), suggesting that the use of constant temperatures may not accurately predict microbial responses to warming and may lead to misleading conclusions about the responses of microbes in the natural environment to environmental warming (Convey *et al*. 2018).

The study here indicated that ECE activities differed significantly between enzymes and fungal isolates. Chitinase and cellulase activities were the highest, with *Acremonium* sp. and *P. pannorum* isolates being the most active producers of these enzymes, in part corroborating other studies showing that *P. pannorum* is one of the most active producers of various enzymes in soils of cold regions (Fenice *et al*. 1997, Krishnan *et al*. 2011, Singh *et al*. 2012) and *Acremonium* sp. is an active keratin decomposer (Friedrich *et al*. 2002). *Mortierella* spp., *Cadophora* sp., *P. lagerbergii* and the Dothideomycete had the lowest enzyme activities. Differences between soil fungal

taxa in their enzymatic capabilities has been previously reported in the literature (e.g., Burns and Dick 2002, Wang *et al*. 2014, Berlemont 2017, Duarte *et al*. 2018), with intraspecific differences in ECE production also being commonplace (Wang *et al*. 2014, Talbot *et al.* 2015, Berlemont 2017). Warming had no main effects on the concentrations of ECEs secreted by the fungal isolates studied here, irrespective of whether data were expressed as enzyme activities or specific enzyme activities, offering no support for Hypothesis 1. This finding corroborates Zhang *et al*. (2014), who found no effects of warming applied *in situ* with open top chambers on the cellulase and phosphatase activities of the total soil microbial community in an alpine meadow soil on the Tibetan Plateau. Furthermore, the findings reported here corroborate those of Wallenstein *et al*. (2009), who showed that chitinase, cellulase and peptidase activities in Low Arctic Alaskan soil were highest at the end of winter and did not increase in summer as soil temperatures rose. The lack of effects of cycling temperatures on ECE activities under controlled conditions, or of warming treatments in the field (Wallenstein *et al*. 2009, Koyama *et al*. 2013) suggests that rising soil temperatures will not have substantial effects on enzyme activities in the natural environment. Increased enzyme activity at higher temperatures has however been found for Arctic and Antarctic isolates of *Pseudogymnoascus* and *Penicillium* spp. grown in a laboratory experiment at constant temperatures, with higher cellulase activity being recorded at temperatures exceeding the growth optima for psychrophiles (25 °C; Krishnan *et al*. 2018).

Water potential was the main factor to influence the growth and activities of the Arctic soil fungi studied here, supporting Hypothesis 2 that changes in water availability would affect the growth rates and enzyme activities of the isolates. In general, the growth rate of the Arctic soil fungi decreased at lower water potentials. This concurs with numerous studies that have found lower growth, typically of crop pathogenic and food spoilage fungi, in response to lower water availability (Luard and Griffin 1981, Hallsworth *et al*. 1998, Jurado *et al.* 2008, Alam *et al.* 2009). Despite water availability being the main factor determining the composition of the fungal decomposer community and the productivity of terrestrial ecosystems in polar regions (Kennedy 1993, Allison and Treseder 2008, Sistla and Schimel 2013), there have been few studies into the effects of water availability on the growth of Arctic soil fungi (Singh and

Singh 2012). The present study found that the effects of water potentials on growth differed between fungal taxa, and that not all fungi responded positively to higher water availability, with a decrease in water potential from -0.2 MPa to -1.6 MPa (equivalent to a reduction in soil moisture concentration from 20 % to 3 %), leading to 8–89 % decreases in the extension rates of *Cadophora* sp., *Leptosphaeria* sp., *Mortierella* sp., *M. alpina*, *M. verticilliata* and *Pseudogymnoascus pannorum* 3. In contrast, the growth rates of *Acremonium* sp., *Pseudogymnoascus pannorum* 1 and 2, the Dothideomycete, *I. farinosa* and *P. lagerbergii* either remained unresponsive to, or were positively affected by, reductions in water availability. These findings partially support those of Geml *et al*. (2015), who studied dry and wet Alaskan tundra soils and found *Acremonium* to be more abundant in dry soil, and *Pseudogymnoascus* sp. to be present in both dry and wet tundra. In studies into the effects of water stress on the growth of filamentous fungi and yeasts isolated from the surfaces of glaciers on the Brøggerhalvøya Peninsula, Singh and Singh (2012) and Singh *et al*. (2016) reported that different isolates grew at a range of osmotic potentials (-4.8 MPa to -19.8 MPa) applied using NaCl solutions (3.5 M, 3 M, 2 M, 1.5 M and 1 M NaCl solutions). As here, the fungi studied had different tolerance ranges, with *Mrakia* sp., *Cryptococcus* sp., *Articulospora* sp., *Rhodotorula* sp. and *Phialophora alba* being able to grow in 1 M – 3.5 M NaCl solutions, and with none of the isolates being able to grow in NaCl solutions with concentrations of > 4 M (Singh and Singh 2012).

The effects of decreases in water potential affected the enzyme activities of the soil fungal isolates, confirming Hypothesis 2. Contrary to expectations, lower water availability tended to increase the enzyme activities of the fungi, indicating that they are well adapted to survive at low water potentials in Arctic soils (Griffin 1963, Gunde-Cimerman *et al.* 2003). Reduced water availability leading to higher enzyme production has been shown in studies of other fungi. For example, acid phosphatase and chitinase activity of *Aspergillus* sp. was shown to be increased at reduced water availability (Alam *et al*. 2009), and increased production of bioactive metabolites has also been found in halophilic and halotolerant fungi at lower water activities (Sepcic *et al*. 2011).

The observations here provided little support for Hypothesis 3, that warming and water availability would interact to determine the hyphal extension rates and enzyme

activities of the 13 Arctic soil fungal isolates studied here. The interactive effect of temperature cycles and water potential on the extension rates of individual isolates was only marginally significant, with significant interactions only being recorded for the *Acremonium*, Dothideomycete and *Leptosphaeria* isolates. In addition, there was a marginally significant effect of temperature cycle and water potential on all three *Pseudogymnoascus pannorum* isolates, with the growth of isolate 2 being decreased by warming at high water availability, most probably associated with a stress response (Krishnan *et al*. 2018). The results here confirm those of Gunde-Cimerman *et al*. (2003), who found that warming and altered water availability had variable effects on the fungi isolated from sea ice water and glacier ice water, but that the highest numbers of isolates were obtained on low water availability media. Over all isolates, the interactive effects of temperature cycles and water potentials on enzyme activities were not statistically robust, with highly significant effects only being found on acid phosphatase, and weak interactive effects of the two treatments being found on specific enzyme activities for chitinase, acid and alkaline phosphatases ($P$ = 0.02–0.04), compared with the robust effects found for the interactions between other factors ($P$ < 0.001), suggesting minimal changes in fungal enzyme activities at different water potentials as Arctic soils warm.

## 6.5 Conclusion

Climate change in the Arctic will lead to increased soil temperatures and altered water availability (AMAP 2012), and thus has the capacity to alter the activities of soil fungal communities (Robinson 2001, Geml *et al.* 2015), with possible effects on nutrient turnover in soil and the respiration of $CO_2$ to the atmosphere (Bokhorst *et al*. 2007, Wallenstein *et al*. 2009, Koyama *et al*. 2013, Talbot *et al*. 2015). Although NGS has been applied previously to provide detailed information on how Arctic soil fungal community composition and richness responds to warming (e.g., Geml *et al*. 2015, Semenova *et al.* 2015, Morgado *et al*. 2016), these studies must be coupled with ecophysiological studies to understand the mechanisms affecting the growth and activities of soil fungi in the region better. Here, using realistic water potentials and diurnally oscillating

temperatures representative of soils in the High Arctic during summer, species-specific responses have been shown, with the growth of *Acremonium* sp*., I. farinosa*, *Leptosphaeria* sp*.*, *P. lagerbergii* and a *Mortierella* spp*.* isolates responding positively to warming, and only that of two *P. pannorum* isolates being negatively affected by increases in temperature from 5–18 °C to 5–24 °C. Water availability also had species-specific effects on fungal growth, with positive effects of increased access to water on the growth of six of the 13 isolates studied here. Water stress induced increased enzyme activities, but varied greatly between enzymes and isolates, highlighting the difficulties in detecting changes to the physiologies of microbes isolated from natural ecosystems, even when studied in controlled environments. The effect of temperature and water potential applied together generally did not affect the growth and activities of the studied fungi. Further species-level studies are needed to decouple complex interactions in soils to gain a better understanding of the probable effects of climate change on Arctic soil processes.

These observations suggest that the rises in air temperature of 3–6 °C that are predicted across the Arctic before the end of the 21$^{st}$ Century under moderate greenhouse gas emission scenarios (Comiso and Hall 2014, Blaud *et al*. 2015) will most probably lead to enhanced fungal growth in soil. Since warmer soil holds less moisture (Carlyle *et al*. 2011), as Arctic soils dry, the growth of specific fungal taxa inhabiting them will be inhibited, but overall, fungal enzyme activities are likely to increase, leading to more rapid breakdown of organic compounds such as cellulose, chitin and amino acids, and possible acceleration of carbon and nitrogen cycling in these soils. However, such effects will depend on fungal community composition (Hawkes *et al.* 2010, Geml *et al*. 2015, Fernandez *et al*. 2016, Treseder *et al*. 2016) and are likely to be offset by increases in precipitation (Bintanja and Andry 2017, Song and Liu 2017).

# 7 Synthesis

Polar regions are subjected to rapid climate change, with increases in air temperatures of up to 0.72 °C per decade currently being recorded on Svalbard in the High Arctic (Førland *et al*. 2011), and, up until the turn of the millennium, 0.2–0.4 °C per decade rises in surface air temperatures in the maritime Antarctic (Bracegirdle *et al*. 2008, Convey *et al*. 2011, Turner *et al*. 2014, Rao *et al*. 2018). Increased rainfall and snowfall, associated with these rises in air temperature, are also predicted across both polar regions as their climates warm (IPCC 2007). Saprotrophic fungi, which are pivotal to the functioning of all terrestrial ecosystems owing to their central roles in nutrient cycling and the decomposition of soil organic matter (Swift *et al*. 1979), are dominant microbes in polar soils owing to their abilities to remain physiologically active at low temperatures and water availabilities (Whitford 1989, Robinson 2001, Duarte *et al*. 2018). Current understanding of the effects of climate warming and associated increases in precipitation on these microbes in polar soils is however limited (Aerts 2006, Siciliano *et al*. 2014, Convey *et al*. 2018). The research reported here aimed to fill this knowledge gap by using a combination of field manipulations and laboratory experiments to investigate the effects of changing temperatures and water availability on the growth and enzymatic capabilities of a range of saprotrophic fungi in Arctic and Antarctic soils.

It has previously been proposed that warming should accelerate the metabolic processes of soil microbes and hence increase the rates of organic matter decomposition in polar soils, returning the significant carbon stocks occurring in these soils - and particularly those in the Arctic (Tarnocai *et al*. 2009, Hugelius *et al*. 2014) - to the atmosphere as $CO_2$, forcing further climate warming (Kirschbaum 1995, Karhu *et al*. 2014). Despite intense research, as yet there is little consensus on the effects of climate warming on the temperature sensitivity of soil carbon breakdown due to the many complex kinetic processes that occur during decomposition (Davidson and Janssens 2006), and a lack of clarity over how soil saprotrophic communities respond to warming. Current literature suggests that climate change should affect microbial communities in Arctic and Antarctic soils (Timling *et al*. 2014, Geml *et al*. 2015, Newsham

*et al*. 2016, Semenova *et al*. 2016). As temperature increases in these regions, more liquid water will become available, which should alter the growth and metabolic responses of fungi to abiotic factors such as temperature, water and nutrient availability, with anticipated effects on soil fungal community composition and diversity in these regions (Aerts 2006, Davidson and Janssen 2014, Morgado *et al*. 2015, Newsham *et al*. 2016).

In Chapter 3, the hypotheses that warming and nutrient amendment would alter the abundances of fungal DNA in soil was tested in a field experiment at Mars Oasis on Alexander Island in the southern maritime Antarctic. The experiment used open top chambers (OTCs) to increase annual mean soil surface temperatures at the site by *ca.* 2 °C, with soil surface temperatures at the experiment frequently exceeding 20 °C in chambered soil during summer. Nutrients (glucose, glycine and tryptic soy broth (TSB)) were also added to soil in factorial combination with the warming treatment to simulate increases in carbon and nitrogen inputs to soil from expanding plant populations (Hill *et al*. 2011). The main focus for the study was *Pseudogymnoascus pannorum*, one of the most commonly isolated saprotrophic fungi from Antarctic soils (Arenz and Blanchette 2011, Ali *et al*. 2013), and the most frequently detected fungal taxon in a DNA-based study of 29 maritime Antarctic soils (Newsham *et al*. 2016). Quantitative (Q-)PCR was used to measure the abundance of the DNA of this species, and of total fungi, in soil at Mars Oasis. Warming with OTCs had substantial effects on the abundance of *P. pannorum* DNA, with 35–99 % decreases in gDNA concentrations in chambered soil to which glycine and tryptic soy broth (TSB) had been applied, relative to unchambered, amended soils. Similar effects were found for total soil fungi, with 21–99 % reductions in the abundance of fungal gDNA in warmed and glycine- or TSB-amended soils. These findings suggested, for the first time, that warming may have deleterious effects on the abundances of fungi in a maritime Antarctic soil in the natural environment. The mechanisms responsible for these reductions in the concentrations of fungal DNA in warmed, nutrient amended Antarctic soils were not identified.

In Chapter 4, the interactive effects of warming and water availability on the growth and enzyme activities of three strains of *P. pannorum* isolated from Mars Oasis soil were studied in detail. Although it has been established for decades that the growth

and enzymatic capabilities of Antarctic psychrophilic fungi, including *P. pannorum*, decline at temperatures exceeding approximately 20 °C (Kerry 1990, Azmi and Seppelt 1997), all of these studies have applied constant temperatures that are unrepresentative of the Antarctic natural environment, in which temperatures at soil, rock or moss surfaces can vary on a diurnal basis by > 30 °C (Longton 1988, Bramley- Alves *et al*. 2014). Given that oscillating temperatures are known to elicit different biological responses to constantly-applied temperatures (Dang *et al*. 2009, Barnes *et al.* 2010), the three *P. pannorum* isolates were exposed to temperature cycles encountered at soil surfaces at Mars Oasis during summer, with temperatures cycling daily from 2–18 °C, 2–21 °C and 2–24 °C. The isolates were also grown at four water potentials (-1.06 MPa, -3.60 MPa, -6.00 MPa and -10.80 MPa), in factorial combination with these temperatures, which were representative of water availabilities in surface soils at Mars Oasis during austral summer. In addition, rather than being grown on nutrient-rich media (*c.f.* Krishnan *et al*. 2016, 2018), the isolates were grown on a weak soil extract medium to more closely predict the responses to the treatments that might occur in the natural environment. These experiments indicated that, relative to the 2– 18 °C temperature cycle, increases in temperature to 2–21 °C, and, in particular, 2– 24 °C, led to substantial (46–66 %) reductions in the hyphal extension rates of the three isolates. The largest reductions in growth rates associated with warming were found at the highest water potential (-1.06 MPa), with few effects of temperature increases on the extension rates of *P. pannorum* at water potentials of -6.00 and - 10.80 MPa, corroborating the view that water availability alters microbial responses to warming (Baldrian *et al.* 2013, Henry 2013, A'Bear *et al*. 2014). Together with the observation, reported in Chapter 3, of reduced concentrations of gDNA of *Pseudogymnoascus* spp. in warmed and nutrient-amended soil at Mars Oasis, these data suggest that the growth of probably the most widespread saprotrophic fungus in the maritime Antarctic is inhibited in moist soils in the natural environment exposed to temperatures exceeding approximately 20 °C. They also suggest that the inhibitory effects of warming on the growth of the fungus most probably occurs in the days just following snowmelt at Mars Oasis, since soils at the site during summertime are dry owing to exceedingly low precipitation. Given that the temperatures of soil surfaces at

the study site already approach 20 °C (Figure 2.4), these observations suggest that the 2–4 °C rises in air temperature, predicted for the maritime Antarctic before the end of the 21st Century by climate models forced with only moderate greenhouse gas emission scenarios (Bracegirdle *et al*. 2008, Bracegirdle and Stephenson 2012), may inhibit the growth of *Pseudogymnoascus pannorum* at the surfaces of moist soils in the region.

Analyses of the enzyme activities of the three *P. pannorum* isolates studied in Chapter 4 suggested that the secretion of five extracellular enzymes (chitinase, cellulase, leucine aminopeptidase and acid and alkaline phosphatase) by hyphae of *P. pannorum* will be reduced by temperatures exceeding approximately 20 °C. As for the growth rates of the isolates, enzyme activities were most strongly inhibited under the 2–24 °C temperature cycle and at the highest water potential (-1.06 MPa), suggesting substantial reductions in the abilities of this taxon to decompose chitin, cellulose, an amino acid and phosphoesters in warmed soil in which water is freely available. Climate warming, previously suggested to have consistently positive effects on maritime Antarctic terrestrial ecosystems (Bokhorst *et al*. 2007, Turner *et al*. 2009, Turner *et al*. 2014), might hence inhibit the ability of this widespread, ecologically important fungus to decompose organic compounds in soil, with deleterious effects on the cycling of nutrients that support the growth of other soil organisms and plants.

In Chapter 5, Q-PCR was used to measure the abundances of the DNA of seven fungal taxa (*Pseudogymnoascus*, *Mortierella*, *Tetracladium*, *Leptosphaeria, Cadophora, Phialocephala* and Dothideomycetes) in soil sampled from an OTC experiment on the Brøggerhalvøya Peninsula on Svalbard in the High Arctic. Unlike the soil at Mars Oasis in Antarctica, the high moisture concentration of the High Arctic soil precluded the direct addition of nutrients, and so water was instead added to soil in factorial combination with warming. In contrast to the results from the Antarctic field experiment, three years of warming with OTCs in the High Arctic failed to elicit effects on the concentrations in soil of gDNA of any of the soil fungal taxa that were studied, including *Pseudogymnoascus* sp. Interactions between OTCs and the watering treatment also did not alter the abundances of any taxa, but watering reduced the summed concentration of all seven taxa, most probably as a result of anaerobic conditions in the soil, or because of competition from bacteria (Steven *et al*. 2006, Lipson *et al*. 2012, Zhang *et al*. 2014).

At present, it is unclear as to why there were so few treatment effects on the abundance of fungal gDNA in the High Arctic soil. One possibility is that by not applying nutrients to the soil, the abundance of fungal gDNA would have remained at low levels, decreasing the likelihood of detecting differences between the treatments. Another is that the warming and watering treatments may not have been applied for long enough to have elicited significant effects, with similar studies in the Low Arctic concluding that 10–15 years of treatment are necessary to generate effects of warming with OTCs on soil fungal and bacterial communities (Rinnan *et al*. 2007b, Deslippe *et al*. 2012). Furthermore, most probably because of the relatively high moisture concentration (*ca*. 4–18 %, mean 12 %) of the Arctic soil compared with that of the Antarctic soil (*ca*. 2– 14 %, mean 3.5 %), soil surface temperatures in OTCs on the Brøggerhalvøya Peninsula in the Arctic only exceeded 20 °C on a single day during the entire three year period of treatment (Figure 2.7), whereas temperatures of > 20 °C occurred regularly in chambered soil during summer at Mars Oasis, Antarctica (Figure 2.3). It is hence possible that the relatively small effect of OTCs on soil surface temperatures in the High Arctic failed to elicit the same effects as those recorded in the Antarctic.

In Chapter 6, 13 isolates of the fungal taxa, six of which were studied in Chapter 5, were grown in laboratory experiments at diurnally cycling temperatures (5– 18 °C, 5–21 °C and 5–24 °C) in factorial combination with three water potentials (- 1.6 MPa, -0.6 MPa and - 0.2 MPa), each of which were representative of the soil on the Brøggerhalvøya Peninsula, Arctic, during the boreal summer. As for the isolates of *P. pannorum* from the maritime Antarctic, the Arctic soil fungi were grown on a weak soil extract medium to increase the likelihood that the observed responses would be more similar to those that might be recorded in the natural environment. In contrast to the results for Antarctic strains of *P. pannorum*, these experiments indicated positive effects of warming on fungal growth rates under the 5–21 °C and 5–24 °C temperature cycles relative to the 5–18 °C cycle, with the growth rates of *Acremonium* sp., *Isaria farinosa*, *Leptosphaeria* sp., *Phialocephala lagerbergii* and *Mortierella* spp. all responding positively to warming. In agreement with the data reported in Chapters 3 and 4, the growth rates of two of three Arctic *P. pannorum* isolates were however inhibited by increases in temperature from 5–18 °C to 5–24 °C. There was also an

absence of main effects of temperature cycles on the activities of the same five enzymes that were studied in Chapter 4, but decreased water availability typically led to higher enzyme activities of the Arctic soil fungal isolates, suggesting that improved access to liquid water will limit the decomposer abilities of soil fungi in the region.

This study highlights several avenues for new research. One of its principal findings was that the main factor affecting the hyphal extension rates and enzyme activities of Arctic fungi was not temperature but water potential, suggesting that saprotrophic fungi inhabiting Arctic soils might be resilient to potential temperature changes predicted from current climate models (*ca*. 3-6 °C rises in surface air temperatures by 21$^{st}$ century (Comiso and Hall *et al*. 2014). The effects of altered water availability on the growth and the enzyme activities of Arctic soil fungi, and particularly those in the active layers above permafrost, is hence a promising area for further research. Accurate predictions for how water availability are likely to alter in active layers, and more accurate projections for changes in future precipitation, which are difficult to measure in polar regions (Turner and Overland 2009) are necessary before such analyses might be made.

The data reported in Chapter 4 strongly suggest that isolates of *P. pannorum* may differ in their responses to oscillating and constant temperatures, with substantial reductions in growth rates being recorded under the 2–21 °C and 2–24 °C temperature cycles, the mean temperatures of which, at just 12 °C and 13 °C, respectively (Section 2.6), are substantially lower than the previously reported upper cardinal growth temperatures for *P. pannorum* (Azmi and Seppelt 1995, Hughes *et al*. 2003, Krishnan *et al*. 2012). These observations suggest that the effects of the peak temperatures to which microbes - and particularly psychrophiles - are exposed may be more important for their growth and activity than temperatures averaged over time. A series of relatively simple experiments could identify whether constant temperature treatments tend to under- or overestimate the inhibitory effects of warming on the growth of *P. pannorum* and other psychrophilic soil fungi, such as the snow moulds *Typhula ishikariensis* and *Pythium* spp. (Tojo and Newsham 2012).

Another avenue for further research might be to identify, at the molecular level, the responses of Antarctic and Arctic isolates of *P. pannorum* to temperatures exceeding

*ca*. 20 °C. High throughput sequencing methods, such as RNA-Seq, could be used to compare the transcriptomes of *P. pannorum* isolates grown at temperatures below and above 20 °C, and to detect differentially expressed genes (Wang *et al*. 2017). It is possible that heat shock proteins (HSPs) play a role in protecting proteins from the deleterious effects of high temperatures in this, and other, psychrophilic fungal species (Reeder *et al*. 2017), and so Q-PCR assays could also be used to identify if HSPs are upregulated in isolates of *P. pannorum* exposed to high temperatures. Such analyses could also be used to determine, as suggested by the research here, whether the responses at the molecular level of *P. pannorum* might be different from those of Arctic saprotrophic fungi such as *Acremonium* sp., *Isaria* sp., *Leptosphaeria* sp., *Phialocephala* sp. and *Mortierella* sp., the growth rates of which were uninhibited by exposure to temperatures cycling daily between 5 °C and 24 °C in the present study.

Arctic soils collectively contain an estimated 1,330–1,580 petagrams ($1.33– 1.58 \times 10^{12}$ tonnes) of organic carbon, which is roughly two to three times the amount of carbon present in the Earth's atmosphere as $CO_2$, and is the largest pool of the element on Earth (Tarnocai *et al*. 2009, Hugelius *et al*. 2014). The majority of this organic carbon is currently present in permafrost and is hence inaccessible to many microbes, but, as the Arctic warms and permafrost thaws, it will become increasingly available to saprotrophic soil fungi. The findings here indicate that increased soil temperature arising from climate warming will lead to faster growth of Arctic saprotrophic fungi, but that enhanced water availability in soil arising from increased precipitation will inhibit the abilities of fungi to decompose soil organic matter. As previously suggested (Oelbermann *et al*. 2008, Koven *et al.* 2011), Arctic soil microbes thus apparently have the capacity to respire huge stocks of organic carbon to the atmosphere as $CO_2$ in warmer soils, but soil water availability will affect their abilities to do so. To further knowledge of the role of saprotrophic soil fungi in this process, studies should quantify the $CO_2$ released to the atmosphere by specific fungal taxa (*c.f.* Newsham *et al*. 2018) at temperatures and water availabilities encountered in the active layer-permafrost zone of Arctic soils.

In summary, given the effects of warming and changes in water availability on the growth and enzyme activities of saprotrophic soil fungi reported here, it is evident

that the activities of these microbes should be incorporated into future models to accurately predict changes to ecosystems associated with climate warming in polar regions.

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# 9 Appendices

## Appendix 1

Details of the layouts of the soil warming experiments at Mars Oasis in the maritime Antarctic (Table 9.1) and on the Brøggehalvoya Peninsula on Svalbard in the High Arctic (Table 9.2).

*Table 9.1 The soil warming experiment at Mars Oasis in the maritime Antarctic consisted of 64 plots to which warming treatment and nutrient amendments were applied in factorial combination. OTCs were used to apply the warming treatment, nutrients (glucose - Glu, glycine - Gly and TSB – Tryptone Soy Broth) were added at 2 mg C $g^{-1}$ dry wt soil, NS - no substrate.*

| | Treatment | | | | | | Treatment | | | | | | Treatment | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Plot** | **OTC** | **Glu** | **TSB** | **Gly** | **NS** | **Plot** | **OTC** | **Glu** | **TSB** | **Gly** | **NS** | **Plot** | **OTC** | **Glu** | **TSB** | **Gly** | **NS** |
| 1 | ✓ | | | ✓ | | 23 | ✓ | ✓ | | | | 44 | ✓ | ✓ | | | |
| 2 | | ✓ | | | | 24 | ✓ | ✓ | | | | 45 | | | | | |
| 3 | ✓ | | | ✓ | | 25 | | ✓ | | | | 46 | | | | ✓ | |
| 4 | ✓ | | | | | 26 | | ✓ | | | | 47 | | | | ✓ | |
| 5 | ✓ | | | | | 27 | ✓ | | ✓ | | | 48 | ✓ | | ✓ | | |
| 6 | | | | | | 28 | ✓ | | ✓ | | | 49 | | ✓ | | | |
| 7 | ✓ | | | | | 29 | ✓ | ✓ | | | | 50 | | | | | ✓ |
| 8 | | | | | ✓ | 30 | ✓ | | | ✓ | | 51 | | ✓ | | | |
| 9 | ✓ | | | | | 31 | ✓ | | | | | 52 | | | | ✓ | |
| 10 | | | ✓ | | | 32 | ✓ | | | ✓ | | 53 | ✓ | | ✓ | | |
| 11 | ✓ | | | ✓ | | 33 | ✓ | ✓ | | | | 54 | ✓ | | ✓ | | |
| 12 | | | ✓ | | | 34 | ✓ | ✓ | | | | 55 | ✓ | | | | |
| 13 | | | | ✓ | | 35 | ✓ | | | | | 56 | | ✓ | | | |
| 14 | ✓ | | | ✓ | | 36 | | | ✓ | | | 57 | | | | | ✓ |
| 15 | ✓ | ✓ | | | | 37 | | ✓ | | | | 58 | ✓ | | | ✓ | |
| 16 | | | ✓ | | | 38 | | | | | | 59 | ✓ | | ✓ | | |
| 17 | | | ✓ | | | 39 | ✓ | | | ✓ | | 60 | | | | ✓ | |
| 18 | | | | ✓ | | 40 | ✓ | | ✓ | | | 61 | ✓ | | | ✓ | |
| 19 | | ✓ | | | | 41 | ✓ | ✓ | | | | 62 | | | | ✓ | |
| 20 | | | | | ✓ | 42 | ✓ | | ✓ | | | 63 | | | | ✓ | |
| 21 | | | ✓ | | | 43 | ✓ | ✓ | | | | 64 | ✓ | | | | |
| 22 | ✓ | ✓ | | | | | | | | | | | | | | | |

*Table 9.2 The High Arctic soil warming experiment consists of 48 plots to which water and warming treatments were applied in factorial combination. OTCs were used to apply the warming treatment, and the watering treatment consisted of adding deionised water (1 L) to polygons twice each summer.*

| Block | Plot | OTC | Water | Block | Plot | OTC | Water | Block | Plot | OTC | Water |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | | | | 17 | | | | 33 | | |
| | 2 | ✓ | | | 18 | ✓ | | | 34 | ✓ | |
| | 3 | | ✓ | | 19 | | ✓ | | 35 | | ✓ |
| | 4 | ✓ | ✓ | | 20 | ✓ | ✓ | | 36 | ✓ | ✓ |
| | 5 | | | | 21 | | | | 37 | | |
| | 6 | ✓ | | | 22 | ✓ | | | 38 | ✓ | |
| | 7 | | ✓ | | 23 | | ✓ | | 39 | | ✓ |
| | 8 | ✓ | ✓ | | 24 | ✓ | ✓ | | 40 | ✓ | ✓ |
| 1 | 9 | | | 2 | 25 | | | 3 | 41 | | |
| | 10 | ✓ | | | 26 | ✓ | | | 42 | ✓ | |
| | 11 | | ✓ | | 27 | | ✓ | | 43 | | ✓ |
| | 12 | ✓ | ✓ | | 28 | ✓ | ✓ | | 44 | ✓ | ✓ |
| | 13 | | | | 29 | | | | 45 | | |
| | 14 | ✓ | | | 30 | ✓ | | | 46 | ✓ | |
| | 15 | | ✓ | | 31 | | ✓ | | 47 | | ✓ |
| | 16 | ✓ | ✓ | | 32 | ✓ | ✓ | | 48 | ✓ | ✓ |

## Appendix 2

Soil moisture curves for Antarctic and Arctic soils showing the relationships between soil water potential and soil moisture concentration.

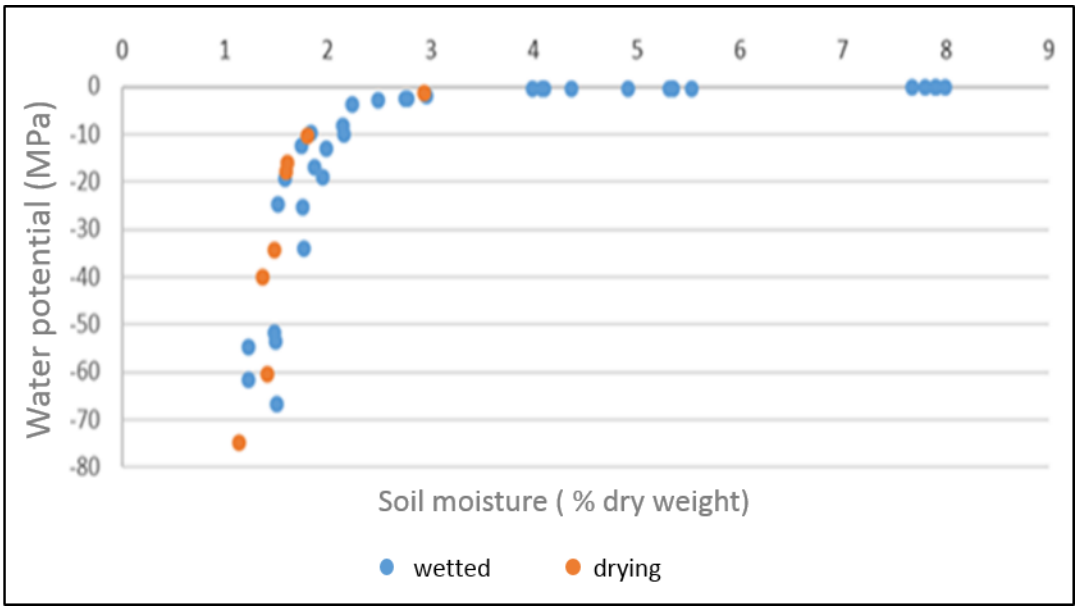


*Figure 9.1 Wetting (blue) and drying (orange) curves for soil at Mars Oasis.*

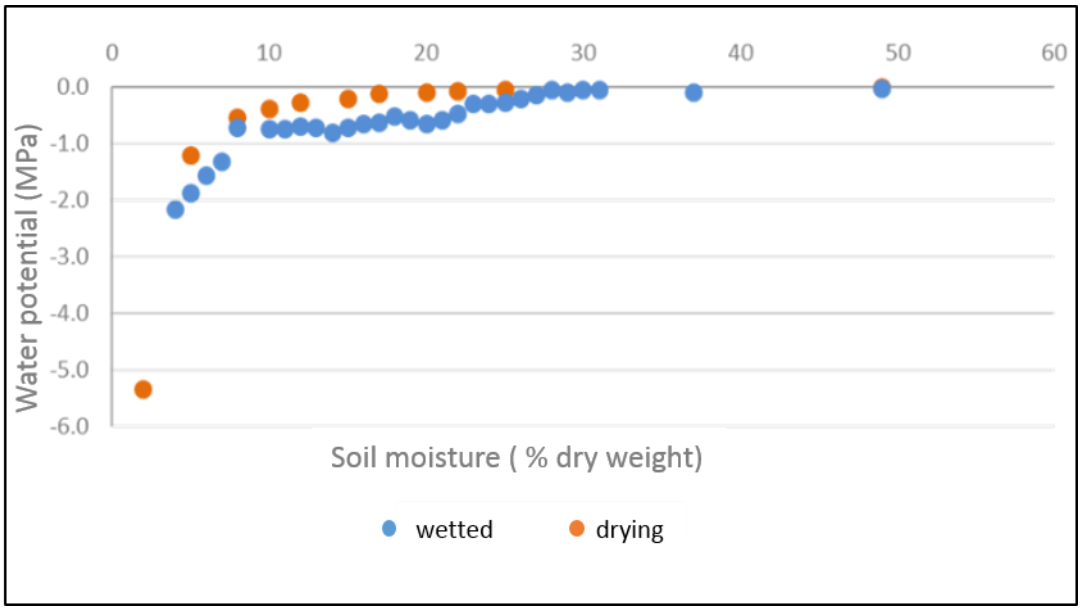


*Figure 9.2 Wetting (blue) and drying (orange) curves for the High Arctic soil. Water potential dropped to < - 100 MPa at 2% soil moisture content on the drying curve (data not shown).*

## Appendix 3

Modified Czapek-Dox agar media used for soil fungal isolation.

Polar soils are low in nutrients, hence modified Czapek-Dox medium was used for soil fungi isolation. The medium had a lower sucrose content. Rose Bengal was added to suppress bacterial extension.

2 g $L^{-1}$ of sucrose (Fisher Scientific, Loughborough, UK)

3 g $L^{-1}$ of $NaNO_3$ (Fisher Scientific, Loughborough, UK)

1 g $L^{-1}$ of $K_2HPO_4$ (SIGMA-ALDRICH, Steinheim, Germany)

0.5 g $L^{-1}$ of $MgSO_4$ (Fisher Scientific, Loughborough, UK)

0.5 g $L^{-1}$ of KCl (Fisher Scientific, Loughborough, UK)

0.01 g $L^{-1}$ of $FeCl_2$ (Fisher Scientific, Loughborough, UK)

0.5 g $L^{-1}$ of yeast extract (OXOID Ltd, Basingstoke, UK)

15 g $L^{-1}$ of bacteriological agar (OXOID Ltd, Basingstoke, UK)

5 mg $L^{-1}$ Rose Bengal (Fisher Scientific, Loughborough, UK)

## Appendix 4

Fungi used in this study were primiraly identified based on fungal cultures DNA extracts, these are listed in Table below. DGGE analysis resulted in identification of mostly either unculturable/unidentified or lichenized fungi, dominated by *Verrucaria* spp. and *Polyblastia* spp. Cloning analysis, similarly resulted in identifying mostly lichenized species, when identifiable, with the exception of *Tetracladium* sp. and *Acremonium* sp.

*Table 9.3 Sequences of fungi used in this study.*

| Region | Fungus identified | Sequence |
|---|---|---|
| Arctic | *Acremonium* sp. | GAGGTCACGTTCAGAAGTCGGGGGTTTTACGGCGTGGCCACGTCGGGGTTCCGGTGCGAGT TGGATTACTACGCAGAGGTCGCCGCGGACGGGCCGCCACTTCATTTCGGGGCCGGCGGTAT ACGGCCGGTCCCCAACGCCGATTTCCCCAAAGGGAAGTCGAGGGTTGAAATGACGCTCGAA CAGGCATGCCCGCCAGAATGCTGGCGGGCGCAATGTGCGTTCAAAGATTCGATGATTCACT GAATTCTGCAATTCACATTACTTATCGCATTTCGCTGCGTTCTTCATCGATGCCAGAACCAAG AGATCCGTTGTTGAAAGTTTTGATTCATTTGTTTTGCCTTGCGGCGGATTCAGAAGATACTGA GAATATAGAGTTTGGGGGTCTCCGGCGGCCGCCTGGATCCAGGCCGCGGCCGGCGCGGGG CCGGCCGGACGCTGGGGCGAGTCCGCCGAAGCAACGATAGGTATGTTCACAAAAGGGTTTG GGAGTTGAAAACTCGGTAATGATCCCTCCGCTGGTTCACCAACGGAGACCTTGTTACGACTT TTACTTCCTCTATNGGACCAAGA |
| Arctic | *Cadophora* sp. | ACCGTGCATCCTTACGGGGAGGCAGCACGACTGAAAATAACGCTGCCGCATGCAAGTCAGG GAACTGGCAACACGATCGAATTGCGGGGACCTCCTAAAGCTTCACATACTACCTACCCTTGA AAGGGGGTAGCACAGTAATAANTAGTGAAGATGCTACAATGGACAATCCGCAGCGAAGAC CCTACAGCGCGAGCCACGGGTAACGTTCACAGACTAAGTGATTGTGGGTAGCTTGCTACTTA AGATATAGTCGAGCCCTGTCCGAAAGGTTAGGGGTAAAGGTCCGTAGGTGAACCTGCGGAA GGATCATTACTAGAGCAAAGGACAGGCAGCGCCCCACAGAAGCTTGCTTCGTGGCGGGCTA CCCTACTCCGGTAGGGTTTAGAGTCGTCGAACCTCTCGGAGAAGTTCGGTCCTGAACTCCAC CCTTGAATAAATTACCTTTGTTGCTTTGGCGGGCCGCCTCGTGCCAGCGGCTTCGGCTGTTGA GTGCCCGCCAGAGGACCACAACTCTTGTTTTTAGTGATGTCTGAGTACTATATAATAGTTAAA ACTTTCAACAACGGATCTCTTGGTTCTGGCATCGATGAAGAACGCAGCGAAATGCGATAAGT AATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACATTGCGCCCTCTGGTAT TCCGGGGGGCATGCCTGTTCGAGCGTCATTATAACCACTCAAGCTCTCGCTTGGTATTGAGG TTCGCGGTTCCGCGGCCCCTAAAATCAGTGGCGGTGCCTGTCGGCTCTACGCGTAGTAATAC TCCTMNGCGATTGAGTCCGGCAGGTCTACTTGCCAGCAACC |
| Arctic | Dothideomycete | TCCGTAGGTGAACCTGCGGAAGGATCATTACCGTGGGGCTTCGGCCCCATTGAGATAGCAC CCTTTGTTTATGAGTACCCTTGTTTCCTCGGCGGGCTTGCCCGCCATGAGGACATCAAAAACC CTTTGTAGTAGCAGTAACTTCAGTTAATAATAAATATTAAAACTTTCAACAACGGATCTCTTG GTTCTGGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAGTGTGAATTGCAGAATTCAG TGAATCATCGAATCTTTGAACGCACATTGCGCCCTTCGGTATTCCGTTGGGCATGCCTGTTCG AGCGTCATTTAAACCTTCAAGCTATGCTTGGTGTTGGGTGTTTGTTCCGCCTTAGCGCGTGGA CTCGCCTCAAATTCATTGGCAGCCGGTATGTTGGCTTCGTGCGCAGCACATTGCAAGCGACT TCAGCAGACCCCCTTCCATTAAGCCCTTTTTTTACTTTGACCTCGGATCAGGTAGGGATACCC GCTGAACT- |
| Arctic | *Isaria farinosa* | TTCCTCCGCTTATTGATATGCTTAAGTTCAGCGGGTWTTCCTACCTGATCCGAGGTCAACGTT CAGAAGTCGGGGGTTTTACGGCGTGGCCACGTCGGGGTTCCGGTGCGAGTTGGATTACTAC GCAGAGGTCGCCGCGGACGGGCCGCCACTTCATTTCGGGGCCGGCGGTATACGGCCGGTCC CCAACGCCGATTTCCCCAAAGGGAAGTCGAGGGTTGAAATGACGCTCGAACAGGCATGCCC GCCAGAATGCTGGCGGGCGCAATGTGCGTTCAAAGATTCGATGATTCACTGAATTCTGCAAT TCACATTACTTATCGCATTTCGCTGCGTTCTTCATCGATGCCAGAACCAAGAGATCCGTTGTT GAAAGTTTTGATTCATTTGTTTTGCCTTGCGGCGGATTCAGAAGATACTGAGAATATAGAGT TTGGGGGTCTCCGGCGGCCGCCTGGATCCAGGCCGCGGCCGGCGCGGGGCCGGCCGGACG CTGGGGCGAGTCCGCCGAAGCAACGATAGGTATGTTCACAAAAGGGTTTGGGAGTTGAAAA CTCGGTAATGATCCCTCCGCTGGTTCACCAACGGAGACCTTGTTACGACTTTTACTTCCTCTA AATTGACCAAGA |
| Arctic | *Leptosphaeria* sp. | TCTTGGTCATTTAGAGGAAGTAAAAGTCGTAACAAGGTTTCCGTAGGTGAACCTGCGGAAG GATCATTAAACATCATCGGGGGGGTTGGATCCAGAATGTAGGGCTTCGGCCTTGCTTTCTGCC CTTCCCTTACTGATTATACCCATGTCTTTTGCGTACTATTTGTTTCCTTGGTAGGCTTGCCTGCC GATAGGACATCATTAAACCTTTTGTAATTGCAGTCAGCGTCAGAAAAACATAATAATTACAA CTTTTAACAACGGATCTCTTGGTTCTGGCATCGATGAAGAACGCAGCGAAATGCGATAAGTA GTGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACATTGCGCCCCTTGGTATT CCATGGGGCATGCCTGTTCGAGCGTCATTTGTACCCTCAAGCTCTGCTTGGTGTTGGGTGTTT GTCCCGCTTTTCGCGTGGACTCGCCTTAAAACAATTGGCAGCCGGCATATTGGCCTGGAGCG CAGCACATTTTGCGCCTCTTGTCATGATTGTTGGCATCCATCAAGACTATTTTTTGCTCTTGAC |

| | | |
|---|---|---|
| | | CTCGGATCNAGGTAGGGATACCCGCTGAACTTAAGCATATCAATAAGCGGAG- |
| Arctic | *Mortierella* sp. | TATGCTTAAGTTCAGCGGGTAGTCTTACTTGATTTGAGATCGAGTTTTACAAGAATCGTTGCC<br>GACCCTTGTGAAATCCTGCATCAGTCAGCCAATACGGTCAAACTCCCTTTATGTTAGCTGCAG<br>CAAAAGTAATAATCCGTTTGACGGGACTAAATAAATATGCTTTTAGCTCAGGAGAATGTCCA<br>GCTGCACCTGCATTTCAAGCAACCCTCCACCGATCGTAAAGACTGGTGTTGGGATTGCTCAA<br>GTCCAAAGCCATTCAAAACAAATTGAAGTCTTTGAGGTGTTTACTGATACTCAAACAAGCAT<br>GCTCTTCGGAATACCAAAGAGCGCAATATGCGTTCAAAGATTCGATGATTCACTGAATTCTG<br>CAATTCACATTACGTATCGCATTTCGCTGCGTTCTTCATCGATGCGAGAGCCAAGAGATCCGT<br>TGTTGAAAGTTGTATTTTGAATTATTTTATTCATAATATTTTTCAGACAAAGAGTTAAAGAATA<br>AGTTGATGTTTGGTCGCTCTCAAAAAAGAGAACGACTGACATTGCACACAAGGTGGATATG<br>GATTTAAAAAGTGCCATAAAACACTTATTAT |
| Arctic | *M. alpina* | ACCTGCGGAAGGNTCATTCATAATCAAAGTGTTTTTATGGCACTTTTAAAAAAATCCATATCC<br>ACCTTGTGTGCAATGTCATCTCACTGGAGGCCAGCGGCTGTAAAAAGCCCGTTTGGTCGCCT<br>TTGGGATTTATATCTACTCAGAACTTTAGTGATTTTGTCTGAAAAATATTATGAATAACTTAAT<br>TCAAAATACAACTTTCAACAACGGATCTCTTGGCTCTCGCATCGATGAAGAACGCAGCGAAA<br>TGCGATACGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCATATTGCG<br>CTCTCTGGTATTCCGGAGAGCATGCTTGTTTGAGTATCAGTAAACACCTCAACTCCCTTTTTCT<br>TTTTTGAAATGAGGGAGCTGGACTTGAGTGATCCCAACACTTTTCTCACTGAAAAGTGGCGG<br>GTCACTTGAAATGCAGGTGCAGCTGGACTTTTCTCTGAGCTATAAGCATATCTATTTAGTCTG<br>CCTAAAAAACAGATTATTACCTTTGCTGCAGCTAACATAAAGGAGATGAGTTCTTGTGCTGA<br>CTGATGCAGGATTCACAGAGACAGCTTTACGGCTGACTTTGTAAACTCGATCTCAAATCAAG<br>TAAGACTACCCGCTGAACTTAAGCATATC |
| Arctic | *M. polygonia* | AAGTGTTTTTATGGCACTTTAAATCCATATCCACCTTGTGTGCAATGTCATCTCACTGGGGTC<br>NCGTTTGTAAAATCTCGTGATCCGTTTGGGATTTATATCTACTCAGAACTTTAGTCATTTTGTC<br>TGAAACAATAACTTAATTCAAAATACAACTTTCAACAACGGATCTCTTGGCTCTCGCATCGAT<br>GAAGAACGCAGCGAAATGCGATACGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATC<br>TTTGAACGCATATTGCGCTCTCTGGTATTCCGGAGAGCATGCTTGTTTGAGTATCANTAAACA<br>CCTCAACTCNNTTTCTTCTTGAAATGGAGCTGGACTTGAGTGATCCCAACGCTTTATTCATAA<br>AGTGGCGGGTTACTTGAAATGCANGTGCAGCTGGACTTTTCTCTGAGCTATAAGCATATCTA<br>TTTAGTCTGCCTCAAAACANATTATTACCTTTGCTGCANCTAACATAAAGGAGATTAGTTCTT<br>GTGCTGACTGATGCANGATTCACAAA |
| Arctic | *M. verticilliata* | AAGTTCAGCGGGTAGTCTTACTTGATTTGAGATCGAGTTGAACAACACATAAAGTGTCGTAA<br>AATCCTGCATCAGTCAGCAAAGAGGACAATTATCCTTTATGTTAGCTGCAGCAAAAGTAATA<br>ATCCTGTTTGACGAGGACTAAATAGATATGCTTTTAGCTCAGATAAAAGTCCAGCTGCACCT<br>GCATTTCAAGCCGCCCGCCGACCGGTAAAGGTTGTTGGGATAGCTCAAGTCCACCCTCCTTT<br>TGAAAAATCAAGAAGAGGTTGAGGTGTTTACTGATACTCAAACAAGCATGCTCTTCGGAATN<br>CCAAAGAGCGCAATATGCGTTCAAAGATTCGATGATTCACTGAATTCTGCAATTCACATTACG<br>TATCGCATTTCGCTGCGTTCTTCATCGATGCGAGAGCCAAGAGATCCGTTGTTGAAAGTTATA<br>TTTTGAATTAAGTATATTCATAATATGTTATCAGACGAATGTTGTTAAAGATATAGGTTGATA<br>TTTATGAAGGGAAAGAAAAGACTTTCCCAACAAACATTGCACACAAGGTGGATATGGATTTT<br>TAAAGAGTGCCATAAAACACTCGTTTGTG |
| Arctic | *Phialocephala lagerberghii* | AGTTCAGCGGGTATCCCTACCTGATCCGAGGTCAACCTGTAAAAATTTGGGGGTTGTTGGCA<br>AGCAACCTACCGGACCCAGACGCGAGGAGTATTACTACGCGTAGAGCCGACAGGCACCGCC<br>ACTGACTTTAGAGGCCGCGGAACCGCGCACCCCAATACCAAGCGAGAGCTTGAGTGGTTAT<br>AATGACGCTCGAACAGGCATGCCCTTCGGAATACCAAAGGGCGCAATGTGCGTTCAAAGAT<br>TCGATGATTCACTGAATTCTGCAATTCACATTACTTATCGCATTTCGCTGCGTTCTTCATCGAT<br>GCCAGAACCAAGAGATCCGTTGTTGAAAGTTTTAACTATTATATAGTACTCAGACATCACTAA<br>AAACAAGAGTTGTGGTCCTCTGGCGGGCGCGCAGCAGCCGAAGCCGCTGGCCGAGACGGC<br>GGCCCGCCAAAGCAACAAGGGTAGTTTATTCAAGGGTTACCTAGGTATTGCTGACATCACGC<br>GAGGGTTGCCCCTATGTACGCGAACGACTAGGTTTATTAATGATCCTTCCGCAGGTTCACCT<br>ACGGAAACCTTGTTACGACTT |
| Arctic | *Pseudogymnoascus pannorum* | CAAGGTTTCNGTAGGTGAMCNTGCGGAAGGATCATTACAGTAGTCATCCGGGATGCCGCA<br>AGGCCTCCCGGGTAACCTACCACCCTTTGTTTATTACACTTTGTTGCTTTGGCAGGCCTGCCCT<br>CGGGCTGCTGGCTCCGGCCGGCGAGCGCTTGCCAGAGGACCTAAACTCTGTTTGTCTATACT<br>GTCTGAGTACTATATAATAGTTAAAACTTTCAACAACGGATCTCTTGGTTCTGGCATCGATGA<br>AGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTT<br>GAACGCACATTGCGCCCCCTGGTATTCCGGGGGGCATGCCTGTCCGAGCGTCATTACAACCC<br>TCAAGCTCAGCTTGGTGTTGGGCCCCGCCGCCCCGGCGGGCCCTAAAGTCAGTGGCGGTGC<br>CGTCCGGCTCCGAGCGTAGTAATTCTTCTCGCTCTGGAGGTCCGGTCGTGTGCTTGCCATCA<br>ACCCCCAATTTTTTTCAGGTTGACCTCGGATCAGGTARGGATACCCGC |
| Arctic | *Pseudogymnoascus* sp. | AACAAGGTTTCCGTAGGTGAACCTGCGGAAGGATCATTACAGTAGTCGCCCGGGTTGCCGC<br>AAGGCCTCCCGGGTAACCTACCACCCTTTGTTTATTACACTTTGTTGCTTTGGCAAGCCTGCC<br>CTCGGGCTGCTGGCTCCGGCCGGCGAGCGCTTGCCAGAGGACCTAAACTCTGTTTGTCTATA<br>CTGTCTGAGTACTATATAATAGTTAAAACTTTCAACAACGGATCTCTTGGTTCTGGCATCGAT<br>GAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATC<br>TTTGAACGCACATTGCGCCCCCTGGTATTCCGGGGGGCATGCCTGTCCGAGCGTCATTACAA<br>CCCTCAAGCTCAGCTTGGTGTTGGGCCCCGCCGCCCCGGCGGGCCCTAAAGTCAGTGGCGG<br>TGCCGTCCGGCTCCGAGCGTAGTAATTCTTCTCGCTCTGGAGGTCCGGTCGTGTGCTCGCCA<br>GCAACCCCCAATTTTTTTCAGGTTGACCTCGGATCAGGTAGGGATACCCGCTGA |
| Arctic | *Pseudogymnoascus* sp. | CTTGGTCATTTAGAGGAAGTAAAAGTCGTWWCAAGGTTTCCGTAGGTGAACCTGCGGAAG<br>GATCATTAAAGTTATAGTCCCCTGGCCTTCTGGCTGGGGTCTTCTATACCACCCTTTGTTTATT<br>ACACCTTTGTTGCTTTGGCAGGCCTGCCCTCGGGCTGCTGGCTCCGGCCGGCGAGCGCCTGC<br>CAGAGGACCTAAACTCTGTTTGTCTATAATGTCTGAGTACTATATAATAGTTAAAACTTTCAA<br>CAACGGATCTCTTGGTTCTGGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGA<br>ATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACATTGCGCTCCCTGGTATTCCGGG<br>GAGCATGCCTGTTCGAGCGTCATTACAACCCTCAAGCTCTGCTTGGTGTTGGGCCCCGCCGC<br>CTCGGCGGGCCCTAAAGTCAGTGGCGGTGCCGTCCGGCTCCGAGCGTAGTAATTTTTCTCGC<br>TTTGGAGGCCCGGGCGCGTGCTCGCCATCAACCCCCAATTTTTTCAGGTTGACCTCGGATCA<br>GNTAGGGATACCCGCTGAACTTAAGCATATC |

| | | |
|---|---|---|
| Antarctica | *P. pannorum 1* | CAGTAGTCGCCCGGGTTGCCGCAAGGCCTCCCGGGTANNNTACCACCCTTTGTTTATTACAC<br>TTTGTTGCTTTGGCAAGCCTGCCCTCGGGCTGCTGGCTCCGGCCGGCGAGCGCTTGCCAGAG<br>GACCTAAACTCTGTTTGTCTATACTGTCTGAGTACTATATAATAGTTAAAACTTTCAACAACG<br>GATCTCTTGGTTCTGGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCA<br>GAATTCAGTGAATCATCGAATCTTTGAACGCACATTGCGCCCCCTGGTATTCCGGGGGGCAT<br>GCCTGTCCGAGCGTCATTACAACCCTCAAGCTCAGCTTGGTGTTGGGCCCCGCCGCCCCGGC<br>GGGCCCTAAAGTCAGTGGCGGTGCCGTCCGGCTCCGAGCGTAGTAATTCTTCTCGCTCTGGA<br>GGTCCGGTCGTGTGCTCGCCAGCAACCCCCAATTTTTTTCAGGTTGACCTCGGATCAGGTAG<br>GGATACCCGCTGAACTTAAGCATATC |
| Antarctica | *P. pannorum 2* | CAGTAGTCGCCCGGGTTGCCGCAAGGCCTCCCGGGTAACCTACCACCCTTTGTTTATTACACT<br>TTGTTGCTTTGGCAAGCCTGCCCTCGGGCTGCTGGCTCCGGCCGGCGAGCGCTTGCCAGAG<br>GACCTAAACTCTGTTTGTCTATACTGTCTGAGTACTATATAATAGTTAAAACTTTCAACAACG<br>GATCTCTTGGTTCTGGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCA<br>GAATTCAGTGAATCATCGAATCTTTGAACGCACATTGCGCCCCCTGGTATTCCGGGGGGCAT<br>GCCTGTCCGAGCGTCATTACAACCCTCAAGCTCAGCTTGGTGTTGGGCCCCGCCGCCCCGGC<br>GGGCCCTAAAGTCAGTGGCGGTGCCGTCCGGCTCCGAGCGTAGTAATTCTTCTCNCTCNGG<br>AGGTCCGGTCGTGTGCTCGCCAGCAACCCCCAATTTTT |
| Antarctica | *P. pannorum 3* | TAGTCGCGCGGGTTGCCGCAAGGCCTCCCGGGTAACCTACCACCCTTTGTTTATTACACTTTG<br>TTGCTTTGGCAAGCCTGCCCTCGGGCTGCTGGCTCCGGCCGGCGAGCGCTTGCCAGAGGAC<br>CTAAACTCTGTTTGTCTATACTGTCTGAGTACTATATAATAGTTAAAACTTTCAACAACGGATC<br>TCTTGGTTCTGGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAA<br>TTCAGTGAATCATCGAATCTTTGAACGCACATTGCGCCCCCTGGTATTCCGGGGGGCATGCC<br>TGTCCGAGCGTCATTACAACCCTCAAGCTCAGCTTGGTGTTGGGCCCCGCCGCCCCGGCGGG<br>CCCTAAAGTCAGTGGCGGTGCCGTCCGGCTCCGAGCGTAGTAATTCTTCTCCCTCNGGAGGT<br>CCGGTCGTGTGCTCGCCAGCAACCCCCAAAATTT |

- *Identification based on sequence comparison against nucleotide NCBI BLAST*
- *Fungal DNA identification based on ITS1 and ITS4 primer*

## Appendix 5

Exemplar melting curve, generated for genomic DNA (gDNA) of *Pseudogymnoascus* spp. in soil samples from Mars Oasis, analysed by Q-PCR.

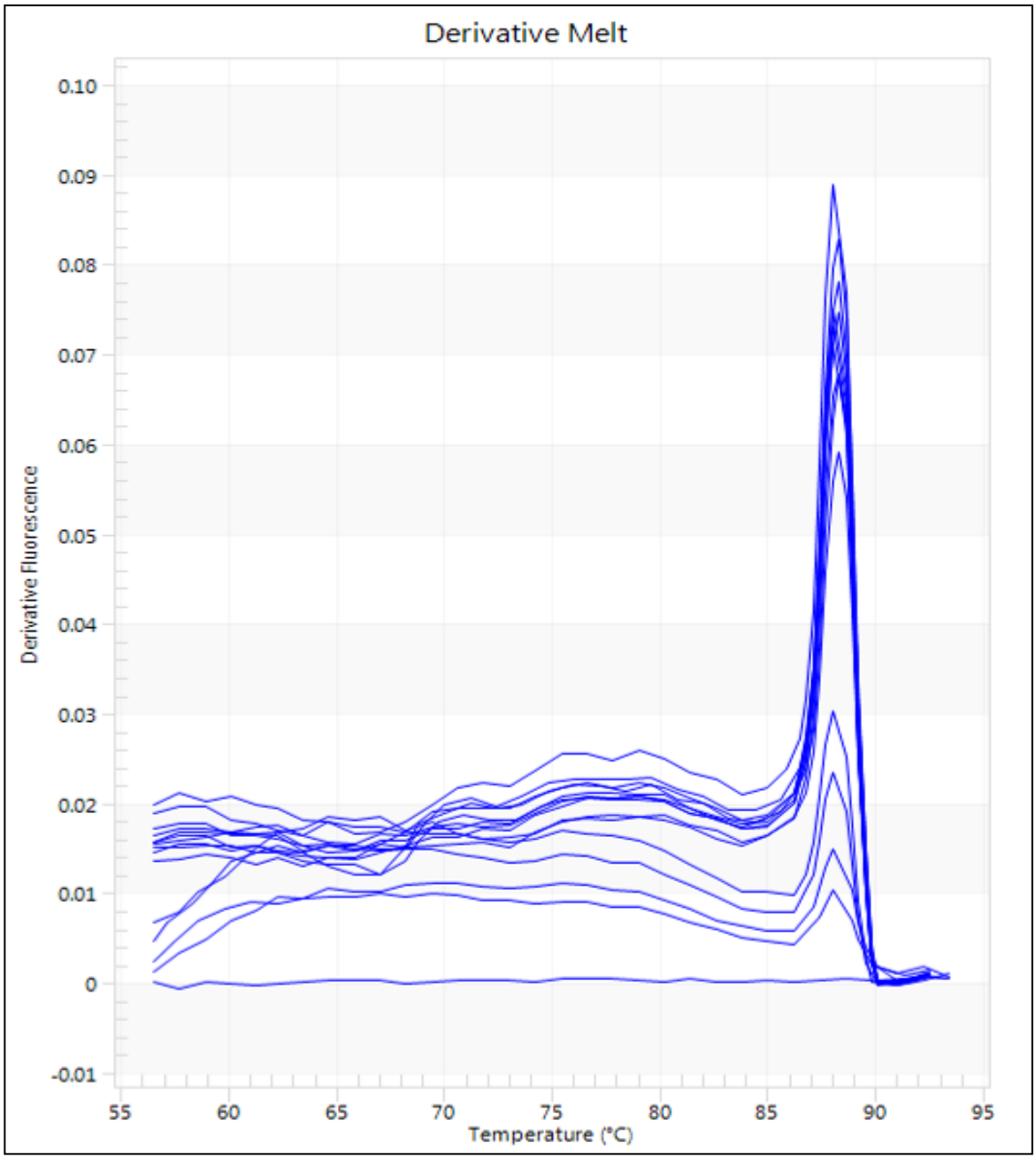


*Figure 9.3 An example of melt curves produced with SYBR green fluorescent dye used for analysis of specific PCR amplicons. Change in fluorescence is plotted against temperature to determine the point at which denaturation of PCR amplicons occurs. A single peak indicates that a single PCR product was amplified during the reaction.*

## Appendix 6

Exemplar extension rate curve

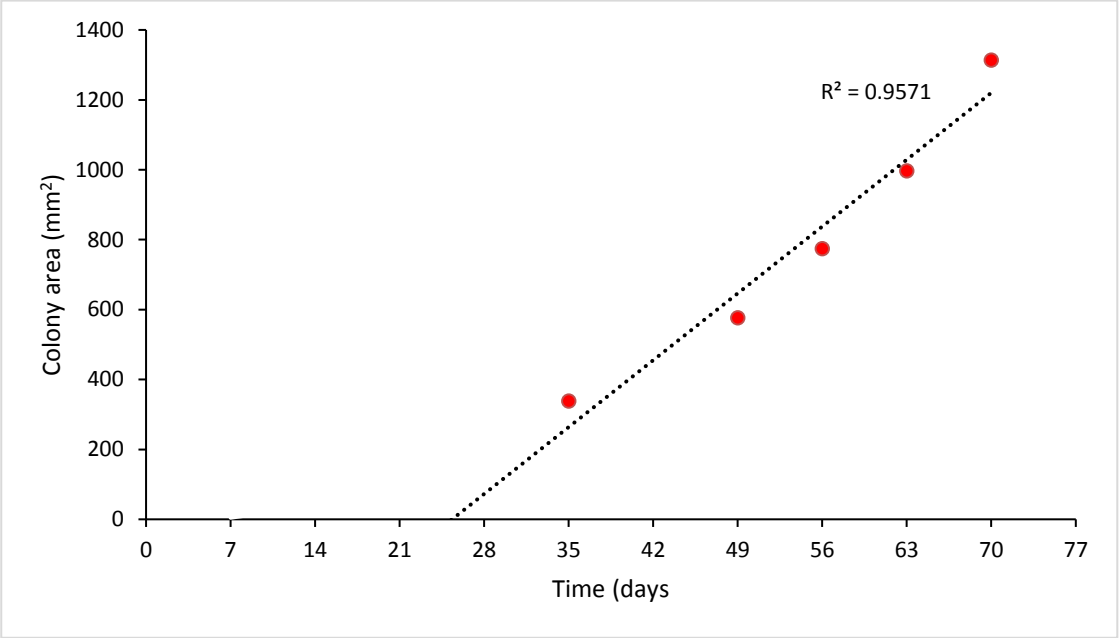


*Figure 9.4 Exemplar extension rate curve of an Arctic fungus used in the laboratory experiments. The dotted line represents the linear trendline ($r^2 \geq 95$ %) association between extension rate and time used to calculate hyphal extension rate ($mm^2\ d^{-1}$). Exponential extension phase of the colony was used for the extension rate calculation (red mark points).*

## Appendix 7

List of enzymes and their substrates in API ZYM kit strips. The test is a fast, semi- quantitative assay that allows identification of 19 enzymes commonly produced by micro-organisms.

*Table 9.4 List of enzymes and their substrates present in API ZYM strips.*

| Test | Enzyme | Substrate |
|---|---|---|
| 1 | control | NA |
| 2 | alkaline phosphatase | 2-napthyl phosphate |
| 3 | esterase | 2- napthyl butyrate |
| 4 | esterase lipase | 2-napthyl caprylate |
| 5 | lipase | 2-napthyl myristate |
| 6 | leucine arylamidase | L-leucyl-2-naphtylamide |
| 7 | valine arylamidase | L-valyl-2napthylamide |
| 8 | cysteine arylamidase | L-cystyl-2-napthylamide |
| 9 | trypsin | N-benzoyl-DL-arginine-2-naphtylamide |
| 10 | α-chymotrypsin | N-glutaryl-phenylalanine-2-napthylamide |
| 11 | acid phosphatase | 2-napthyl phosphate |
| 12 | napthol-AS-BI-phosphohydrolase | Napthol-AS-BI-phosphate |
| 13 | α-galactosidase | 6-Br-2-napthyl-αD-galactopyranoside |
| 14 | β-galactosidase | 2-naphtyl-βD-galactopyranoside |
| 15 | β-glucuronidase | Napthol-AS-BI-βD-glucoronide |
| 16 | α-glucosidase | 2-napthyl-αD-glucopyranoside |
| 17 | β-glucosidase | 6-Br-2-napthyl-βD-glucopyranoside |
| 18 | N-acetyl-β-glucosaminidase | 1-napthyl-N-acetyl-βD-glucosaminide |
| 19 | α-mannosidase | 6-Br-2-napthyl-αD-mannopyranoside |
| 20 | α-fucosidase | 2-napthyl-αL-fucopyranoside |

## Appendix 8

Exemplar API ZYM kit test strip results.

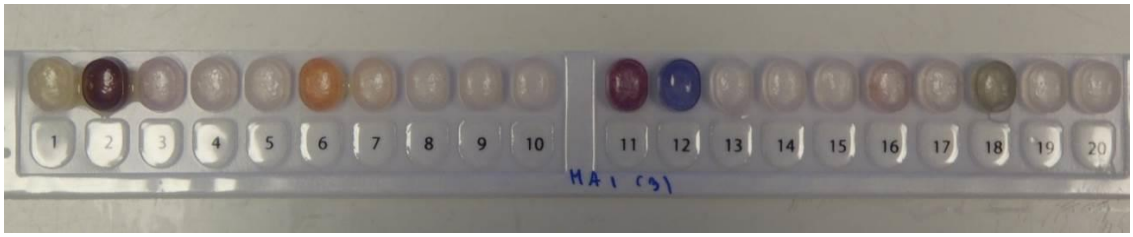


*Figure 9.5 API ZYM kit test results for* Mortierella alpina*. A change in colour relative to the control (well 1) indicates the presence of the enzymes shown in Appendix 7.*

## Appendix 9

Effect of warming with open top chambers (OTCs) and nutrients application on the abundance of *Pseudogymnoascus* spp. gDNA (Table 9.5) and total fungal gDNA (Table 9.6) in Antarctic soil.

*Table 9.5 One way ANOVA analyses outputs showing the main effects of glucose, glycine and TSB application and warming with open top chambers (OTCs) on the abundance of* Pseudogymnoascus *spp. genomic DNA (expressed as ng genomic DNA $g^{-1}$ dwt soil) over four years. Data were log(*n*+1) transformed prior to analyses. Significant* P *values* (P < *0.05*) *are marked in bold*, $F_{\text{degrees of freedom}}$.

| | | Treatment | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | CONTROL | | | | OTC | | | |
| **Statistic** | **Year** | **no substrate** | **Glucose** | **Glycine** | **TSB** | **no substrate** | **Glucose** | **Glycine** | **TSB** |
| mean | | 0.00012 | 0.00467 | 4.12000 | 0.50300 | 0.00010 | 0.00026 | 0.01970 | 0.00315 |
| $F_{1, 16}$ | 2009 | | 5.42 | 7.97 | 8.93 | 19.36 | 0.50 | 15.60 | 10.88 |
| *P* | | | **0.024** | **0.007** | **0.004** | **<0.001** | 0.483 | **<0.001** | **0.002** |
| Mean | | 0.00011 | 0.00070 | 0.66300 | 0.41100 | 0.16600 | 0.00026 | 0.31600 | 2.54000 |
| $F_{1, 16}$ | 2010 | | 25.31 | 68.44 | 133.32 | 0.15 | 2.97 | 0.66 | 0.65 |
| *P* | | | **<0.001** | **<0.001** | **<0.001** | 0.701 | 0.09 | 0.42 | 0.423 |
| Mean | | 0.55500 | 0.64100 | 19.90000 | 6.07000 | 0.28900 | 0.00672 | 0.95600 | 0.93800 |
| $F_{1, 16}$ | 2011 | | 0.34 | 57.71 | 1.69 | 1.03 | 1.28 | 4.83 | 0.44 |
| *P* | | | 3.88 | **<0.001** | 0.199 | 0.315 | 0.263 | **0.032** | 0.512 |
| mean | | 0.00024 | 0.00018 | 5.48000 | 0.51500 | 0.00048 | 0.00013 | 3.63000 | 7.93000 |
| $F_{1, 16}$ | 2012 | | 0.10 | 115.47 | 74.56 | 1.29 | 0.01 | 3.58 | 0.38 |
| *P* | | | 0.749 | **<0.001** | **<0.001** | 0.260 | 0.914 | 0.064 | 0.538 |

*Table 9.6. One way ANOVA analyses outputs showing the main and interactive effects of glucose, glycine and tryptic soy broth (TSB) application and warming with open top chamber (OTCs) on the concentration of total genomic fungal DNA (ng of genomic DNA $g^{-1}$ dwt soil) over four years. Data were log (n+1) transformed prior to analyses. Significant P values* (P<0.05) *are marked in bold,* $F_{degrees\ of\ freedom}$.

| | | Treatment | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | CONTROL | | | | OTC | | | |
| **Statistic** | **Year** | **no substrate** | **Glucose** | **Glycine** | **TSB** | **no substrate** | **Glucose** | **Glycine** | **TSB** |
| mean | | 1.369 | 1.554 | 224.400 | 196.500 | 2.960 | 14.710 | 1.147 | 1.198 |
| $F_{1, 16}$ | 2009 | | 10.86 | 7.97 | 8.93 | 19.36 | 0.50 | 15.60 | 10.88 |
| *P* | | | 0.188 | **0.007** | **0.004** | **<0.001** | 0.483 | **<0.001** | **0.002** |
| mean | | 0.294 | 0.646 | 42.800 | 418 | 0.002 | 39.700 | 11.130 | 473.000 |
| $F_{1, 16}$ | 2010 | | 25.31 | 68.44 | 133.32 | 0.15 | 2.97 | 4.19 | 0.65 |
| *P* | | | **<0.001** | **<0.001** | **<0.001** | 0.701 | 0.09 | **0.045** | 0.423 |
| mean | | 3.090 | 42.370 | 434.000 | 859.000 | 2.720 | 175.900 | 172.400 | 488.000 |
| $F_{1, 16}$ | 2011 | | 56.51 | 57.71 | 142.34 | 1.03 | 1.28 | 4.31 | 0.14 |
| *P* | | | **<0.001** | **<0.001** | **<0.001** | 0.315 | 0.263 | 0.057 | 0.709 |
| mean | | 1.946 | 129.200 | 397.000 | 750.000 | 0.487 | 164.700 | 314.000 | 765.000 |
| $F_{1, 16}$ | 2012 | | 100.71 | 100.68 | 171.88 | 1.29 | 0.67 | 6.63 | 0.18 |
| *P* | | | **<0.001** | **<0.001** | **<0.001** | 0.26 | 0.415 | **0.020** | 0.676 |

## Appendix 10

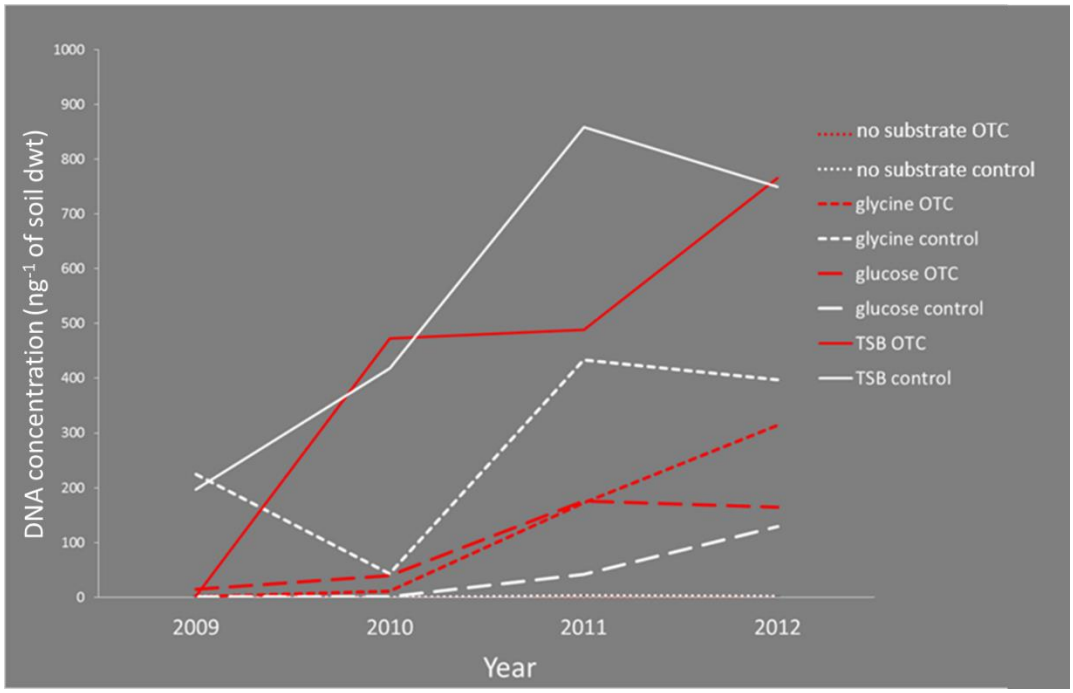


*Figure 9.6 The combined effects of warming with open top chambers (OTCs, red) and glycine, glucose or TSB amendment on the concentration of total genomic fungal DNA in soil at Mars Oasis. Values are means of eight measurements.*

*Table 9.7 Outputs of GLMs (three way ANOVA) analysis of the main effects and interactive effects of temperature and matric water potential on the enzyme activities on chitinase, cellulase, leucine aminopeptidase and acid and alkaline phosphatase of three P. pannorum isolates. Bold - treatments that were statistically significant ($P < 0.05$); $log_{10}$ (n+1) data were used for analyses; $F_{2, 47}$ for temperature, $F_{3,47}$ for water and $F_{6,47}$ for water x temperature effect.*

| | | | Isolate 1 | | | | | Isolate 2 | | | | | Isolate 3 | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Enzyme activity | Factor | Statistics | N | C | L A | Ac P | Al P | N | C | L A | Ac P | Al P | N | C | L A | Ac P | Al P |
| EAC | $\Psi_m$ | *F value* | **403.69** | **850.45** | **488.33** | **37.85** | **168.28** | **194.2** | **563.5** | **432.48** | **26.19** | **11.58** | **153.03** | **1632.69** | **363.22** | **18.05** | **115.73** |
| | | *P value* | **<0.001** | **<0.001** | **<0.001** | **<0.001** | **<0.001** | **<0.001** | **<0.001** | **<0.001** | **<0.001** | **<0.001** | **<0.001** | **<0.001** | **<0.001** | **<0.001** | **<0.001** |
| | T | *F value* | **69.52** | **21.74** | **51.84** | **53.49** | **161.63** | **37.62** | 3.19 | **57.02** | **21.94** | **87.07** | **74.36** | **31.16** | **52.78** | 2.32 | **27.48** |
| | | *P value* | **<0.001** | **<0.001** | **<0.001** | **<0.001** | **<0.001** | **<0.001** | 0.053 | **<0.001** | **<0.001** | **<0.001** | **<0.001** | **<0.001** | **<0.001** | 0.113 | **<0.001** |
| | $\Psi_m$ x T | *F value* | **16.41** | **9.76** | **23.23** | **9.14** | **31.2** | **7.63** | **5.6** | **9.08** | **9.48** | **24.06** | **6.94** | **16.49** | **22.33** | 1.88 | **11.86** |
| | | *P value* | **<0.001** | **<0.001** | **<0.001** | **<0.001** | **<0.001** | **<0.001** | **<0.001** | **<0.001** | **<0.001** | **<0.001** | **<0.001** | **<0.001** | **<0.001** | 0.111 | **<0.001** |
| SEAC | $\Psi_m$ | *F value* | **245.12** | **382.7** | **123.67** | **461.99** | **44.31** | **49.49** | **160.19** | **73.2** | **591.28** | **26.23** | **69.16** | **2755.95** | **232.48** | **435.64** | **23.51** |
| | | *P value* | **<0.001** | **<0.001** | **<0.001** | **<0.001** | **<0.001** | **<0.001** | **<0.001** | **<0.001** | **<0.001** | **<0.001** | **<0.001** | **<0.001** | **<0.001** | **<0.001** | **<0.001** |
| | T | *F value* | **57.75** | **17.64** | **108.23** | **43.35** | **67.96** | **5.84** | 2.21 | **62.04** | **63.68** | **26.69** | **41.58** | **83.52** | **66.49** | **49.87** | **3.93** |
| | | *P value* | **<0.001** | **<0.001** | **<0.001** | **<0.001** | **<0.001** | **0.006** | 0.124 | **<0.001** | **<0.001** | **<0.001** | **<0.001** | **<0.001** | **<0.001** | **<0.001** | **0.029** |
| | $\Psi_m$ x T | *F value* | **42.92** | **30.56** | **23.83** | **19.55** | **11.46** | **3.9** | **4.46** | **12.22** | **10.76** | **10.07** | **6.67** | **13.99** | **26.88** | **22.04** | **2.55** |
| | | *P value* | **<0.001** | **<0.001** | **<0.001** | **<0.001** | **<0.001** | **0.004** | **0.002** | **<0.001** | **<0.001** | **<0.001** | **<0.001** | **<0.001** | **<0.001** | **<0.001** | **0.037** |
| EA | $\Psi_m$ | *F value* | **7.4** | **26.68** | **79.28** | **20.13** | **32.79** | **5.37** | **65.96** | **155.17** | **15.98** | **14.59** | **27.76** | **319.25** | **97.39** | **4.75** | **15.1** |
| | | *P value* | **<0.001** | **<0.001** | **<0.001** | **<0.001** | **<0.001** | **0.004** | **<0.001** | **<0.001** | **<0.001** | **<0.001** | **<0.001** | **<0.001** | **<0.001** | **0.007** | **<0.001** |
| | T | *F value* | **139.73** | **66.03** | **74.28** | **41.91** | **75.71** | **36.73** | **23.14** | **146.63** | **26.65** | **24.6** | **182.72** | **27.14** | **100.92** | 3.02 | **20.08** |
| | | *P value* | **<0.001** | **<0.001** | **<0.001** | **<0.001** | **<0.001** | **<0.001** | **<0.001** | **<0.001** | **<0.001** | **<0.001** | **<0.001** | **<0.001** | **<0.001** | 0.061 | **<0.001** |
| | $\Psi_m$ x T | *F value* | **14.82** | **5.85** | **26.24** | **8.54** | **20.15** | 1.86 | **7.61** | **21.78** | **8.23** | **5.36** | **14.54** | **13.24** | **22.17** | 1.94 | **6.64** |
| | | *P value* | **<0.001** | **<0.001** | **<0.001** | **<0.001** | **<0.001** | 0.114 | **<0.001** | **<0.001** | **<0.001** | **<0.001** | **<0.001** | **<0.001** | **<0.001** | 0.102 | **<0.001** |
| SEA | v | *F value* | **52.95** | **46.47** | **103.09** | **84.56** | **26.57** | **12.81** | **31.22** | **672.68** | **20.08** | **17.3** | 162.96 | **940.03** | **322.23** | **10.63** | **30.95** |
| | | *P value* | **<0.001** | **<0.001** | **<0.001** | **<0.001** | **<0.001** | **<0.001** | **<0.001** | **<0.001** | **<0.001** | **<0.001** | <0.001 | **<0.001** | **<0.001** | **<0.001** | **<0.001** |
| | T | *F value* | **197.95** | **88.29** | **19.83** | **144.73** | **53.22** | **16.55** | **4.82** | **141.97** | **40.73** | **32.08** | **161.17** | **4.01** | **97.96** | **5.12** | **43.63** |
| | | *P value* | **<0.001** | **<0.001** | **<0.001** | **<0.001** | **<0.001** | **<0.001** | **0.014** | **<0.001** | **<0.001** | **<0.001** | **<0.001** | **0.027** | **<0.001** | **0.011** | **<0.001** |
| | $\Psi_m$ x T | *F value* | **51.74** | **31.74** | **8.52** | **23.05** | **15.38** | 1.13 | **4.98** | **28.07** | **10.34** | **5.43** | **18.31** | **7.74** | **18.75** | **3.02** | **11.93** |
| | | *P value* | **<0.001** | **<0.001** | **<0.001** | **<0.001** | **<0.001** | 0.367 | **0.001** | **<0.001** | **<0.001** | **<0.001** | **<0.001** | **<0.001** | **<0.001** | **0.018** | **<0.001** |

*Abbreviations: T, temperature, $\Psi_m$, matric water potentials, EAC, enzyme activity per colony, SEAC, specific enzyme activity per colony, EA, enzyme activity per biomass, SEA, specific enzyme activities per biomass, N, chitinase, C, cellulase, LA, leucine aminopeptidase, AcP, acid phosphatase, AlP, alkaline phosphatase*

## Appendix 12

*Table 9.8 Outputs from GLMs (three way Anova) testing for the effects and interactive effects of OTC, water application and block on soil properties at the Arctic field experiment. Values in bold indicate statistically significant effects ($P < 0.05$). $F_{1\text{-}3,\ 43\text{-}47}$ for all blocks and $F_{1,\ 14\text{-}16}$ for idividual blocks.*

| Block | Factor | Statistic | Soil moisture (% dwt soil) | pH | Soil organic C (% dwt soil) | Total C (% dwt soil) | Total N (% dwt soil) | C:N ratio |
|---|---|---|---|---|---|---|---|---|
| All | OTC | *F* | 0.66 | 0.46 | 0.51 | 0.63 | 0.83 | 0.32 |
| | | *P* | 0.422 | 0.503 | 0.478 | 0.433 | 0.368 | 0.574 |
| | W | *F* | 0.08 | 0.63 | 0.02 | 0.01 | 0.43 | 1.04 |
| | | *P* | 0.779 | 0.432 | 0.879 | 0.911 | 0.514 | 0.314 |
| | B | *F* | 9.21 | 0.68 | 8.75 | 6.25 | 1.71 | 1.04 |
| | | *P* | **< 0.001** | 0.512 | **0.001** | **0.004** | 0.193 | 0.362 |
| | OTC x W | *F* | 0.24 | 0 | 0.49 | 0 | 0.31 | 0.17 |
| | | *P* | 0.624 | 0.949 | 0.487 | 0.944 | 0.581 | 0.684 |
| | OTC x B | *F* | 1.25 | 0.15 | 0.93 | 0.87 | 0.36 | 0.07 |
| | | *P* | 0.296 | 0.862 | 0.402 | 0.427 | 0.702 | 0.936 |
| | W x B | *F* | 0.32 | 1.11 | 0.08 | 1.03 | 0.04 | 0.18 |
| | | *P* | 0.725 | 0.338 | 0.92 | 0.366 | 0.962 | 0.837 |
| | OTC x W x B | F | 0.11 | 0.09 | 0.05 | 0.2 | 0.73 | 0.46 |
| | | P | 0.895 | 0.916 | 0.955 | 0.818 | 0.487 | 0.634 |
| 1 | OTC | *F* | 0.28 | 0.01 | 0.41 | 1.69 | 0 | 0.15 |
| | | *P* | 0.606 | 0.924 | 0.536 | 0.217 | 0.968 | 0.706 |
| | W | *F* | 0 | 0.07 | 0 | 0.98 | 0.51 | 0.45 |
| | | *P* | 0.97 | 0.8 | 0.962 | 0.342 | 0.489 | 0.515 |
| | OTC x W | *F* | 0.01 | 0.19 | 0.08 | 0.14 | 0.08 | 0.13 |
| | | *P* | 0.925 | 0.67 | 0.778 | 0.718 | 0.785 | 0.724 |
| 2 | OTC | *F* | 0.47 | 0.03 | 0.65 | 0.25 | 0.7 | 0.24 |
| | | *P* | 0.504 | 0.859 | 0.434 | 0.623 | 0.417 | 0.635 |
| | W | *F* | 0.11 | 1.49 | 0.05 | 1.13 | 0.02 | 0.01 |
| | | *P* | 0.741 | 0.242 | 0.826 | 0.306 | 0.889 | 0.905 |
| | OTC x W | *F* | 0.63 | 0.04 | 0.1 | 0.3 | 0.85 | 0.72 |
| | | *P* | 0.444 | 0.842 | 0.761 | 0.591 | 0.376 | 0.413 |
| 3 | OTC | *F* | 2.84 | 1.19 | 3.27 | 0.38 | 0.17 | 0 |
| | | *P* | 0.114 | 0.294 | 0.092 | 0.547 | 0.682 | 0.956 |
| | W | *F* | 0.67 | 0.02 | 0.39 | 0.03 | 0.46 | 1.1 |
| | | *P* | 0.428 | 0.889 | 0.544 | 0.871 | 0.507 | 0.312 |
| | OTC x W | *F* | 0.02 | 0.01 | 1.4 | 0 | 0.07 | 0 |
| | | *P* | 0.879 | 0.918 | 0.259 | 0.987 | 0.794 | 0.985 |

*Abbreviations: dwt, dry weight soil, OTC, open top chamber, W, watering treatment, B, block, C, carbon, N, nitrogen.*

# Appendix 13

*Table 9.9 Outputs from General Linear Models (three way ANOVA) showing the main and interactive effects of isolate, temperature cycles (T) and water potential ($\Psi_m$) on enzyme activity and specific enzyme activity of chitinase, cellulase, leucine aminopeptidase and acid and alkaline phosphatase secreted by Arctic soil fungi. Bold text indicates significant (P < 0.05) effects. Data were $log_{10}$ (n+ 1)-transformed prior to analyses,* $F_{\text{degrees of freedom}}$.

| | | Enzyme activity (EA) | | | | | Specific enzyme activity (SEA) | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **Factor** | | **N** | **C** | **LA** | **AlP** | **AcP** | **N** | **C** | **LA** | **AlP** | **AcP** |
| Isolate | $F_{12, 541}$ | 2525.7 | 237.08 | 138.43 | 137.3 | 173.94 | 33.82 | 38.65 | 11.61 | 9.72 | 35.24 |
| | *P* | **<0.001** | **<0.001** | **<0.001** | **<0.001** | **<0.001** | **<0.001** | **<0.001** | **<0.001** | **<0.001** | **<0.001** |
| T | $F_{2, 541}$ | 1.99 | 1.8 | 2.47 | 0.41 | 1.88 | 2.99 | 0.69 | 1.59 | 1.22 | 2.12 |
| | *P* | 1.38 | 0.167 | 0.086 | 0.666 | 0.154 | 0.051 | 0.502 | 0.205 | 0.296 | 0.121 |
| $\Psi_m$ | $F_{2, 541}$ | 10.94 | 55.27 | 96.51 | 51.97 | 54.87 | 10.6 | 33.44 | 26.46 | 16.42 | 22.22 |
| | *P* | **<0.001** | **<0.001** | **<0.001** | **<0.001** | **<0.001** | **<0.001** | **<0.001** | **<0.001** | **<0.001** | **<0.001** |
| Isolate x T | $F_{24, 541}$ | 4.17 | 2.82 | 4.44 | 5.88 | 6.28 | 4.36 | 1.79 | 1.71 | 2.85 | 4.67 |
| | *P* | **<0.001** | **<0.001** | **<0.001** | **<0.001** | **<0.001** | **<0.001** | **0.013** | **0.02** | **<0.001** | **<0.001** |
| Isolate x $\Psi_m$ | $F_{24,541}$ | 33.91 | 7.4 | 8.88 | 7.84 | 9.2 | 4.56 | 15.44 | 3.82 | 2.64 | 6.59 |
| | *P* | **<0.001** | **<0.001** | **<0.001** | **<0.001** | **<0.001** | **<0.001** | **<0.001** | **<0.001** | **<0.001** | **<0.001** |
| T x $\Psi_m$ | $F_{4, 541}$ | 2.05 | 2.32 | 1.69 | 2.2 | 4.55 | 2.58 | 1.49 | 0.51 | 2.59 | 2.93 |
| | *P* | 0.087 | 0.056 | 0.152 | 0.068 | **0.001** | **0.037** | 0.204 | 0.73 | **0.036** | **0.021** |
| Isolate x T x $\Psi_m$ | $F_{48, 541}$ | 2.94 | 1.91 | 1.91 | 1.47 | 2.41 | 3.28 | 2.16 | 2.01 | 2.13 | 2.68 |
| | *P* | **<0.001** | **<0.001** | **<0.001** | **0.026** | **<0.001** | **<0.001** | **<0.001** | **<0.001** | **<0.001** | **<0.001** |

*Abbreviations: T, temperature cycles, $\Psi_m$, matric water potential, N, chitinase, C, cellulase, LA, leucine aminopeptidase, AcP, acid phosphatase, AlP, alkaline phosphatase*