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Effect of climate variability and extreme events on microbial activity in soils

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Effect of climate variability and extreme events on microbial activity in soils

A thesis submitted to the Bangor University

by Maki Miura

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Thesis Summary

Climate change is expected to alter important process operating in soil ecosystems such as microbial activity, biogeochemical cycling, and hydrological processes, and thus soil functioning and the delivery of a range of ecosystem services. Under future climate change scenarios, extreme weather events are predicted to become ever more frequent globally, with rising temperatures and concentrated rainfall events having an impact on soil functioning. It is necessary to study how changes in soil moisture status and/or temperature will affect soil respiration for predicting future changes in soil carbon (C) storage. In the scientific literature, previous studies have frequently observed a CO_2 pulse from soil after a freeze-thaw or dry-wet event; however, the mechanisms underlying these effects are not well understood. The first experiment of this thesis (Chapter 3) investigated how a single freeze-thaw or dry-wet event affected microbial C dynamics using ^{14}C tracking. Our results revealed that freeze-thaw or dry-wet events altered the allocation of C into labile and structural microbial C pools. The next experiment (Chapter 4) investigated how the C budget of an intact plant-soil system responded to freeze-thaw and dry-wet events. The presence of plants resulted in significantly greater total CO_2 flux following freeze-thaw or dry-wet events in comparison to the unplanted soil. The greater CO_2 efflux seen after thawing or rewetting was caused by a disruption of the microbial biomass, rather than a stimulation of soil organic matter turnover (Chapter 5). This was supported by a decrease in extracellular enzyme activity immediately after freeze-thaw or dry-wet event (Chapter 5). We also showed that soil microbes accumulated osmotic solutes (i.e. sugars and polyols) in response to extreme freeze-thaw or dry-wet events. In this thesis, the microbial community quickly responded to freezing or drying events by altering cellular metabolism (Chapter 6). The final experimental chapter (Chapter 7) investigated how future climate scenarios may affect arctic ecosystems. We monitored greenhouse gas (GHG) emissions and nutrients in soil solution throughout a year in response to a 2050 and 2100 climate warming scenario. A simulated warmer winter led to enhanced microbial decomposition of soil organic matter with increased CO_2 efflux and more N becoming available to roots and associated mycorrhiza in the Arctic soils. We hypothesize that this could lead to a potential future shift in plant communities. In conclusion, this thesis present events showing that under future climate scenarios, an increase in freeze-thaw or dry-wet events will alter soil C and N processing in soils and disrupt biogeochemical cycling. We also conclude that the presence of plants is key in determining how ecosystems respond to these extreme events.

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Abbreviations

˚C	Centigrade	**M**	Molar
µM	Micromolar	**m**	Metre (s)
µmol	Micromole	**ml**	Millilitre (s)
µL	Microliter	**mg**	Milligram (s)
ANOVA	Analysis of variance	**min**	Minute(s)
C	Carbon	**Min^{-1}**	Per minute (s)
CH_4	Methane	**mM**	Millimolar
cm	Centimetre (s)	**mm**	Millimetre (s)
CO_2	Carbon dioxide	**N**	Nitrogen
DNA	Deoxyribonucleic acid	**N_2O**	Nitrous oxside
DOC	Dissolved organic carbon	**NaOH**	Sodium hydroxide
DON	Dissolved organic nitrogen	**NH_4^+**	Ammonium
DW	Dry-weight	**NO_3^-**	Nitrate
EC	Electrical conductivity	**P**	Phosphorus
g	Gravitational acceleration	**PC**	Principal Components
GC	Gas chromatograph	**PCA**	Principal component analysis
GHG	Greenhouse gas	**PLFA**	Phospholipid fatty analysis
h	Hour (s)	**s^{-1}**	Per second
h^{-1}	Per hour	**SEM**	Standard error of mean
HMW	High molecular weight	**SOM**	Soil organic matter
IPCC	Intergovernmental Panel on Climate Change	**TOC**	Total organic carbon
kBq	Kilobecquerel	**TXRF**	Total reflection x-ray fluorescence
kg	Kilogram	**UK**	United Kingdom
L	Liter (s)	**w/v**	Weight to volume
LMW	Low molecular weight		

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Chapter 1

Introduction

1.1 General Introduction

Under future climate change scenarios extreme weather events are predicted to become ever more frequent globally, with rising temperatures and concentrated rainfall events having an impact on soil functioning (IPCC. 2017). Here I examine how altered dry-wet and/or freeze-thaw cycles can impact nutrient cycling and GHG balance in two contrasting ecosystems (temperate and polar).

Grasslands (including arable and pasture systems) are an important part of the global ecosystem, covering 37 % of the earth's terrestrial area (O'Mara, 2012). They are globally important as a sink for carbon (C) (Leahy *et al*., 2004), and play a crucial role in C and nitrogen (N) cycling (Jones *et al*., 2004). Climate change impacts to grasslands are largely associated with increased in seasonal temperature, changing precipitation patterns, a greater frequency of extreme weather events and increases in ground level ozone and CO_2 (Loarie *et al*., 2009; Craine *et al*., 2012). These ongoing changes can both positively and negatively affect plant growth and productivity depending on the magnitude of the response and geographical location (Osakabe *et al*., 2014; Zampieri *et al*., 2017). For example, approximately 67 % of crops in the USA. have been lost over the last 50 years due to drought (Comas *et al*., 2013). Drought is also likely to lead to loss of grassland habitats (Tilman and Haddi, 1992). Grassland vegetation supports high densities of grazing animals (i.e. sheep, cattle and deer) as well as providing a wide range of other ecosystem services (Bilotta *et al*., 2007). Vegetation shifts in grassland communities may cause changes in ecosystem and species composition as well as plant productivity, which may also impact the human communities that rely on agricultural production in temperate regions (Sebastià *et al*., 2008).

In polar ecosystems, northern terrestrial soil and permafrost contains

approximately half of the global belowground organic C (~ 1700 Pg C) (Tarnocai *et al*., 2009), accumulated over thousands of years due to slow microbial decomposition under frozen conditions (Schuur *et al*., 2015). Due to rapid climate warming (IPCC, 2013), some soils in the region are experiencing unprecedented thawing during the winter season. Thawing of soils can enhance microbial activity, increasing the decomposition of old organic matter, potentially resulting in the release of large quantities of greenhouse gases (CO_2 and CH_4 in particular) to the atmosphere (Schuur *et al*., 2015). This induces a positive climate feedback exacerbating the impact of climate change. Increases in microbial activity can also increase the N mineralization rate, which affects nutrient availability for plants with knock-on effects likely to be seen right through the food chain (Chapin *et al*., 1994).

Changes in temperatures and moisture alter important processes operating in soil ecosystems such as microbial activity, biogeochemical cycling, and hydrological processes (Schimel *et al*., 2007). This thesis explores how climate variability and extreme environmental conditions affect microbial activity in soils and the mechanisms that control C and N cycling in soil, and is therefore critical for predicting ecological responses to climate change.

1.2 Thesis structure including aims and objectives

This thesis is divided into several chapters as shown in Figure 1.1. Chapter 3, 4, 5, and 6 focus on the effects of freeze-thaw or dry-wet events on microbial activity in soil. Chapter 7 investigates future winter warming on C and N cycling in Arctic soils.

Chapter 3 aimed to compare the relative effect of mild freeze-thaw (-5 ˚C / +5 ˚C), extreme freeze-thaw (-20 ˚C / +5 ˚C) and dry-wet events on microbial activity and

C/N cycling in soils collected from two different ecosystems (temperate grassland and polar grassland).

To better understand the impact of freezing or drought on the CO_2 balance of arable soils, wheat (*Triticum aestivum* L.) cropping systems (planted versus un-planted soil mesocosms) were exposed to a freeze-thaw or dry-wet events, with CO_2 efflux monitored throughout (Chapter 2).

In chapter 5, the aim of this study was to directly compare the effects of a single dry-wet or freeze-thaw (-5 °C or -20 °C) cycle on extracellular enzyme activity and soil organic matter turnover. We measured the activity of six exoenzymes before and after imposing the freeze-thaw or dry-rewet events. We also added ^{14}C-labelled plant residues to the soil prior to imposing the same thermal or moisture stress events.

Chapter 6 aimed to compare the relative effects of a single freeze-thaw or dry-wet event on soil microbial activity, with specific emphasis on shifts in the microbial community's metabolite profile.

In chapter 7, we investigated the effects of winter warming on arctic climate and ecosystems. We set winter temperature regimes to simulate a range of climate scenarios: (i) current (present-day) climate in which the soils were frozen in winter and thawed in the summer, (ii) a year 2050 regime in which the soils were both frozen and thawed in winter), and (iii) a 2100 regime in which the soils remained unfrozen during winter. We monitored GHG emissions and nutrient dynamics in soil solution throughout a year.

In the concluding chapter, I set out how the findings of each chapter contribute to our current understanding of C and N soil cycling, and also provide a range of new ideas for future research.

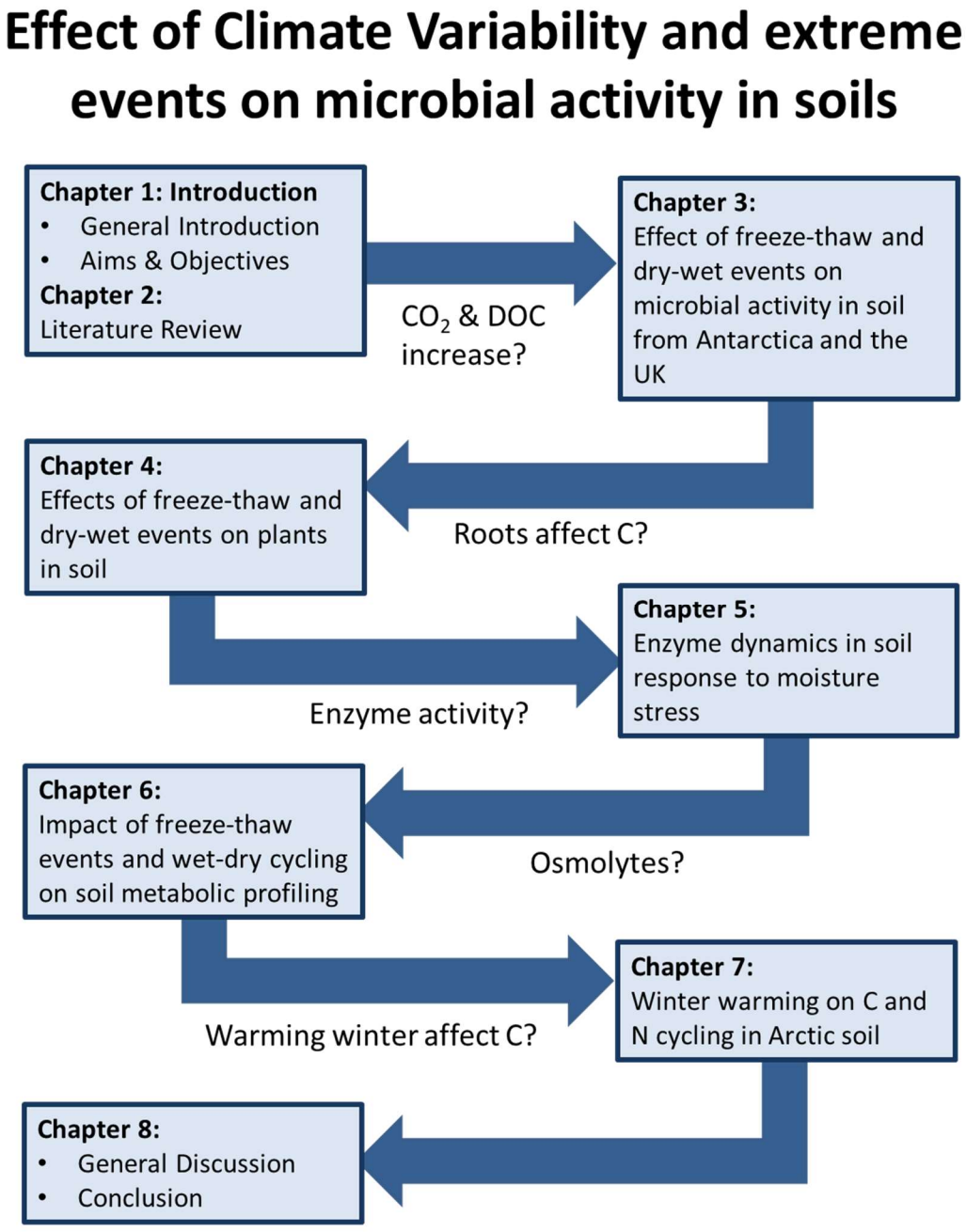

Figure 1.1. Schematic of the chapter content of this thesis and the links between the individual chapters.

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Chapter 2

Literature Review

2.1 Introduction

This literature review presents a summary of previous research about how freeze-thaw or dry-wet events affect soils, in particular soil nutrients dynamics, GHG emissions and microbial activity, as well as the general effects on plant and soil ecosystems. The first part of this review focuses on the plants and soil ecosystems of the polar regions. The second part of this review summarises the physical, chemical and biological effect of freeze-thaw events on soils. Finally, the physical, chemical and biological effects of dry-wet events on in soils are reported.

2.2 Climate change in polar regions

Future climate change is predicted to alter important processes operating in soil ecosystems such as microbial activity, biogeochemical cycling, and hydrological processes (Lawrence and Swenson, 2011). Plants and microbial communities of cold regions, in particular the poles, are believed to be highly sensitive to environmental change (Pearson *et al*., 2013). In Antarctica, soil temperature in most areas are well below 0 °C throughout the year with temperatures at Vostok station going down to -68 °C (Figure 1). In the Arctic, soil temperatures in winter are below 0 °C (-50 to -30 °C) but thaw in summer, reaching temperatures of 15 ˚C (Figure 2.2). Freeze-thaw events represent a period of stress for all living organisms inhabiting cold areas. Small soil animals are highly susceptible to damage by freezing process (Bokhorst *et al*., 2012). Impacts of climate change on polar ecosystems can be significant because many polar soils contain high amounts of organic matter (Tarnocai *et al*., 2009). A warming climate is expected to accelerate the decomposition of soil organic matter (SOM) resulting in enhanced microbial soil respiration and the loss of CO_2 to the atmosphere (Schuur *et al*., 2015). A warmer climate may also be associated with a greater N mineralization rate, with an

increase in plant available N (Chu and Grogan, 2009). Increases of soil temperature up to 2 °C appear to have little effects on net mineralization in tundra soils, however, when soil temperature rises up to 4-5 °C, the rate of decomposition and nutrient mineralization will increase (Jonasson *et al*., 1993).

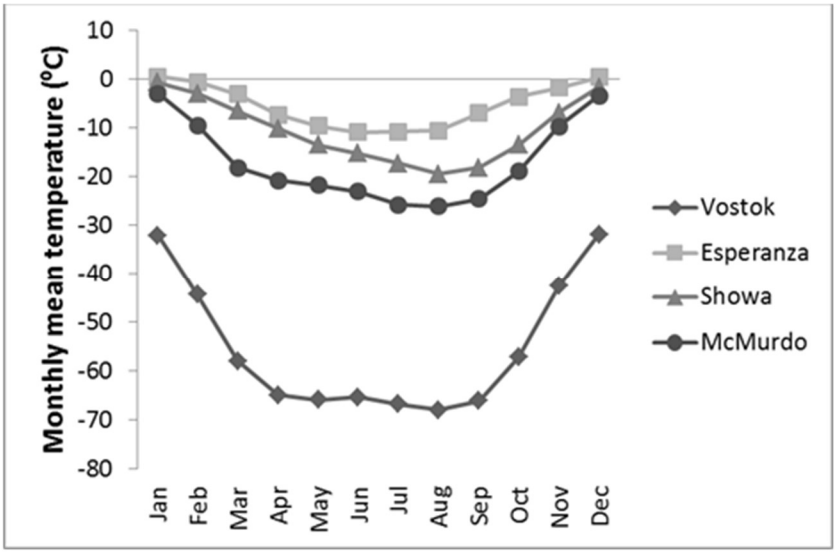

Figure 2.1. Typical climate for Antarctica; Vostok station, Esperanza Base, McMurdo station(https://web.archive.org/web/20101009114203/http://coolantarctica.com/Antarctica%20fact%20file/antarctica%20environment/climate_graph/vostok_south_pole_mcmurdo.htmia) and Showa station (Japan Meteorological Agency, 2013).

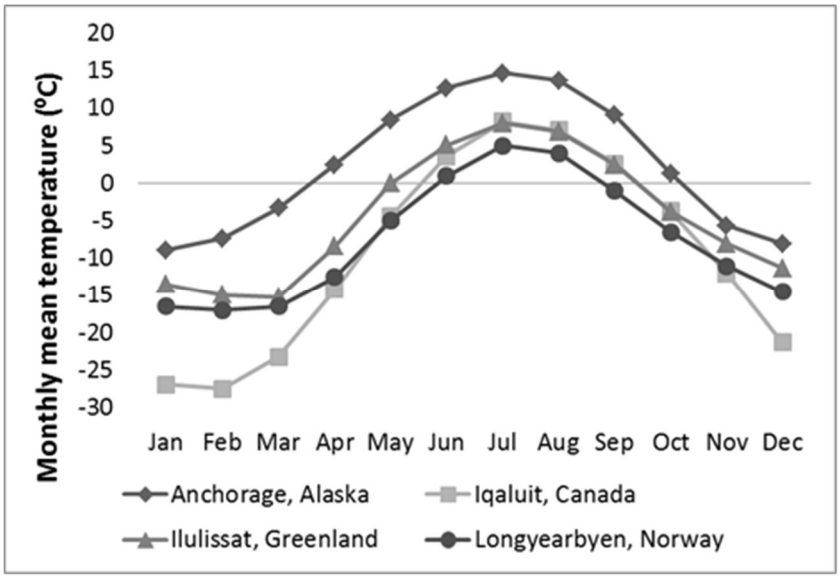

Figure 2.2. Typical climate for Arctic; Anchorage(https://www.timeanddate.com/weather/usa/anchorage/climate), Iqualuit(https://en.climate-data.org/north-america/canada/nunavut/iqaluit-1251/), Illulissat(https://www.timeanddate.com/weather/greenland/ilulissat/climate) and Longyearbyen(https://www.timeanddate.com/weather/norway/longyearbyen/climate).

2.3 Polar Plant and Soil Ecosystem

2.3.1 Antarctic plant and soil ecosystems

In Antarctica, there are only two species of flowering plants: Antarctic hair grass (*Deschampsia antarctica*) and Antarctic pearlwort (*Colobanthus quitensis*). They grow mainly in the penguin colonies on the South Orkney Islands, the South Shetland Islands and along the western Antarctic Peninsula. Both species are angiosperms and do not support symbiotic N_2-fixing bacteria (Hill *et al*., 2011). Primary productivity in these ecosystems is largely limited by N availability (Table 2.1). Plants in Antarctica can take up inorganic N (NH_4^+ and NO_3^-). However, they tend to prefer organic N (peptide and amino acid) (Hill *et al*., 2011). Hill *et al.* (2011) reported that soil microbes in fellfield site (lichen dominated tundra) took up peptide, amino acid and NH_4^+ but did not take up NO_3^-, and *D antarctica* took up these N forms faster than NO_3^-. N_2-fixing genes in soil microbes were detected in fellfield sites, which are dominated by lichens (Yergeau *et al*., 2007). However, there was no correlation between N_2-fixing genes and the concentration of NH_4^+ or NO_3^- in the soil (Yergeau *et al*., 2007). High level of nitrite and nitrate reductase genes were also found in soils from Signy Island. The abundance of nitrite reductase genes was correlated with soil temperature and high NO_3^- concentration was observed on lower latitude sites such as adjacent to Lake Vanda (Yergeau *et al*., 2007; Wada *et al*.,1981).

Table 2.1. N concentration (μmol N l^{-1}) in Antarctic soils (adapted from Hill *et al*., 2011)

Site	Total soluble N	Ammonium	Nitrate
All moss and grass (n=39)	346 ± 42	12 ± 2	238 ± 37
***D. antarcrica* (n=20)**	428 ± 75	11 ± 2	336 ± 63
***S. uncinata* (n=11)**	262 ± 16	14 ± 2	124 ± 20
Mixed moss and grass (n=8)	255 ± 59	10 ± 5	151 ± 42
Fellfield (n=6)	54 ± 9	9 ± 5	18 ± 5

2.3.2 Arctic plant and soil ecosystems

The Arctic ecosystem is dominated by tundra soils, classified as young as they were covered by glaciers until 10,000 year ago (Kaplan, 1996). Soils in young ecosystems tend to have high pH, low organic matter content, high bulk density, and low cation exchange capacity (Crocker and Major, 1955; Ugolini and Mann, 1967). Chapin *et al*. (1994) studied soil development in response to primary successional change at different stages (Pioneer, *Dryas* (evergreen dwarf shrubs), Alder and Spruce) after glacial retreat at Glacier Bay, Alaska. They observed increases in soil organic matter, soil moisture, total N, and declines in bulk density, pH, and total phosphorus (P) with increasing successional age. Plant growth in early-successional soils is limited due to N and P availability as well as a lack of mycorrhizas (Chapin *et al.*, 1994). Although N availability in *Dryas* was less than alder and spruce, it contributes to N accumulation (Chapin *et al*., 1994). Kielland *et al.* (2007) reported physical and chemical parameters of boreal forest soils in Alaska across different successional stage, (1) willow (*Salix* sp), (2) alder (*Alnus tenuifolia*), (3) balsam polar (*Populus balsamifera*), (4) white spruce (*P. glaucea*), and (5) black spruce (*P. mariana*) underlain by permafrost. In their study, C and N concentration in soils increase with increasing successional stages from willow to black spruce (Figure 2.3 and

2.4).

N turnover in soil regulates ecosystem development (Ollivier *et al*., 2011) with total N concentration in soil less than 0.1 % at the start of ecosystem development. When the total N concentration in soil exceeds 0.2 %, plant colinisation starts, C inputs increase and the soil surface develops (Ollivier *et al*., 2011). N concentration in soil at this early successional stage is still much lower than in grassland soils (Chronáková *et al*., 2009). Pioneer species compete with soil microbes for N (Schimel and Bennett, 2004; Hämmerili *et al*., 2007) and the N fixation activity associated with rhizosphere of the pioneer species increases. Less nitrifying bacteria and denitrifying bacteria in soil are present at this stage (Smith and Ogram, 2008). When total N concentration is above 0.7 % (as typical of well-developed grasslands), nitrification activity is enhanced (Ollivier *et al*., 2011).

In some arctic ecosystems, high concentrations of soluble organic N are present (Chapin *et al*., 1993; Kielland, 1995; Näsholm *et al.,* 2009) due to slow soil N mineralisation rates (Jones *et al*., 2005). The soluble organic N concentration is higher than the concentration of N in inorganic forms such as NH_4^+ and NO_3^-, and contains great amounts of free amino acids (Jones and Kielland, 2002). Organic N is produced through proteolytic activity from enzymes by free living microbes, mycorrhizal fungi and plant roots. Amino acid turnover is associated with soil protease activity at soil temperature below 10 °C (Kielland *et al.*, 2007). In Alaska, the rate of amino acid mineralisation in soil L and O1 horizons is faster than Ah horizons, and the amino acid (Glycine and glutamate) are rapidly taken up by soil microbes (Jones and Kielland, 2002). Uptake of amino acid capacity of fine roots is higher than in thick roots (Kielland, 1994). In Arctic tundra soils, there is a correlation between dissolved organic nitrogen (DON) and NH_4^+ (Keilland, 1997; Jones and Kielland, 2002), while in the taiga forests of Alaska, there was

an inverse relationship between DON and NO_3^- (Jones and Kielland, 2002). The level of DON in the organic surface soils were high and the level of NO_3^- in the Ah horizon mineral zone which has a low organic content was higher than organic surface soil (Jones and Kielland, 2002).

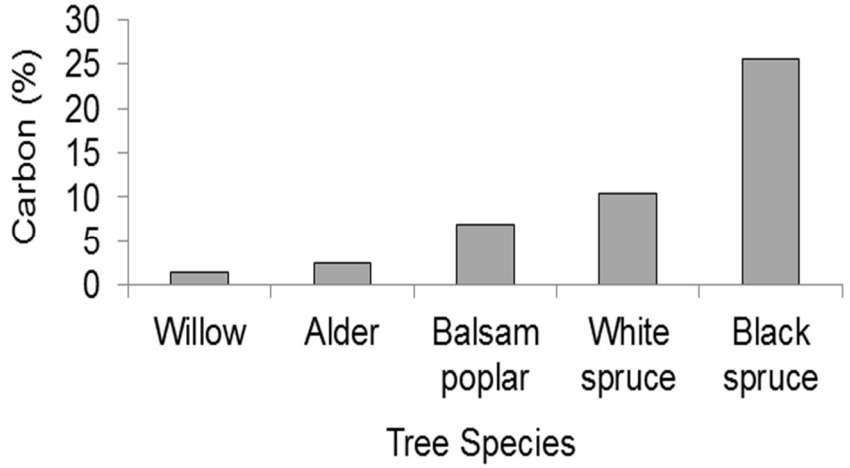

Figure 2.3. % of C concentration in soils across the successional stages on the Tanana River Flood Plain in Alaska (adapted from Kielland *et al*., 2007).

Figure 2.4. % of N concentration in soils across the successional stages on the Tanana River Flood Plain in Alaska (adapted from Kielland *et al*., 2007).

2.4 Effect of Soil Freeze-Thaw events on microbial activity in soil

2.4.1 Physical Effect of Soil Freeze-Thaw

Land forms created by frost heaving

Freeze-thaw cycles affect water movement in landscapes and produce regular ground patterns such as polygon, pingo, palsas, circles and tundra hummocks. These unique landscapes are created by freeze-thaw events and appear in very cold areas (Gallagher *et al*., 2011).

Influence of physical factors

Temperature

Deluca *et al*. (1992) demonstrated the effect of freeze-thaw event on N mineralization in central Iowa, USA. Two types of soils were stored for 7 days at temperature 5°C and -20 °C, then thawed for 0 day, 3 days, 10 days and 20 days at 5 °C and 25 °C. A significant increase in N mineralisation rates was observed in freeze-thaw treated soils compared with non-frozen soils.. Nielsen *et al.* (2001) studied the effect of freeze-thaw events on soil respiration, nitrous oxide (N_2O) flux, and ammonification and nitrification in soils dominated by American beech (*Fagus grandifolia Ehrh*.), sugar maple (*Acer saccharum* Marsh), and yellow birch (*Betula alleghaniensis*) in New Hampshire, USA. After thawing, they observed that higher CO_2 and N_2O flux and NH_4^+ concentration from soils exposed to temperatures of -13 °C than soils exposed at -3 °C (Nielsen *et al*., 2001). Hentschel *et al.* (2008) studied the effect of frost temperature on DOC, DON, NH_4^+ and NO_3^- in a forest soil (O horizons) in Norway using three freezing temperatures -3°C, -8 °C, and -13 °C. They observed the frost effect on concentration of DOC and DON was the greatest at -8 °C, while concentration of NH_4^+ and NO_3^- was at -

13 °C. Anderson *et al.* (2000) investigated how soil pH and temperature affect microbial activity by leaching of DOC and DON in mor humus (O horizon) in Sweden. Concentration of DOC, DON and NH_4^+ in soils was increased when soil pH decreased. They explained that pH was the most important factor and temperature was more important after pH.

Snow

Snow cover influence on soil freezing (Osokin *et al*., 2000) and GHG emission from soil (Groffman *et al.,* 2006). Groffman *et al*. (2006) found that there were low fluxes of CO_2 soil with snow cover but N_2O flux significantly increased at snow melt. Rienmann *et al*. (2012) observed DOC increased after snowmelt due to induced denitrification.

2.4.2 Chemical Effect of Soil Freeze-Thaw

Chemical transport and ion exchange reaction

The soil cations have positive charge. NH_4^+, Ca^{2+}, Mg^{2+} and K^+ are essential for plant growth while Na^+, $Al^{3+}$and hydrogen affect soil pH. In contrast, anions are negatively charged. The soil anions such as PO_4^{3-}, S^{2-}, and Cl^- have negative charge. Anion exchange capacity is depending on the soil pH. Clay particles usually have a negative charge, so they attract and retain cations. Soil organic matter has both positive and negative charges, so it can hold both positively and negatively charged ions. Brown *et al.* (1962) studied exchangeable cations at different types of geological materials. High concentrations of calcium and magnesium at calcareous streams were influenced by their bedrock and soil materials (Woodin and Marquiss, 1997). Edwards *et al.* (1986) investigated that the effect of freeze-thaw on concentration of exchangeable cations (K^+

Na^+ and Ca^{2+}) in organic soils in two catchment areas in north Scotland. Significant increases in potassium were observed whilst there were not significant in Na^+ and Ca^{2+}. In contrast, no change in K^+ and $Ca^{2+}$was presented (Allen and Grimshaw, 1962). Hinman (1970) studied the effect of freeze-thaw on chemical properties of soils (heavy clay, clay loam and wood Mountain loam) in southwestern Saskatchewan. Significant decrease in exchangeable K and no change in the cation exchange in cation exchange capacity or exchangeable Ca and Mg were observed (Hinman, 1970). Pulubesova and Shirshova (1992) reported no significant change in cation exchange capacity or exchangeable Ca, Mg, K and Na. After pulse of water electrical conductivity in Ca in cryoconite hole in Antarctica (Fountain *et al.,* 2008) Telling *et al.* (2004) observed high amount of Na, K, during the initial melt.

Nutrient availability and decomposition

Increased in total organic carbon (TOC) concentration in organic soils from north Scotland were observed after short period of freezing at temperature of -12 °C (Edwards *et aI*., 1986). Significant increase of dissolved organic carbon (DOC) in arable soils after the freeze-thaw events has been observed (Wang and Bettany, 1993). Feng *et al.* (2007) studied effect of freeze-thaw events on organic matter composition in grassland soil from Canada. Fungi and bacteria showed different degradation patterns of SOM and Fungi-dominated soil may have a higher C storage capacity than a bacteria-dominated (Feng *et al*., 2007). Bacteria were more influenced by rising temperature, while fungi were affected by the presence of vegetation cover and freeze-thaw cycle frequency (Yargeau and Kowalchuk, 2008). Yargeau and Kowalchuk (2008) observed changed in fungal community structure at the DNA level after the freeze-thaw frequencies. The change in

fungal community structure was influenced by combined vegetation of freeze-thaw event (Yargeau and Kowalchuk, 2008). The vegetation presence influences availability of organic matter (Yargeau and Kowalchuk, 2008). Fan *et al.* (2012) studied effect of multiple freeze-thaw cycles on water soluble organic C and N in alpine meadow soil and alpine steppe soil from China. They observed both water soluble organic C and N increased in soils with alpine meadow and alpine steppe after the first cycle and then decreased after the second cycle.

The effect of freeze-thaw events on nutrient availability is important for plant nutrient uptake because N content in polar regions is limited (Marion, 1995). The major processes of the N cycle include N_2 fixation, ammonification, nitrification, denitrification, immobilisation, plant uptake, cation exchange of NH_4^+ and NO_3^- leaching (Bowman and Paul, 1998). A number of studies observed that freeze-thaw events enhanced concentration of NH_4^+ and NO_3^- in the soil (Soulides and Allison, 1961; Hinman, 1970; DeLuca *et al.*, 1992, Schimel and Clein, 1996; Urakawa *et al*., 2014). Schimel and Clein (1996) determined the effect of freeze-thaw cycles on N mineralization in four contrasting soil types typical of the Alaskan region (: (1) alder (*Alnus tenuifolia*) - balsam poplar (*Populus balsamifera*), (2) birch (*Betula papyrifera*)), (3) tussock tundra dominated by *Eriophorum vaginatum* and (4) wet meadow dominated by the sedges (*Carex aquatalis* and *Eriophorum angustifolium*)) with net N mineralization increased immediately after the first freeze-thaw cycle. Yergeau and Kawalchuk (2008) also studied the effect of freeze-thaw cycles on soils with and without vegetation from Signy Island in Antarctica, with greater concentrations of NH_4^+ and NO_3^- from both soil types observed immediately after thawing. Telling *et al.* (2014) observed the impact of freeze-thaw event on microbial activity in cryoconite holes on McMurdo Dry Valley glaciers in Antarctica from frozen

conditions to melted holes during spring thaw. There was high nutrient availability (NH_4^+, NO_3^-) during the initial snow melt period when soil temperatures increased to above xx C. On the other hand, Read and Cameron (1979) observed increases of NO_3^- between autumn and spring, but decreases of NH_4^+ in soils from southwestern Saskatchewan, Canada.

Greenhouse gas emission

Soil-atmosphere fluxes of carbon dioxide (CO_2), nitrous oxide (N_2O) and methane (CH_4) are important to global GHG balance (Karl and Trenberth, 2003), with over 300 focusing on the impact of re-wetting and/or thawing on soil GHG flux (Kim *et al*., 2012). Previous studies found GHG emission (CO_2, N_2O and CH_4) increased from Antarctica soil (Zhu *et al.*, 2009) and from forest soils (Groffman *et al*., 2006) after freeze-thaw cycles.

CO_2 emission from soil is the result of combined root and microbial respiration (Mooney *et al*., 1987) with a correlation between respiration and soil temperature common in cold or temperate ecosystems Grogan *et al*. (2004). A flush of CO_2 in both field and laboratory settings after thawing has been observed in agricultural soils (Priemé and Christensen, 2001; Dörsch *et al*., 2004; Kurganova *et al*., 2004), forest soils (Coxson and Parkinson, 1987; Nelsen *et al*., 2001), and arctic heath soils (Schimel and Clein, 1996; Elberling and Brandt, 2003). Comparing soils across ecosystems, CO_2 flux change after thawing in tundra is the highest (Kim *et al*., 2012), however there are relatively few studies of CO_2 efflux in tundra soils.

N_2O is a product of N transformations such as nitrification and denitrification (Mooney *et al*., 1987). Increases of N_2O emission in field after a freeze-thaw event have

been observed in various soils, including agricultural soils (Flessa *et al.*, 1995; Dörsch *et al*., 2004) grassland soil (Müller *et al*., 2003) and forest soils (Goodroad and keeney, 1984; Boutin and Robitaille, 1995; Mitchell *et al.,* 1996; Fitzhugh *et al*., 2001; Fitzhugh *et al.*, 2003; Kurganova *et al*., 2004; Callesen *et al*., 2007), alpine tundra soil (Brooks *et al*., 1997). Similar results have been reported in laboratory studies of agricultural soil (Kurganova *et al*., 2004), grassland soil (Yao *et al*., 2007), forest soil (Kurganova *et al*., 2004; Goldburg *et al*., 2008), coastal Antarctica (Zhu *et al*., 2009) and permafrost (Elberling *et al*., 2010). On the other hand, decrease of N_2O emission from forest has been observed in laboratory experiment (Hentschel *et al*., 2008).

CH_4 is produced by microorganisms under anaerobic condition or by microbial consumption (Mooney *et al*., 1987). Increases of CH_4 emissions after seasonal thawing from permafrost (Wagner *et al*., 2003) and wetland (Bubier and Moore, 1993; Christensen *et al*., 1996; Song *et al*., 2012), and forest (Groffman *et al*., 2006; Luo *et al*., 2013) are commonly observed. The methane fluxes are influenced by moisture content and temperature (Luo *et al*., 2013).

2.4.3 Biological Effect of Soil Freeze-Thaw

Soil microbial community

Soil microbes play an important role in terrestrial ecosystems and their contributions include decomposition of organic matter, biochemical cycling and regulation of plant-available nutrients (Wild, 1993). Freeze-thaw events may influence soil microbial activity as well as soil microbial populations (Soulides and Allison, 1961). Freeze-thaw events can also trigger soil microbial death with nutrients released from dead microbial cells by survival decomposers upon thawing (Soulides and Allison,1961;

Ivarson and Snowden, 1966; Skogland *et al*.,1988; DeLuca *et al*.,1992). Microbial death is due to damage to the cell walls as a result of ice formation during the freezing period (Soulides and Allison, 1961). Increases in soil nutrients were associated with increased activity of surviving microbes (Christensen and Tiedje, 1990; Koponen *et al*., 2006). The surviving microbes produce a pulse of CO_2 (Schimel and Clein, 1996) on thawing indicating an ability to recovery from damage (caused by freezing) for a subset of the microbial community (Schimel and Clein, 1996). The rate of freezing had little effect on mortality (Hains, 1938). A single freeze-thaw event was suggested to kill up to 50% of the viable soil microbial population (Soulides and Allison, 1961; Skogland *et al*., 1988; DeLuca *et al*., 1992). Mortality was recorded after a single freeze-thaw event (-27°C, thawed to 23°C) was 40-60% (Morley *et al*., 1983). On the other hand, microbes are able to survive under subzero temperature if unfrozen water is available (Rivkina *et al.*, 2000).

After Soulides and Allison (1961) suggested that freeze-thaw events may affect the composition and function of the microbial community, many studies examined how freeze-thaw events affected microbial biomass using a chloroform-fumigation and extraction method. This is a popular method to estimate microbial biomass. Some studies observed a decline in microbial biomass after freeze-thaw, for example in arable soils (Herrmann and Witter, 2002; Yanai *et al.*, 2003; Dörsch *et al*., 2004). However, this effect was not seen in alpine or arctic soils (Lipson and Monson, 1998; Grogan *et al*., 2004), while microbial biomass C and N from alpine soil increased after first freeze-thaw cycle (Fan *et al*., 2012). Larsen *et al.* (2002) observed microbial biomass C in arctic heath soils from Abisko, Sweden decreased after thawing. Grogan *et al*. (2004) observed small change of microbial biomass in soils from sub-arctic heath tundra in Sweden. Many studies concluded that the microbial community can adapt to freeze-thaw events because

(1) Microbial communities living at the surface of soil from desert to polar region are highly stress tolerant under extreme environmental conditions (Schaaf *et al*., 2011). (2) Freeze-thaw event might have small impact on soil microbes (Grogan *et al*., 2004; Yanai *et al*., 2004). (3) Microbial biomass recovered quickly (Stenberg *et al*., 1998; Pesaro *et al*., 2003; Matzner and Borken, 2008). (4) Microbes may have special strategies such as anti-freeze substance. Some studies conducted effect of freeze-thaw events on soil microbial biomass in Colorado alpine in different seasons. Increases of microbial biomass in the autumn and winter (Brooks *et al*., 1996; Jaeger *et al*., 1999; Lipson *et al*., 1999; Lipson *et al*., 2000) and decreases in the spring and summer (Brooks *et al*., 1998; Lipson *et al*., 1999) have been observed. This different response between summer and winter was suggested to due to a shift in the species composition (Lipson *et al*., 2000).

Bacteria and fungi

Fungi and bacteria in polar soils are able to adapt to extreme climatic conditions (Glanville *et al*., 2012). Psychrophilic bacteria live in the cold environment (below 0 ˚C). Small species have special strategies (freeze tolerance and freeze avoidance) to survive harsh conditions. Psychrophilic microbes have successfully adapted to cold environments from the deep ocean to high mountains, and the polar region. The substitution patterns of amino acid between the orthologous proteins of psychrophiles and mesophiles are significantly different (Metpally and Reddy, 2009). Larkin and Stokes (1968) determined the ability of psychrophilic bacteria to grow at subzero temperatures by adding six antifreeze compounds; glycerol, lactose, ethyl alcohol, dimethyl sulfoxide, ethylene glycol and salt. Three species of Bacillus grew at −2 °C, at −4.5 °C with one antifreeze compound, and at −5 to −7 °C with five antifreezes. Moreover, they found glycerol as the

antifreeze compound had the best results in the experiment. Psychrophilic bacteria were also isolated from cold marine environments (Morita, 1996) and the high mountain (Morita and Burton, 1970). Maximal growth rates at 2 °C (*Moritella profunda*) and 4 °C (*Moritella abyssi*) with maximum temperatures of 12 °C (*M. profunda*) or 14 °C (*M. abyssi*) were observed (Xu *et al*., 2003). Panikov (2009) reviewed on metabolic activity of microorganisms in permafrost and frozen tundra soils. Gas exchange and unfrozen water in permafrost are sufficient to support activity of microorganisms in the temperature down to -40°C. Several bacteria and fungi are able to grow in frozen soils without antifreezes. They were isolated from Alaskan permafrost including bacterial species (*Polaromonas hydrogenovorans*, *Pseudmonas*, and *Arthrobacter*) as well as fungal species (*Stereaceae*). Some bacteria and fungi have proteins associated with their membranes which have ability act for ice crystal formation in their fluid (Warton, 2002). The protein induces ice crystal formation (Lorv *et al*., 2014). There are ice nucleating soil bacteria (*Pseudomonas syringae*) (Vandervee *et al*., 2014) and fungi (*Fusarium*) (Warton, 2002). Some species produce antifreeze during freezing conditions. Normally, the freezing point and melting point occur at the same time. The supercooling point is lower than these. However, the antifreeze-substance depresses melting point below that of the freezing point. The antifreezes prevent formation of ice crystal in the cell. Yanai *et al.* (2003) estimated numbers of ammonia-oxidizing bacteria and nitrite-oxidizing bacteria by the most probable number method and the results showed both bacteria were not significantly influenced by the soil freeze-thaw events. Feng *et al.* (2007) studied effect of freeze-thaw event on microbial community composition in grassland soil from Canada using phospholipid fatty acid (PLFA) analysis. Fungal biomass decreased after the freeze-thaw cycles but bacterial biomass did not change (Feng *et al*., 2007). Bacterial community

structure changed with various factors such as soil depth, vegetation cover, nutrient status and soil pH (Eilers *et al*., 2012; Frank-Fahle *et al*., 2014). Bacterial community in soils is dominated by *Alphaproteobacterial* and *Acidobacterial* lineages (Thomson *et al.,* 2010). The abundance of relative Alphaproteobacteria increased in vegetation soil, whereas *Acidobacteria* present in bare soils (Thomson *et al.,* 2010). *Alphaproteobacteria* dominated in upper roots and *Acidobacteria* increased in lower root-free soil (Griffiths *et al.*, 2006). *Alphaproteobacteria* grow fast and are present in nutrient-rich soil (Thomson *et al*., 2010). *Acidobacteria* grow slowly and prefer low nutrient availability in soil (Fierer *et al*., 2007). Bacterial community composition in the arctic and the subarctic soils was more influenced by the acidity of parent materials than changes in soil temperature (Chu *et al*., 2010; Männistö *et al*., 2007). *Acidobacteria*, *Alphaproteobacteria*, and *Gammaproteobacteria* are less abundant with rising soil pH, whereas *Betaproteobacteria* and *Chloroflexi* are abundant with rising pH (Kim *et al*., 2014). Higher amount of C and N concentrations is exhibited in vegetated soils (Thomson *et al.*, 2010). The ratio of *Alphaproteobacteria* to *Acidobacteria* correlated with C turnover (Thomson *et al.*, 2010). Yergeau *et al.* (2012) studied impact of field warming on soil microbial community using open-top chambers in Antarctica: site (1) Falklands Island: *Empetrum rubrum* (vascular plant, meancover 68%); Falkland fell-field*: Poa annua* (vascular plant, 26%) site (2) Signy Island: *Chorisodontium aciphyllum* (moss, 76%) site (3) Anchorage Island: *Sanionia uncinata* (moss, 48%); Anchorage fellfield: *Buellia latemarginata* (lichen, 30%). The abundance of fungi and bacteria were higher in open-top chambers treated soils than control, but bacterial abundance was only in vegetation covered soils. Decreases in the relative abundance of *Acidobacteria* and increases in the relative abundance of *Alphaproteobacteria* were observed in open-top chambers treated soils. However, no

consistent changes in N contents between open-top chambers and control were observed.

Soil Fauna (Rotifers, Nematodes, and Tardigrades)

Soil fauna such as rotifers, nematodes, and tardigrades play an important role in nutrient cycling (Gebremikael *et al*., 2016). Sohlenius *et al.* (1995) found seven species of tardigrades four species of nematodes, sixteen species of rotifers in several types of soil from East Antarctica (where soil temperatures routinely reach -20 ˚C in winter). On the other hand, all tardigrades in fresh water from the lake at Signy Island were killed after rapidly freezing at -20 °C (McInnes and Ellis-Evans, 1990). Newsham *et al.* (2006) studied tolerance of soil fauna (nematodes, tardigrades, and rotifers) in Antarctica. After freeze-thaw events 31 % of nematodes, 13 % of tardigrades, and 2 % of rotifers survived.

Although three species tardigrades (*Echiniscus jenningsi*, *Macrobiotus furciger* and *Diphascon chilenense*) from Müihlig-Hofmannfjella, Dronning Maud Land, Antarctica survived at -22 °C for 600 days (Sømme and Meier, 1995), some tardigrades form a tun (suspended animation) (Baumann, 1992) to protect internal organs from freezing (Figure 2.7). It requires slow dehydration (Crowe, 1972) and metabolic activity (Sømme, 1996). When an anaesthetized tardigrade is under drought conditions, it does not form a tun (Sømme, 1996).

Although many organisms accumulate disaccharide trehalose during dehydration, the trehalose level is significantly different between the species ((Westh and Ramløv, 1991; Ramløv and Westh, 1992; Hengherr *et al*., 2007; Jönsson and Persson, 2010). Trehalose level in tardigrade (*Adorybiotus coronifer*) has increased (from 0.1% to 2.3 % within 5-7 h) during dehydration and declined rapidly during rehydration even though it was still in the tun stage (Westh and Ramløv, 1991). On the other hand, there

was no change in any species of Heterotardigrada were observed (Hengherr *et al.*, 2007). Trehalose also has been observed in nematode *Aphelenchus avenae* (Madin and Crowe, 1975; Crowe *et al*., 1977) in the cryptobiotic state. There was no trehalose in bdelloid rotifers *Philodina roseola*, *Adineta vaga* (Lapinski and Tunnacli, 2003) and *Macrotrachela quadricornifera* (Caprioli *et al.*, 2004) in anhydrobiosis state, while 0.35% trehalose in resting egg of the monogonont rotifer (*B. plicatilis*) has been observed (Caprioli *et al.*, 2004).

Lots of studies of cold strategies for an Antarctic nematode, *Panagrolaimus davidi*, have been conducted by David Warton. The optimum growth temperature of *P. davidi* is 25 °C and it does not grow when exposed to temperatures below 6.8 °C (Warton, 2002). *P. davidi* can survive if it contacts ice when water is frozen (Warton, 2002). It survives intracellular freezing (Wharton and Ferns, 1995). Some insects survive freezing in their bodies (Warton, 2002). Intercellular freezing was observed in part of the cell of *Eurosta solidaginis* (Salt, 1962) and between muscles and under the skin in frogs (Storey and Storey, 1992). Warton and Ferns (1995) observed intracellularly ice formation in all parts of the body of *P. davidi*. Warton *et al.* (2003) studied the effect of freezing at different temperature (range -1 to -6°C) on survival of *P. davidi*. A negative correlation between freezing and survival has been observed (Warton *et al*., 2003).

Some studies of effect of freeze–thaw cycles on soil nematode have been reported. Pickup (1990) studied cold strategies in three species of nematodes (*Eudorylaimus coniceps*, *Eudorylaimus. spaulIi* and *Eudorylaimuspseudocarteri*) in Signy Island. The temperature (estimated supercooling point) at 50% survival nematodes was -10.4 for *E.coniceps*, -7.1 for *E.pseudocarteri*, and -6.1 for *E. spaulli* (Pickup, 1990). *E. coniceps* and *E. spaulIi* had capable to survive up to -20 °C (Pickup, 1990). Sulkava

and Huhta (2003) studied effect of freeze-thaw event on nematode in boreal forest dominated by Scots pine (*Pinus sylvestris*) in central Finland. 63 nematodes were extracted from the soil using wet funnels. They decreased in population of nematodes during the winter and recovery after spring have been observed (Sulkava and Huhta, 2003). As a similar result, Knox *et al.* (2015) also studied effect of freeze–thaw cycles on soil nematode (*Scottnema lindsayae*) in Taylor Valley, Antarctica. Decreases of adult populations of *S. lindsayae* in December have been observed (Knox *et al.*, 2015). *S. lindsayae* inhabit more dry soil than *E. antarcticus* and *P. murrayi* (Knox *et al.*, 2015). As survival at freezing temperatures, anhydrobiotic proportion of *S. lindsayae* was correlated with daily temperature changes (Treonis *et al*., 2000; Treonis and Wall, 2005).

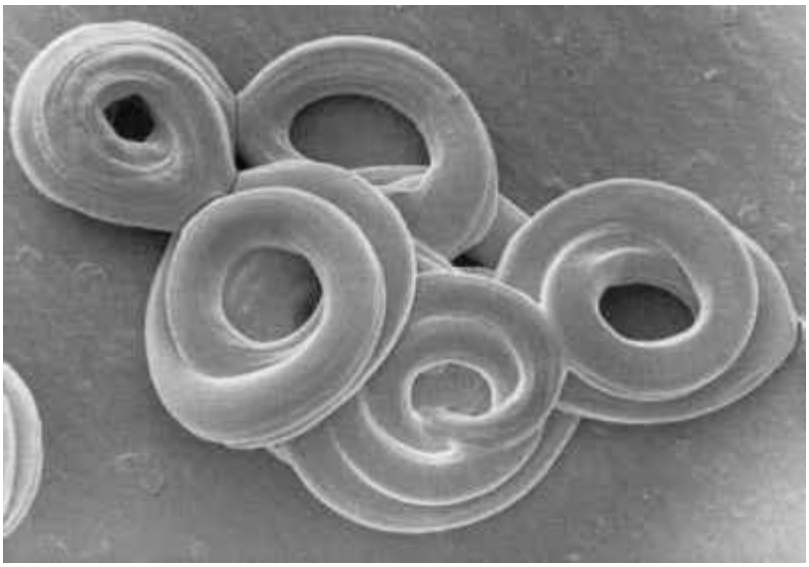

Figure 2.5. Coiled nematodes (*Ditylenchus dipsaci*) in dry state (Warton, 2002).

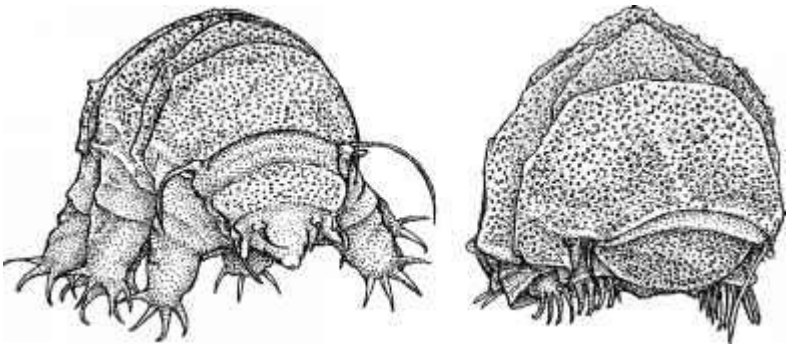

Figure 2.6. A tardigrade in active state (left) and tun forming during degydration (right) (Crowe and Cooper, 1971).

2.5. Effect of dry-wet events on microbial activity in soils

2.5.1 Physical effects of dry-wet on soil

Moisture content

Soil moisture content influences soil respiration (Orchard and cook, 1983; Schimel *et al*., 1999; Harms and Grimm, 2012; Kim *et al*., 2012). On the other hand, Ruser *et al*. (2006) reported that CO_2 production from a fine-loamy soil fertilized with nitrate was not strongly affected by soil moisture content.

Length of drying and rewetting

Muhr *et al*. (2010) examined the effect of dry-wet events with different drying intensity on soils from forested soils. They had three different drought treatments which were 16, 35 and 47 days. CO_2 flux from the soil was greatest? after the longest drying period. There was no significant effect on CO_2, N_2O, or CH_4 soil effux with intensity of rewetting (Muhr *et al.*, 2008).

2.5.2 Chemical effects of dry-wet on soil

Soil nutrient availability

Dry-wet events affect decomposition rates of organic C in soil (Fierer and Schimel, 2002). Decomposition rate after drying and rewetting depends on amount of C in the soil (Birch, 1958). Previous studies found that soil DOC concentration increased after rewetting in northern alpine forests (Muhr *et al*., 2010) and agricultural land (Barton *et al*., 2008) but decreased in deciduous (oak) forest (Fierer and Schimel, 2002).

In field and laboratory studies, NH_4^+ and NO_3^- concentrations increased after rewetting of dry soils in agricultural land (Barton *et al*., 2008) and northern alpine forest (Muhr *et*

al., 2010). NH_4^+ and NO_3^- concentration tended to be greater in the surface soil (0-50mm) than at greater soil depths and increased in response to the application of N fertilizer and/or rainfall (Barton *et al*., 2008).

Greenhouse gas emission

In field studies, CO_2 flux increased after rewetting of dry soils have been observed in soils from agricultural land (Kessavalou *et al*., 1998), pasture (Xu and Baldocchi, 2004), grassland (Joos *et al*., 2010), forest (Kim *et al*., 2010; Sakabe *et al*., 2016), savannas (Castaldi *et al*., 2010), and desert (Sponseller and Fisher, 2008). CO_2 emission increased quickly after rain fall (Sakabe *et al*., 2016). In laboratory studies, CO_2 flux increases after rewetting of dry soils have been observed in soils from agricultural land (Beare *et al*., 2009), pasture (Wu *et al*., 2010), grassland (Fierer and Schimel, 2003; Xiang *et al*., 2008), forest (Muhr *et al.,* 2008; Fierer and Schimel, 2003), peatland (Goldhammer and Blodau, 2008) and desert (Sponseller and Fisher, 2008). CO_2 flux from soil reduced during drought periods (Muhr *et al.,* 2008; Muhr *et al*., 2010). andrapidly increased after rewetting of soil (Muhr *et al*., 2010) and quickly recovered back to pre-drought levels (Muhr *et al.,* 2008).

In field studies, N_2O flux increased after rewetting of dry soils in agricultural land (Barton *et al*., 2008), pasture (Kim *et al*., 2010), fen (Goldburg *et al*., 2010), grassland (Hao *et al*., 1988), tropical forest (Butterbach-Bahl *et al*., 2004) and savannah (Martin *et al*., 2003). In laboratory studies, N_2O flux increased after rewetting of dry soils in agricultural land (Beare *et al*., 2009), peat (Dinsmore *et al*., 2009), grassland (Yao *et al*., 2010) and northern alpine forest (Muhr *et al.*, 2008). N_2O emissions from agricultural soils increased with elevation of water-filled pore space (WFPS), soil mineral N

concentrations,surface soil temperatures, immediately following the planting of the crop and following summer rainfall events but were not affected by the application of N fertiliser (Barton *et al*., 2008). N_2O emissions following rewetting events were related to increasing NH_4 concentration (Barton *et al*., 2008).

In laboratory studies, no response or small CH_4 increases after rewetting of dry soils have been observed in soils from Northern alpine forest (Muhr *et al.,* 2008), alpine tundra (West and Schmidt, 1998) and forest (Sakabe *et al*., 2016).

2.5.3 Biological effects of dry-wet on soil

To better understand how dry-wet events affect soil microbial, soil C and N processes is a major challenge (Xiang *et al*., 2008). The amount of C and N from soils after the drying-wet events generally increases (Winsor and Polard, 1956). The increase of C and N was probably the result of (1) substrate supply (Xiang *et al*., 2008) (2) releasing of labile substrates of microbial cell lysis (Fierer *et al*., 2003) or (3) osmoregulation (Fierer *et al*., 2003; Schimel *et al*., 2007).

Previous studies found that dry-wet events dramatically influence microbial activity and biomass in soils (Bottner, 1985). In an early study, the dry-wet events generally were thought to enhance decomposition of organic matter, because the dry-wet events increased the availability of decomposable substances due to release at microbial death into the soil (Soulides and Allison, 1961; Denef *et al*., 2001). Numerous studies have been conducted to explore the effects of dry-wet events on microbial biomass using chloroform fumigation technique (Bottner, 1985, Schimel *et al*., 1999; Fierer and Schimel, 2002). Microbial biomass C declined significantly as a result of air-drying of soil (Bottner, 1985; Sparling *et al*., 1985). Soil drying damaged 1/3 to l/4 of biomass (Bottner, 1985).

After rewetting, the biomass recovered quickly to approximately the same size as before drying (Bottner, 1985). Drying event killed more of the younger microbial cells than the older ones (Soulides and Allison, 1961). On the other hand, no change in soils under oak tree and grassland soil has been observed (Fierer and Schimel, 2002). Fierrer *et al*. (2003) investigated the effects of dry-wet events on microbial community composition and structure using the terminal restriction fragment length polymorphism methods. They found that dry-wet events influenced bacterial community composition in soil under oak tree from California, USA, but there was no change in soil from grassland (Fierrer *et al*., 2003). Pesaro *et al*. (2004) also studied the effect of dry-wet events on microbial soil characteristics using the terminal restriction fragment length polymorphism method. They also observed a shift in bacterial community composition in soils under oak trees from Switzerland d after several dry-wet cycles.

Microbes must accumulate high concentrations of solute (osmolytes) to retain water inside of the microbe's cell and to avoid dehydrating and drying (Schimel *et al*., 2007). Bacteria typically accumulate amino compounds such as proline, glutamine, glycine betaine (Csonka, 1989; Schimel *et al*., 2007). These amino compounds may account for between 7% and 20 % of total C (Koujima *et al*., 1978; Killham and Firestone 1984; Schimel *et al*., 2007) and between 11 % and 30 % of total N (Schimel *et al*., 2007). Fungi typically accumulate polyols such as glycerol, erythritol, mannitol (Witteveen and Visser, 1995; Schimel *et al*., 2007). These polyols may account for over 10 % of cell mass (Tibbett *et al*., 2002; Schimel *et al*., 2007). Besides the above, other molecules (e.g., trehalose and ectoine) are produced under extreme conditions (Schimel *et al*., 2007; Bouskill *et al*., 2016). During times of drought, total cytoplasmic constituents can account for 30-40 % of total C and 20-60 % for both fungi and bacteria and 20 % and 60 % of

total N for fungi and bacteria, respectively, compared to only 3-6 % of C and N under unstressed conditions (Schimel *et al*., 1989, 2007). When water rewets, enzyme lysozyme hydrolyzes the cell wall polysaccharide, thereby weakening the cell. Water molecules can pass through the wall from low concentration to high concentration to equal a balance in a process called osmosis. However, osmolytes do not penetrate the cell. When water flows into the cell, it swells and bursts. To avoid rupture and death, microbes need to dispose of osmolytes rapidly on rewetting either by respiration, polymerizing or transporting them across cell membrane (Schimel *et al*., 1989, 2007). The large pulse of CO_2 release on rewetting is of results that osmolytes is metabolized (Schimel *et al*., 2007). In contrast, there was no evidence of a large pulse of osmolytes in soil solution on rewetting (Warren, 2014). Concentration of TON decreased within 1-3 h (Warren, 2014) and LMW organic N compounds are rapidly taken by microbes and plants (Jones and Murphy, 2007; Boddy *et al*., 2008; Jones and Kielland, 2012; Roberts and Jones, 2012). Moreover, it is unclear whether osmolyte accumulation under drought condition, given that previous studies observed significant increase osmolytes production (Warren, 2014; Bouskill *et al*., 2016) or no effect (Williams and Xia, 2009; Boots *et al*., 2013; Göransson *et al.*, 2013; kakumanu *et al.*, 2013).

Enzyme activity tends to decline during drought conditions (Toberman *et al*., 2008; Steinweg *et al*., 2012; Manzoni *et al*., 2014), however Hydrolytic enzyme activity responsible for degradation of organic C was higher in soils under drought conditions (Alster *et al*., 2013; Bouskill *et al*., 2016), while oxidative enzyme activity did not change or declined (Alster *et al*., 2013). The concentration of organic compounds was lower under treatment comparing to control soils (Bouskill *et al*., 2016). Extra-cellular enzyme may still able to break down organic matter (Manzoni *et al*., 2014; Bouskill *et al*., 2016)

even if microbial activity is low.

2.6 Conclusions

Previous studies have frequently observed significant changes in nutrient availability, GHG emissions and microbial biomass in soils after freeze-thaw or dry-wet events. However, the mechanisms underlying these effects are not well characterized. Understanding of how freeze-thaw or dry-wet events affect C and N cycling in soils is crucial.

2.7 References

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Chapter 3

Extreme freeze-thaw and dry-wet events alter microbial C pools

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Abstract

Freeze-thaw and dry-wet events are common stressors to soil microbes. Further, many studies have frequently observed a pulse release of CO_2 release from soil after thawing or rewetting of soils. The enhanced release of CO_2 to the atmosphere may have important implications for soil carbon (C) storage and GHG emissions. This study aims to investigate the effect of freeze-thaw and dry-wet events on microbial activity in two contrasting soils collected from either the UK or Antarctica. We applied three treatments 1) freeze-thaw (-5 ˚C), 2) freeze-thaw (-20 ˚C), and 3) dry-wet. We added ^{14}C-labelled and non-labelled C-glucose to the soils and measured organic C and N, inorganic N and ^{14}C in soil solution. Our results showed a significant increase of DOC, TON, and ^{14}C after rewetting and thawing of the soils. This suggests that a large proportion of soil microbes were killed by freeze-thaw or dry-wet events, leading to the supply of necromass to the organic matter pool. Alternatively, it could be associated with the accumulation of high concentration of solutes (osmolytes) inside of the cell during freezing or drying conditions and their subsequent release into soil upon stress removal. NH_4^+ and NO_3^- increased after thawing or rewetting, likely due to the ability of surviving microbes to process NH_4^+ and NO_3^- from microbially-derived organic N. We also observed a significant increase of $^{14}CO_2$ from soils. This suggests that surviving microbes rapidly mineralized the C released into the soil. In conclusion, the results suggest that freeze-thaw or dry-wet events significantly altered C and N cycling.

Keywords: Soil microbes, Freezing, Drying, CO_2, ^{14}C, Carbon cycling

3.1 Introduction

Freeze-thaw and dry-wet events are important and frequent phenomenon in terms of soil hydrology and thermodynamics (Spaans and Baker, 1996). Both freeze-thaw and dry-wet events are changes which are commonly observed under natural environmental conditions and may induce stress within the soil microbial community. Thawing or rewetting generally increases microbial activity in soils (Edwards and Cresser, 1992; Muhr *et al*., 2010). Previous studies have frequently observed a pulse of CO_2 from topsoil after freeze-thaw (Herrmann and Witter, 2002; Schimel and Clein, 1996) or dry-wet events (Kessavalou *et al*., 1998; Beare *et al*., 2009; Wu *et al*., 2010). The enhanced release of CO_2 to the atmosphere may have important implications for soil C storage and GHG emissions. However, the origin of this C and the mechanisms responsible for its release have not been well characterized.

Freeze-thaw or dry-wet events may affect soil microbial biomass (Soulides and Allison, 1961; DeLuca *et al*., 1992) due to cell damage and/or degradation of cell materials in response to latent heat. At freezing temperature, solidification of lipid membranes (Methe *et al*., 2005) and rupture of cells by ice crystals (Rivkina *et al*., 2000) are potentially fatal to soil microbes (Schimel *et al*., 2007). It was suggested that soil freezing killed up to 1/2 of the microbial biomass (Soulides and Allison, 1961; Skogland *et al*., 1988; DeLuca *et al*., 1992) and soil drying damaged 1/3 to 1/4 of biomass (Bottner, 1985). However, a number of studies have observed an increase in respiration and nutrient availability after freeze-thaw or dry-wet events with no significant reduction in microbial biomass (Grogan *et al*., 2004; Butterly *et al*., 2009).

Soil microbes have evolved a range of strategies to be able to overcome extreme environmental conditions. For example, it is widely known that soil microbes can

accumulate high concentrate of solutes (osmolytes) when cells are exposed to stressful conditions (Killham and Firestone, 1984; Kol *et al*., 2010). Bacteria and Archaea accumulate many different organic osmolytes, including amino acids (e.g. proline, glutamine, and glycine betaine), ectoine, and trehalose (Burg and Ferraris, 2008). Fungi produce different polyols such as erythritol, mannitol, glycerol (Witteveen and Visser, 1995) in response to stress. Microbes are also known to synthesize protective molecules (e.g. trehalose) (Koide *et al*., 2000) and antifreeze proteins (Bae *et al*., 2004) in response to freezing conditions. Although the microbial community has the potential to adapt to freezing or drying stresses, their physiological responses remain poorly characterized.

Our aim was therefore to investigate the effects of a single freeze-thaw or dry-wet event on soil C and nitrogen (N) cycling. We subjected soils to three treatments freezing (-5 °C and -20 °C) and drying, and determined 1) soluble C and N availability, 2) microbial activity in soils by pre-labelling the microbial biomass with ^{14}C prior to imposing a stress. We hypothesized that C and N in soil solution would increase after freeze-thaw or dry-wet events as a result of damage to microbial cells or disposing of accumulated osmolytes into the soil. Furthermore, the increased C in soil solution after either freeze-thaw or dry-wet event would be rapidly mineralized by surviving microbes leading to an increased CO_2 efflux.

3.2 Materials and Methods

3.2.1 Sample collection

Soil samples were taken from two different grasslands (Table 3.1). The first soil was obtained under *Lolium perenne* L. from a Eutric Cambisol in a temperate agricultural

grassland located at Abergwyngregyn, Gwynedd, North Wales (53°N14'N, 4°N01'W). The mean annual soil temperature at 10 cm depth is 10.6 °C and the mean annual rainfall is 1055 mm yr^{-1}. Fertilizer was regularly added (50 kg N ha^{-1} y^{-1}, 10 kg P ha^{-1} y^{-1}, 10 kg K $ha^{-1}y^{-1}$). The soil was sieved to pass 2 mm, removing stones, roots and earthworms.

The second location was Signy Island, South Orkney Islands (60°N63'S, 45°N35'W). The mean annual soil temperature is ca.-2 °C (Gugliemin *et al*., 2012). Immediately prior to use, samples were collected under swards of Antarctic hair grass (*Deschampsia antarctica*) where they had been maintained in a climate controlled growth room at temperature 7 °C in Bangor University.

pH and electrical conductivity (EC) were determined in a 1:5 (w/v) soil-distilled water extract (Smith and Doran, 1996). Soil moisture and organic matter content were determined by oven-drying for 24 h at 105 °C and 450 °C, respectively.

3.2.2 Freeze-thaw or dry-wet treatments

Either single freeze-thaw or dry-wet events were applied to the soil samples. The samples were exposed to either freezing (-5 ˚C or -20 ˚C) or air-drying. Samples for freezing treatments were placed in incubators at -5 ˚C or -20 ˚C for 24 h then thawed at +5 ˚C. Temperatures of -5 ˚C and -20 ˚C were chosen to reflect temperatures likely to be experienced by soils under natural conditions in the UK (-5 ˚C) and Antarctica (-20 ˚C).

Soil samples in the drying treatments (kept at +5 ˚C throughout) were dried by passing air over the samples at a rate of 0.85 - 0.88 m s^{-1} (Figure S1) until they had reached an air-dry state (ca. 3 - 6.5 h) they were then maintained in a dry state for 21 h. After drying for 24 h, samples were re-wetted by adding distilled water back to the soil to reach initial water content (36.4 ± 0.10 % for temperate grassland soils, 257.8 ± 15.7% for polar

grassland soils). On either thawing or rewetting, the samples were transferred to an incubator where the temperature was maintained at +5 ˚C. Soil samples that were kept at +5 ˚C were used as a control. The experiment therefore consists of four main treatments (4 replicates per treatment (n = 4)):i) Control (+5 ˚C), ii) Dry-wet (+5 ˚C), iii) Freeze-thaw (-5 ˚C / +5 ˚C), iv) Freeze-thaw (-20 ˚C / +5 ˚C).

3.2.3 Labelling of microbial biomass

We chose ^{14}C-labelled D-glucose for this study as it represents one of the simple C compounds commonly found in soil and rapidly taken up by the soil microbial community (Hill *et al*., 2008). In order to determine which portions of microbial C are affected by dry-wet and freeze-thaw events, we performed three types of experiment (Figure 3.1):

(1) Experiment A: the ^{14}C glucose solution (1 μM; 3.5 kBq ml^{-1}) was added 5 min before applying freeze-thaw or dry-wet treatments (labelling the rapidly cycled labile C pool).
(2) Experiment B: the ^{14}C glucose solution (10 mM; 3.5 kBq ml^{-1}) was added 24 h before applying freeze or dry-wet treatments (labelling the microbial structural C pool).
(3) Experiment C: the ^{14}C glucose solution (10 mM; 3.5 kBq ml^{-1}) was added after applying freeze-thaw or dry-wet treatments (labelling the DOC pool).

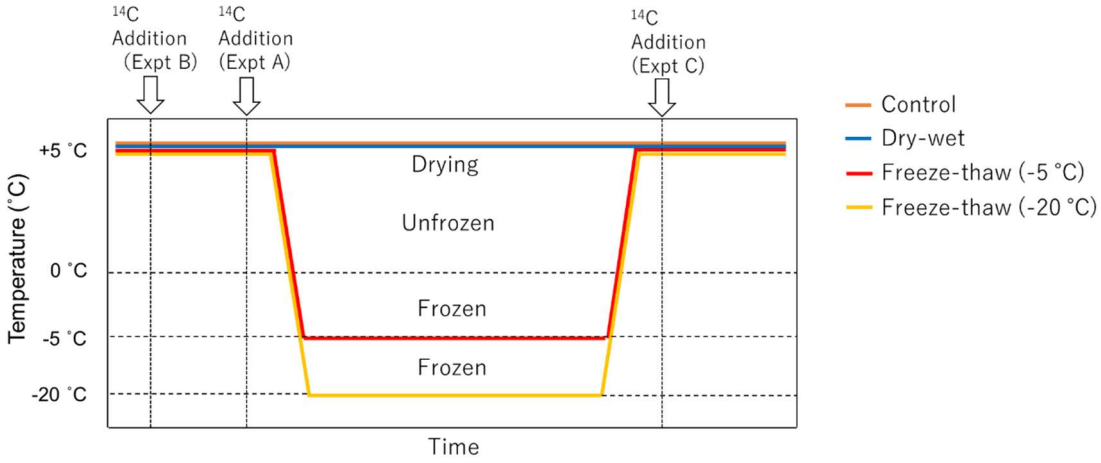

Figure 3.1. Schematic of experimental design. Control (+5 ˚C), Dry-wet (+5 ˚C), Freeze-thaw (-5 ˚C / +5 ˚C), and Freeze-thaw (-20 ˚C / +5 ˚C).

3.2.4 Microbial uptake of glucose in soil

Four replicate samples of each soil type were weighed (1 g for temperate grassland soil and 0.5 g for polar grassland soil) and placed into a 1.5 ml micro-centrifuge tube with a hole pierced at the bottom. This tube was then placed inside another intact micro-centrifuge tube. ^{14}C-labelled glucose solution (10 mM) was added to the soil surface and the soils were incubated at +5 °C and either freeze-thaw or dry-wet treatments were applied as described in the section 2.2. The amount of ^{14}C in the soil solution was measured at various times. The soil samples were centrifuged (6850 *g*, 5 min) and the soil solution was passed into the lower tube. 0.75 ml of soil solution was added to a scintillation vial and the amount of ^{14}C-glucose remaining in the soil was determined by liquid scintillation counting using a Wallac 1404 scintillation counter (Wallac EG&G, Milton Keynes, UK) after adding 800 µl of distilled water and 4 ml of scintillation liquid.

3.2.5 Glucose mineralization

Four replicate samples of each soil type were weighed (1 g for temperate grassland soil and 0.5 g for polar grassland soil) and placed into 50 cm^3 polypropylene tubes. ^{14}C-labelled glucose solution (100 µl for temperate grassland soil and 50 µl for polar grassland soil) was added to the soil surface and a 5 M NaOH trap (200 µl) was placed into each tube to capture any evolved $^{14}CO_2$. The traps were exchanged at varying times after adding ^{14}C-glucose solution. At each time point, $^{14}CO_2$ in the NaOH trap was determined by liquid scintillation counting as described in section 2.4. After the final NaOH trap was removed, the amount of ^{14}C glucose remaining in the soil was determined by extracting soils with 0.5 M K_2SO_4 (1:5 w/v). The soil samples were shaken (15 min, 200 rev min^{-1}) and centrifuged (18,000 g, 5 min) and measured by liquid scintillation counting as described above.

3.2.6 Chemical analysis

The amount of soluble C and N were measured [according to Hill *et al*. (2008)] at various time points before (1, 3, 6, and 24 h) and after (0, 3, 6, 24, 48, and 72 h) applying freeze-thaw or dry-wet treatments. DOC and TDN analysis was determined (following centrifuging at 6850 *g* for 5 minutes) on soil solution (1:1 w/v extract) using a multi N/C 2100 (Analytik Jena AG, Jena, Germany).

3.2.7 Statistical and data analysis

To describe ^{14}C glucose mineralization, Exponential equations were fitted to the experimental results using Sigmaplot 14. Microbial uptake of ^{14}C in the soil solution was described by a first-order single exponential decay equation (Figure S2):

$y = a1 \exp(-k1t)$

where y represents the amount of ^{14}C remaining in the soil, a1 describes initial pool in microbial biomass (Glanville *et al*., 2016), k1 describe the exponential coefficient describing depletion by the soil microbial community, *t* is time. The half-life of the soil solution glucose pool (a1) can defined as:

$T1/2 = In(2)/k1$

A double first-order exponential decay equation was used to describe substrate ^{14}C glucose mineralization (Figure S2):

$y = [a2 \exp(-k2t)] + [a3 \exp(-k3t)]$

where a2 and a3 represent the size of pools (first pool and slower pool, respectively), k2 is the exponential coefficient describing the primary mineralization phase, and k3 is the exponential coefficient describing the secondary mineralization of the microbial biomass. The first rapid phase (k2) of $^{14}CO_2$ production as substrate is immediately use for catabolic processes (i.e. respiration) (Body *et al*., 2007), the remaining substrate is immobilized within the microbial biomass (i.e. cell growth or storage C polymers) (Hill *et al*., 2008). The slower second phase (k3) of $^{14}CO_2$ production is attributable to the subsequent turnover of the soil microbial community or storage C polymers (Hill *et al*., 2008). The half-life of the soil solution glucose pool (a2) can defined as:

$T1/2 = In(2)/k2$

T-tests were carried out to evaluate differences between the temperate and polar soil for a suite of soil characteristics (pH, EC, soil moisture content, organic matter content, DOC, TDN, ammonium, nitrate). A one-way analysis of variance (ANOVA) was performed to determine the effect of the three stress treatments on microbial activity in soils. To compare differences between pairs of treatments, multiple comparisons were

carried out by Tukey post-hoc test at significance level of 0.05. Repeated measures analysis of variance (ANOVA) randomized block design was carried out to evaluate cumulative $^{14}CO_2$ efflux over time. All statistical analyses were performed using RStudio 0.99.486 (R Development Core Team, 2004).

3.3 Results

3.3.1 Soil extractable C

The response of soil extractable DOC pools to freeze-thaw or dry-wet events were different for the temperate and polar grassland soils. For the temperate grassland soil, increases in DOC occurred immediately after thawing and rewetting in all treatments (Figure 3.2). The greatest flush in DOC occurred in the dry-wet treated soil, where up to 176 ± 5 mg/L was released after rewetting. This was significantly different from DOC concentrations in the control treatment ($p < 0.001$). In the freeze-thaw (-20 °C) treated soil, released DOC concentration after thawing was 57 ± 2 mg/L ($p < 0.001$) and the least impacting treatment was the freeze-thaw (-5 °C) treated soil, at 27 ± 1 mg/L ($p < 0.05$). In most cases, any induced increase in the DOC concentration decreased gradually over time after thawing and rewetting. In contrast, for the polar soil, there was no significant difference in DOC between groups after freeze-thaw or dry-wet events.

Table 3.1. Soil properties. Values represent means ± SEM (n = 4). *** indicates a significant difference between soils ($p < 0.001$) while *NS* indicates no significant difference ($p > 0.05$). Where appropriate, values are expressed on a dry weight basis.

	Temperate grassland	Polar grassland	p value
pH	5.47 ± 0.02	5.71 ± 0.02	***
EC (μS cm^{-1})	121 ± 9	166 ± 27	*NS*
Moisture content (%)	36.4 ± 0.10	257.8 ± 15.7	***
Organic matter content (%)	6.53 ± 0.02	49.2 ± 4.18	***
Dissolved organic C (mg C/L)	13.8 ± 1.2	183.0 ± 12.9	***
Dissolved organic N (mg N/ L)	55.5 ± 1.3	29.2 ± 1.4	***
Ammonium (mg N/L)	0.18 ± 0.01	3.77 ± 0.03	***
Nitrate (mg N/L)	59.8 ± 1.9	25.0 ± 0.8	***

3.3.2 Soil extractable N

TDN, NH_4^+, and NO_3^- in soil solution for all location-stress treatment combinations is shown in Figure 3.2. For the temperate grassland soil, TDN and NH_4^+ concentration in soil solution was significantly greater than the control treatment 3 h after dry-wet events ($p < 0.001$), with NO_3^- greater than the control after 48 h ($p < 0.01$). TDN and NO_3^- in soil solution after freeze-thaw (-20 °C) events were greater than the control after 48 h ($p < 0.05$). NO_3^- in soil solution after freeze-thaw at -5 °C were significantly different to the control treatment ($p < 0.05$) after 24 h, but TDN and NH_4^+ remained similar to the control treatment.

In contrast, TDN and NO_3^- in the polar grassland soil were greater than the control 48-72 h after rewetting ($p < 0.05$). However, TDN, NH_4^+, and NO_3^- in soil solution were not influenced by freeze-thaw events. Overall, the effect of dry-wet events on nitrogen cycling for both soils was greater than freeze-thaw events.

3.3.3 Microbial uptake of ^{14}C in soil solution

Microbial uptake of ^{14}C-glucose in soil solution for Experient A is shown in Figure 3.3C (temperate grassland soil) and Figure 3.3D (polar grassland soil). ^{14}C in all treatments was removed from soil solution rapidly (within 5 min) by microbes following addition of the ^{14}C-glucose substrate (over 99% for both temperate and polar grassland soils). Following rewetting of soils, ^{14}C in the temperate grassland soil (6-8 %, $p < 0.001$) and (10 %, $p < 0.001$) in polar soil were greater than in the control. In contrast, after freeze-thaw (-20 ˚C) events, ^{14}C in polar soil (5 %, $p < 0.05$) was greater than in the control, however, there was no effect on ^{14}C in the temperate soil ($p > 0.05$). The ^{14}C production after rewetting or thawing in both soils declined over time. For both soils, there was no significant difference in ^{14}C between freeze-thaw (-5 ˚C) events and the control treatment.

Microbial uptake of ^{14}C-glucose in soil solution for Experient B is shown in Figure 3.4C (temperate grassland soil) and Figure 3.4D (polar grassland soil). ^{14}C in all treatments was removed from soil solution rapidly by microbes following addition of ^{14}C-glucose substrate (over 99% for both temperate and polar soils). After dry-wet events, ^{14}C in the temperate grassland soil (18 %, $p < 0.001$) and polar soil (30 %, $p < 0.001$) were greater than the control. After freeze-thaw (-20 ˚C) events, ^{14}C increased 3 % in the temperate soil ($p < 0.05$) and increased 5 % in the polar soil ($p < 0.05$). The released of ^{14}C in both soils after either rewetting or thawing was removed over time. There was no significant effect of freeze-thaw (-5 ˚C) events on both soils ($p > 0.05$).

Microbial uptake of ^{14}C-glucose in soil solution for both soils after freeze-thaw or dry-wet events (Experiment C) is shown in Figure 3.5C (temperate grassland soil) and Figure 3.5D (polar grassland soil). After dry-wet events, the rate of ^{14}C glucose removal

from soil solution for the temperate and polar was slower than control ($p < 0.05$ and $p < 0.001$, respectively). In contrast, after freeze-thaw (-5 ˚C and -20 ˚C) events, ^{14}C-glucose removal from soil solution for both soils were quite similar to control ($p > 0.05$).

Overall, effects of freeze-thaw or dry-wet events on ^{14}C in soil solution was different between treatments, with the effects of treatments in order of dry-wet > freeze-thaw (-20 ˚C) > freeze-thaw (-5 ˚C). The rate of ^{14}C depletion in the polar grassland soil after treatments tended to be slower than for the temperate grassland soil ($p < 0.01$).

3.3.4 Kinetics of $^{14}CO_2$ evolution

Cumulative $^{14}CO_2$ evolution from the temperate and polar grassland soil for Experiment A is shown in Figure 3.3A and (temperate grassland soil) and Figure 3.3B (Polar grassland soil). After addition of ^{14}C-glucose substrate to the soils within 5 min, ^{14}C-glucose was respired rapidly from the fast pool (a2, Figure S3) in all treatments from both soils, respiring ~5 % and ~1% (temperate and polar soil, respectively). After thawing or rewetting, cumulative $^{14}CO_2$ evolution in all treatments for both soils was less than control ($p < 0.001$ and $p > 0.001$, temperate and polar soil, respectively).

Cumulative $^{14}CO_2$ evolution from the temperate and polar soil for Experiment B is shown in Figure 3.4A and (Temperate grassland soil) and Figure 3.4B (polar grassland soil). After addition of ^{14}C-glucose substrate to the soils, ^{14}C-glucose was respired rapidly from the fast pool (a1) and the remaining respiration was from the slower pool (a3, Figure S3).

For the temperate grassland soil, cumulative $^{14}CO_2$ evolution from freeze-thaw (-20 ˚C) treated soil and dry-wet treated soil were significantly greater than control ($p < 0.001$ and $p < 0.01$, respectively). However, there was no change from freeze-thaw (-5

˚C) treated soil ($p > 0.05$). For polar grassland soil, cumulative $^{14}CO_2$ evolution did not differ from the control following freeze-thaw (-5 and -20 ˚C) or dry-wet events ($p > 0.01$).

Cumulative $^{14}CO_2$ from the temperate and polar soil for Experiment C is shown in Figure 3.5A (temperate grassland soil) and Figure 3.5B (polar grassland soil). Following addition of ^{14}C-glucose substrate to the soils, ^{14}C-glucose respiration in all treatments for both soils was rapid. For the temperate soil, in all treatments, mean $^{14}CO_2$ evolution within 1 hour was similar, respiring ~5%. However, $^{14}CO_2$ evolution 3 h after thawing (from -20 ˚C) and re-wetting was greater than control ($p < 0.001$). For the polar soil, cumulative $^{14}CO_2$ evolution 24 h after thawing (from -20 ˚C) was greater than the control ($p < 0.001$), but 24 h after re-wetting was less than observed in the control ($p < 0.001$).

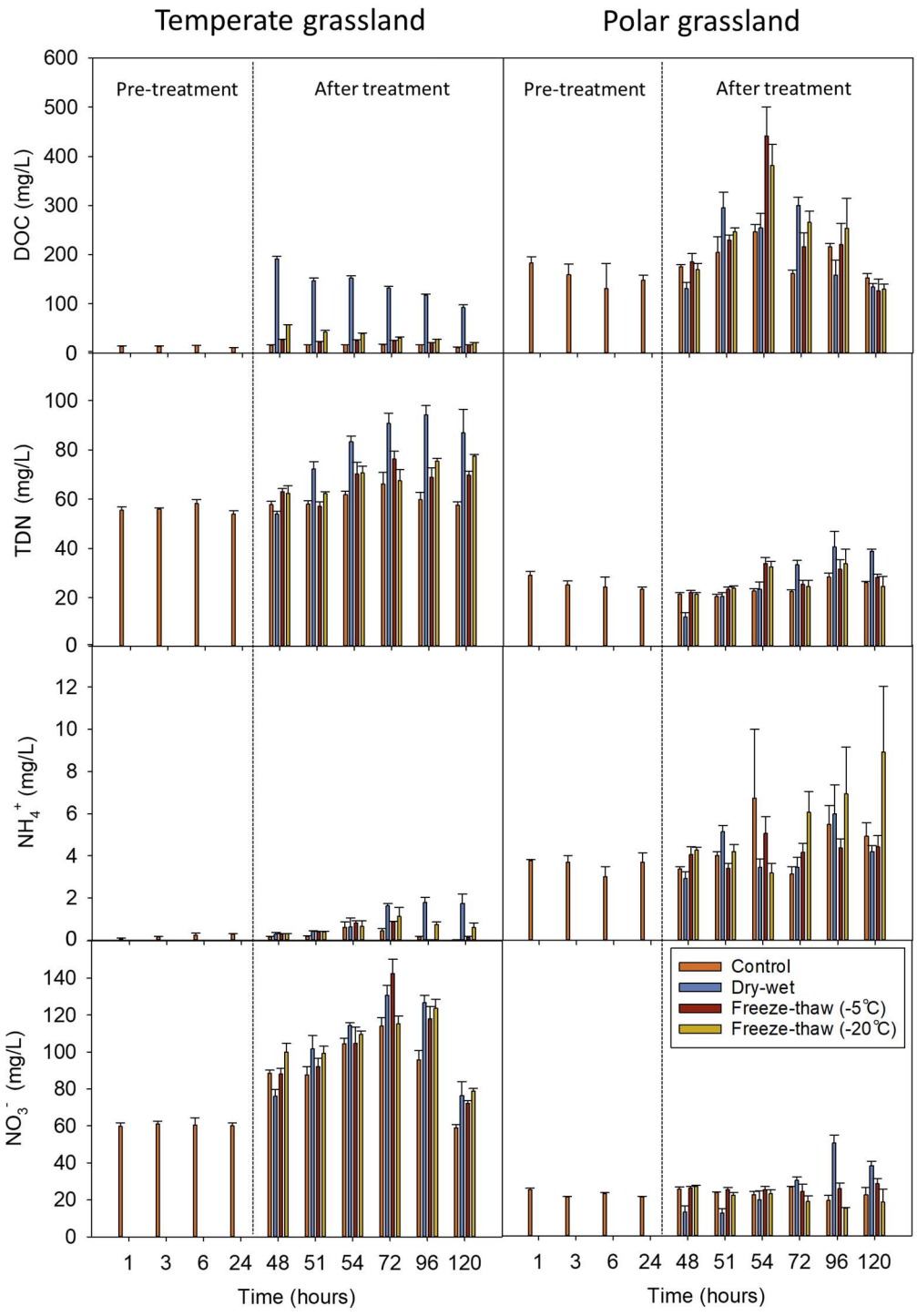

Figure 3.2. Dynamics of dissolved organic C (DOC), Total dissolved N (TDN), NH_4^+, and NO_3^- before and after applying a single freeze-thaw (-5 or -20 °C) or dry-wet event for the temperate and polar grassland soils. Values represent means ± SEM (n = 4). The dotted lines denote the times at which the conditions were changed.

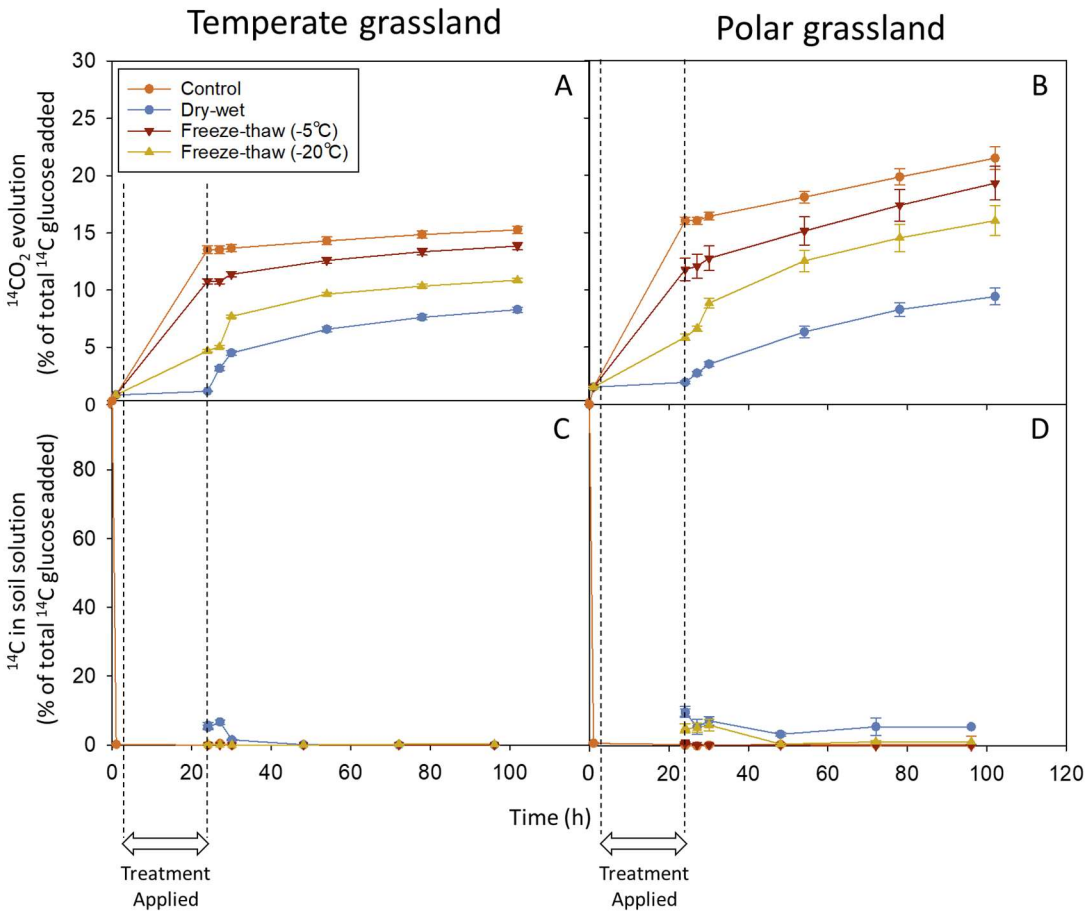

Figure 3.3. Effects of a single freeze-thaw (-5 or -20 °C) or dry-wet event on the cycling of ^{14}C held in the labile microbial C pool (Experiment A). Cumulative $^{14}CO_2$ evolution from the temperate grassland soil (A) and polar grassland soil (B). Amount of ^{14}C-label in soil solution after the addition of ^{14}C-glucose to the temperate grassland soil (C) and polar grassland soil (D). Values represent means ± SEM (n = 4). The dotted lines denote the times at which the conditions were changed.

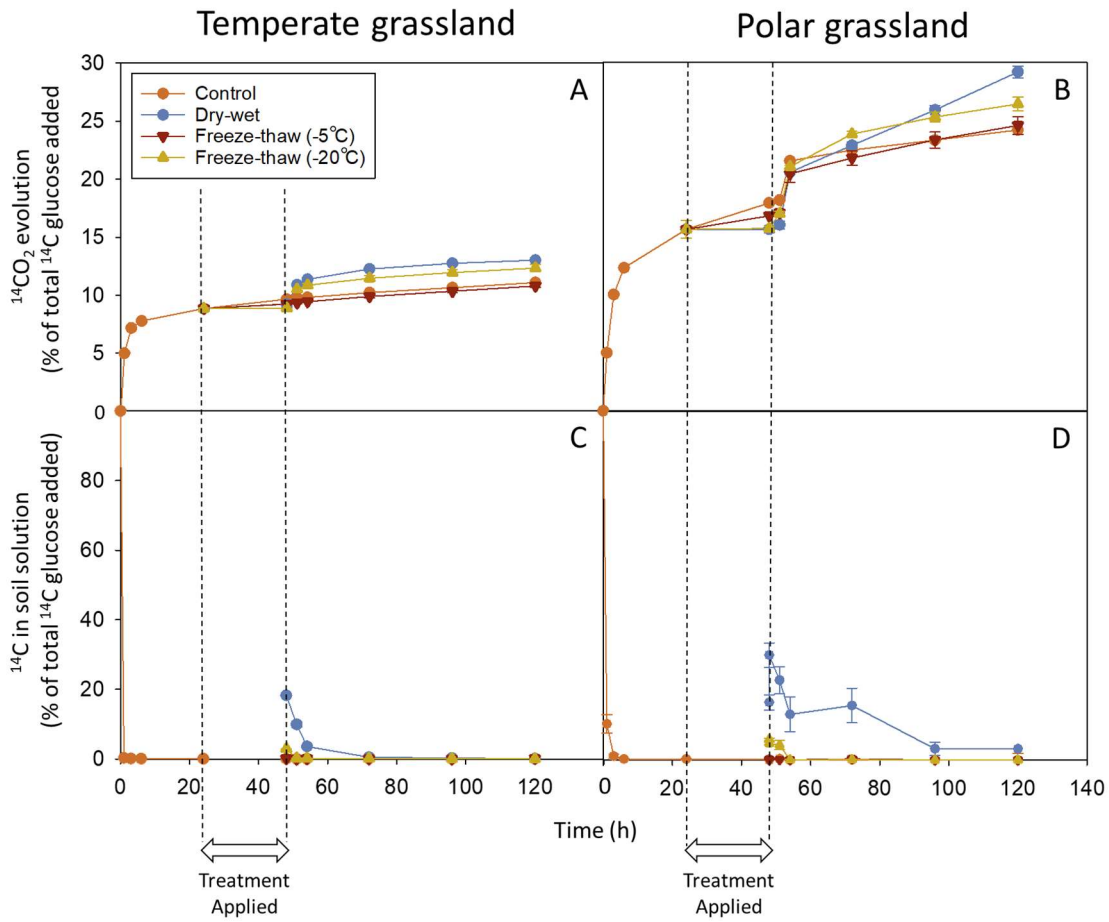

Figure 3.4. Effects of a single freeze-thaw (-5 or -20 °C) or dry-wet event on ^{14}C turnover held in the microbial structural C pool (Experiment B). Cumulative $^{14}CO_2$ evolution from the temperate grassland soil (A) and polar grassland soil (B). Amount of ^{14}C-label in soil solution after the addition of ^{14}C-glucose to the temperate grassland soil (C) and polar grassland soil (D). Values represent means ± SEM (n = 4). The dotted lines denote the times at which the conditions were changed.

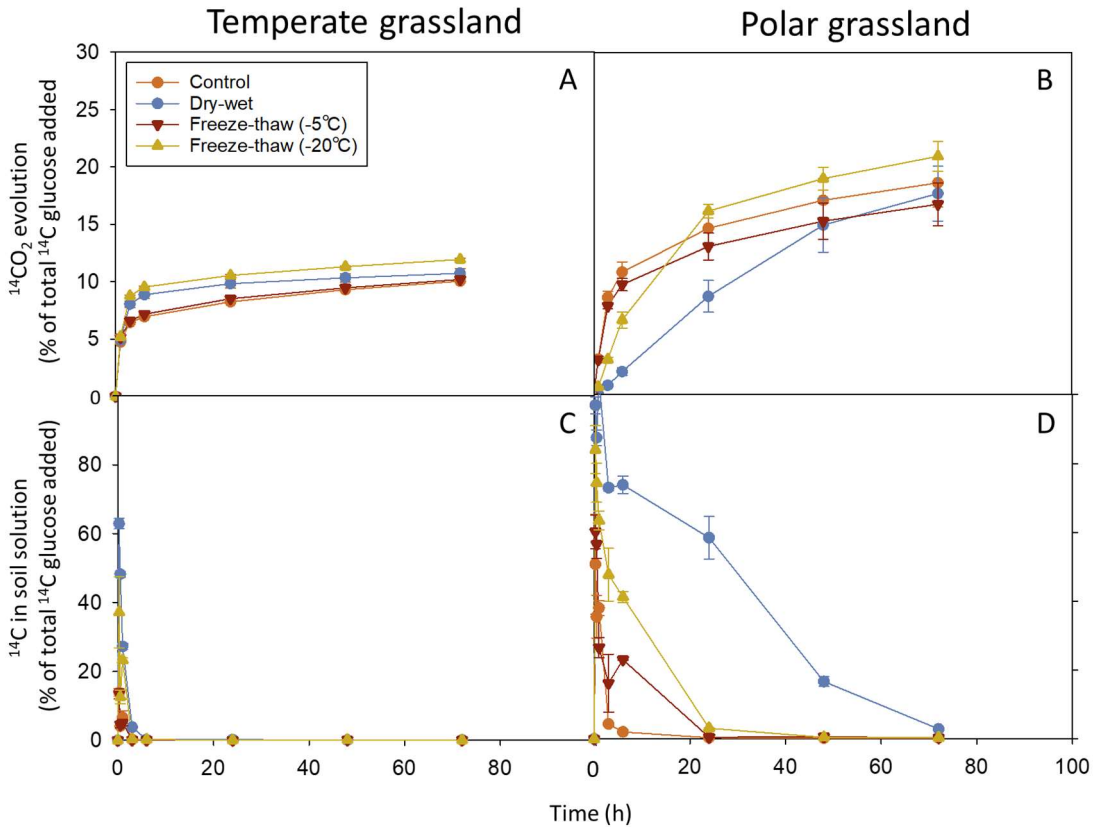

Figure 3.5. Effects of a single freeze-thaw (-5 or -20 °C) or dry-wet events on microbial activity (^{14}C DOC pool) in soil. (Experiment C). Cumulative $^{14}CO_2$ evolution from the temperate grassland soil (A) and polar grassland soil (B). Amount of ^{14}C-label in soil solution after the addition of ^{14}C-glucose to the temperate grassland soil (C) and polar grassland soil (D). Values represent means ± SEM (n = 4).

3.4 Discussion

3.4.1 C and N dynamics

As expected, DOC concentration in the temperate grassland soil substantially increased after freeze-thaw or dry-wet events. This is consistent with other studies in grassland soil after dry-wet cycling (Merckx *et al*., 2001). Increased DOC in soils after thawing or rewetting, suggesting that there are three main reasons: 1) A large proportion of soil microbes could be killed by freeze-thaw (Soulides and Allison, 1961) or dry-wet events (Clein and Schimel, 1994), which could increase organic matter. 2) soil microbes could accumulate high concentration of solutes (osmolytes) inside of the cell during freezing or drying conditions (Schimel *et al*., 2007). Disposal of the accumulated osmolytes into soil during thawing or rewetting could increase DOC concentration (Schimel *et al*., 2007). 3) Freeze-thaw or dry-wet events could disrupt soil structure. Disruption of soil aggregates or soil pores resulting increase exposure of organic C (Utomo and Dexter, 1982; Lundquist *et al*. 1999; Xian *et al*., 2008). We investigated the biological effects on soils using ^{14}C labelled glucose (as discussed in section 3.4.2-3.4.4), but we did not measure the physical effects on soils. Here we observed an increase in DOC which then decreased over time, presumably due to decomposition by the surviving microbial community (Kieft *et al*., 1987; Van Gestel *et al*., 1993).

Our results revealed that N mineralization rate increased in the temperate grassland soil after freeze-thaw (-20 ˚C) or dry-wet event. This is consistent with other studies in agricultural soil (DeLuca *et al*., 1992). Increases of soluble organic N concentration in the temperate grassland soil after freeze-thawing (-20 ˚C) or dry-wet event was probably due to denatured microbial protein and/or disruption of microbial cells providing a substrate for ammonification and nitrification. Another possible

explanation is that microbes may rapidly dispose of accumulated N-containing osmolytes into soil (e.g. amino acids). These would have a low C:N ratio and would favour mineralization.

After thawing (following freezing at -20 ˚C) or rewetting, we observed NH_4^+ increased rapidly, followed by slower increase of NO_3^- in the temperate grassland soil. Soluble organic N (proteins, peptides, and amino acids) were probably taken up rapidly by surviving microbes and processed to NH_4^+ (Jones and Kielland, 2012; Roberts and Jones, 2012). Transformation of NH_4^+ to NO_3^- was slow because sorption of NH_4^+ to cation exchange sites could decrease rates of nitrification (Jiang *et al*., 2011; Jones and Kielland, 2012). More transformation of NO_3^- from NH_4^+ could potentially lead to greater N_2O or N_2 losses, especially at high soil water contents (Jones *et al*., 2018).

On the other hand, after dry-wet event, there was little or no change in DOC and TDN in polar soils after freeze-thaw event, suggesting that soil microbes in polar soils have frequently experienced to extreme temperature and probably more tolerant to freeing conditions. This is consistent with the minimum effects on DOC in other grassland soil (Foster *et al*., 2016). We also observed increase of NO_3^- in polar grassland soil, which is consistent with temperature grassland soil (Figure 3.2). Nitrification of ammonium ions to nitrite and thence to nitrate is commonly a rapid process in soil (Lees, 1951). This explanation could be why we did not observe pulse of NH_4^+.

3.4.2 Dynamics of microbial C pools

Microbial labile ^{14}C pool

Almost all of the added ^{14}C-glucose was rapidly taken up (within 5 min) by the soil microbial community in all treatments for both soils (Figure 3.3C and D). This pattern

is consistent with the results of Hill *et al*. (2008) in temperate grassland. ^{14}C-glucose was respired from fast pool (a2, Figure S3) immediately from both soils (Figure 3.4A and B), which occurred probably due to a lag between uptake and biosynthesis of the added ^{14}C-glucose (Bremer and van Kessel, 1990). This indicates that most of the added ^{14}C has been taken into the microbial cytosol before freeze-thaw or dry-wet events (Bremer and van Kessel, 1990).

Microbial structural ^{14}C pool

After the soil microbes had taken up the ^{14}C-glucose from the soils (Figure 3.5A and B), ^{14}C-glucose was respired immediately from fast pool (a2), while the remaining ^{14}C was attributed to the slow cycling C pool (a3, Figure S3). This pattern is also in agreement with the results Hill *et al*. (2008) in temperate grassland. The acquired ^{14}C by soil microbes is progressively transfered from the soluble cytoplasm pool into insoluble structural polymers over time (Glanville *et al*., 2016). Most of ^{14}C was probably incorporated in microbial structural components before applying freeze-thaw or dry-wet events (Bremer and van Kessel, 1990; Boddy *et al*., 2007).

3.4.3. Soil microbes are intolerant of freeze-thaw or dry-wet event

Our study of the effects of a single freeze-thaw or dry-wet on the ^{14}C-labelled soils is consistent with the conclusions of several reports that a freeze-thaw or dry-rewet event can kill up to 1/2 of the microbial biomass (Soulides and Allison, 1961; Bottner, 1985; Skogland *et al*. 1988, DeLuca *et al*., 1992). We observed that there was a ^{14}C flush from microbial structural pool in soil solution following rewetting. This suggests that these stress events significantly increased turnover of the microbial biomass. Microbial

structural ^{14}C were probably lysed upon rewetting (Fierer and Schimel, 2003) and ^{14}C was released into soil. The released ^{14}C cell materials could be available for surviving microbes to decompose. The pulse of ^{14}C in soil solution decreased over time, suggesting the ^{14}C was taken up by surviving microbes.

We also found that the rate of uptake of ^{14}C-glucose in DOC pools by soil microbes was slower than the control treatment after rewetting, suggesting that dry-wet events could be fatal to some soil microbes, probably due to protein denaturation (Steger, 1994). Loss of some microbes could reduce mineralization rate of soil organic matter (Fierer and Schimel, 2002).

We also observed that ^{14}C from microbial structural pool in soil solution after freeze-thaw (-20 ˚C) immediately increased and then decreased over time. The pattern is consistent with dry-wet event. Soil microbes can be damaged by ice crystals during freezing or thawing processes (Steponkus, 1984).

For the temperate grassland soil, the increase in respiration rate from fast pool above the control treatment suggests that surviving microbes which suffered from stress significantly increased ability to metabolize C. This hypermetabolic response to thawing or rewetting could have occurred after significant suffering from freezing or drying stress that could induce an increase of catabolism of glucose, metabolic rate, oxygen consumption, and lipolysis (Chioléro *et al*., 1997).

3.4.4 Soil microbes adapted to freeze-thaw or dry-wet event

Many studies have concluded that a pulse of CO_2 or mineralization after freeze-thaw or dry-wet events is largely due to biological disruption (DeLuca *et al*., 1992; Schimel and Clein, 1996; Van Gestel *et al*., 1993). Here we found that a large amount of

^{14}C was released from the microbial labile C pool soil solution after thawing (-20 ˚C) and rewetting. The flush of ^{14}C in soil solution could be rapidly increased turnover of labile C, probably due to damaged microbes by stress or disposing osmolytes (Schimel *et al*., 2007).

3.4.5 Control on C and N cycling

The different response to the three treatments in terms of C and N cycling indicates that the effects became more pronounced with increasing stress intensity. In our study, dry-wet event appeared to have the most significant effects on C and N cycling. Our drying treatment was probably less water in soil than freezing, which could inactive soil microbes (Borken and Matzner, 2009). Our results also showed that the effects of dry-wet event were greater in polar soil than temperate soil, probably reflecting the local climate conditions. In Antarctica, the soils are exposed to more extreme freeze-thaw events in summer than dry-wet events (Guglielmin *et al*., 2012). On the other hand, the soils in the UK are often exposed to mild freeze-thaw (-5 ˚C) (5 or 6 times a year), the soils frequently receive dry-wet events (100 times a year), whereas the soils very rarely experience extreme freeze-thaw (-20 ˚C) events. Another explanation is high soil organic matter (SOM) components in polat grassland soil may influence wettability (Bachmann *et al*., 2008) as high SOM contents is known to enhance water retention (Rawls *et al*., 2003; Jones *et al*., 2018).

Our results appeared mild freeze-thaw (-5 °C) was less effects than extreme freeze-thaw (-20 °C), suggesting more soil microbes could survive at -5 °C than -20 °C. Soil water is relatively mobile within the ice zone at temperatures of 0-2 °C and the proportion of water that remains unfrozen as thin film around soil particles decreases with

temperature (Edwards and Cresser, 1992; Yoshikawa and Overduin, 2005). Significant liquid water could exist in both soils at -5 °C (Anderson, 1970; Brooks *et al*., 1997) and ice crystal did not form in intracellular bacteria at this temperature (Measures, 1975). This explanation is supported by Lipson *et al*., (2000) who observed no significant reduction in soil microbial biomass after freeze-thaw events (-5 °C). Probably -5 °C was chilled to microbes and not fatal temperature.

3.5 Conclusions

Freeze-thaw (-20 °C) or dry-wet conditions alter microbial labile and structural C pools, but soil microbes are less affected by -5 °C. Soil water content before and after treatments is a key factor controlling microbial activity and C and N cycling. The results presented suggests that soil microbes are damaged by freeze-thaw (-20 °C) or dry-wet events. Freeze-thaw (-20 °C) and dry-wet events result in significant fluctuations in available C and N in soil. These events increase nutrient supply to microbes that metabolized and mobilized. However, the mechanisms that control C during thawing and rewetting events are not fully understood. Further investigation is needed to understand how microbial metabolism response to stressful conditions.

3.6 Acknowledgement

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Chapter 4

Role of plants in determining the soil response to either a single freeze-thaw or dry-wet event

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Abstract

In a changing climate, freeze-thaw and dry-wet events are predicted to increase in frequency and magnitude. The change in environmental conditions associated with these events may induce stress within plants and soil microbial communities. Although many studies have described stress tolerance mechanisms in plants, the impact of climate extremes on root-soil interactions remains poorly understood. The enhanced release of CO_2 to the atmosphere, triggered by freeze-thaw and dry-wet events, may be linked to greater root exudation and may thus influence soil carbon (C) storage and net GHG emissions. To better understand the response of wheat (*Triticum aestivum* L.) cropping systems to freezing (-5 or -10 °C) and drought, a freeze-thaw or dry-wet cycle was imposed on planted and unplanted soil mesocosms. We measured GHG fluxes (CO_2, N_2O, CH_4) (daytime, night-time, and total), plant-available nutrients and soil microbial community structure before, during and after the stress event. Total CO_2 flux from planted soil reduced during the drought period and the decreased CO_2 flux was negatively correlated with water availability. Freeze-thaw events (particularly at -10 °C) damaged plants, increasing microbial activity and rhizodeposition, which resulted in increased CO_2 flux. The presence of plants resulted in significantly greater total CO_2 flux following freeze-thaw or dry-wet events than observed in unplanted soil. This implies that freeze-thaw or dry-wet events induced strong physiological response in soil-plant C cycling, when plants are present.

Keywords: Soil microbes, Freezing, Drying, CO_2, Carbon cycling, Stress

4.1 Introduction

Climate change is likely to alter the frequency of freeze-thaw or dry-wet events with wide-reaching implications for the functioning of temperate grassland (Kreyling *et al*., 2007; Donat *et al*., 2016). Freeze-thaw or dry-wet events are important ecological processes that affect soil hydrology and thermodynamics (Harrison-Kirk *et al*., 2013; Yang and Wang, 2019), and can trigger a stress response in both plant (Harp *et al*., 2010; Skinner, 2015) and soil microbes (Schimel *et al*., 2007). Freezing and drying processes can damage soil microbes and roots cells, releasing organic C and N, resulting in an increase in soil solution concentrations (Schimel *et al*., 2007). These changes in soil C and N concentration may directly affect native soil organic matter (SOM) turnover rates, which may stimulate an increase in GHG emissions (Kim *et al*., 2012). Field and laboratory studies of the effects of freeze-thaw or dry-wet events on soil gas fluxes have been widely reported (Kim *et al*., 2012), with an increase in CO_2, N_2O, and CH_4 following thawing or rewetting, commonly observed in grassland soils (Xiang *et al*., 2008; Wu *et al*., 2010 Kim *et al*., 2012). GHG emissions caused by freeze-thaw or dry-wet events may have important implications in soil C and N cycling.

The root system plays an important role in the response of the soil environment to freeze-thaw or dry-wet events (Whitmore and Whalley, 2009; Hosokawa *et al*., 2017). Root exudates are primary derived from recently assimilated photosynthate (Jones and Darrah, 1993; van Hees *et al*., 2005) and represent a semi-continuous input of labile C to soil (Jones *et al*., 2009). Most exudates consist of sugars, amino acids, and organic acids (Badri and Vivanco, 2009; Carvalhais *et al*., 2011) and these low molecular weight (MW) organic compounds can be released into soil by passive diffusion under non-stressed conditions (Jones *et al*., 1998). The release of organic compounds from roots is a key

factor in mediating nutrient availability in the rhizosphere (Pierret *et al*., 2007) and stimulating microbial activity (Meier *et al*., 2017). Freeze-thaw or dry-wet events may induce root activity and enhance rhizodeposition process, which in turn may affect microbial activity and subsequently soil GHG emissions (Bais *et al*., 2006; Hinsinger *et al*., 2009).

Soil CO_2 flux results from a combination of heterotrophic (microbial) and autotrophic (plant) respiration in the soil (Wang *et al*., 2014). Although separating heterotrophic and autotrophic respiration may help to understand C cycling under extreme environmental change (Casals *et al*., 2011; Zhou *et al*., 2009), root-soil interactions are rarely characterized. In the field, catching natural freeze-thaw events in temperate grassland is difficult. Although freeze-thaw cycles using buried heating wire was studied in grassland (Kreyling *et al*., 2010), methods of simulating freeze-thaw events are not well established.

The aim of this experiment was (1) to investigate how the C budget of the combined plant-soil system responded to freeze-thaw and dry-wet events, and (2) to discover how planted and non-planted treatments differed in their CO_2 flux response (daytime, night-time, and total). We hypothesized that CO_2 flux would reduce during simulated drought conditions due to water limitation. We also hypothesized that a large CO_2 pulse would be released into the atmosphere following freeze-thaw events. We also hypothesized the effects of freeze-thaw or dry-wet events on CO_2 flux from planted soil would be greater than from unplanted soil.

4.2 Materials and methods

4.2.1 Sample collection

Soil samples (5-10 cm depth, Ah horizon) under *Lolium perenne* L. were collected (Table 4.1) from a Eutric Cambisol in a sheep-grazed grassland located at the Henfaes Experimental Station at Abergwyngregyn, Gwynedd, North Wales (53°14'N, 04°01'W). Mean annual soil temperature at 10 cm depth is 10.6 °C and the mean annual rainfall is 1055 mm yr^{-1}. Mean winter air temperature is approximately 5 °C. Fertilizer is regularly added to the grassland (50 kg N ha^{-1}, 10 kg P ha^{-1}, 10 kg K ha^{-1}). Prior to use, the soil was sieved to pass 1 cm and any roots and stones removed. pH and electrical conductivity (EC) were determined in a 1:5 (w/v) soil-distilled water extract (Smith and Doran, 1996). Soil moisture and organic matter content were determined by oven-drying for 24 h at 105 °C and 450 °C, respectively. Soil chemical analysis is described in section 4.2.4.

Table 4.1 Properties of the soil used in the experiments. Values represent means ± SEM (n = 4).

Measurements	Values
pH	5.47 ± 0.02
EC (μS cm^{-1})	121 ± 9
Moisture content (%)	36.4 ± 0.10
Organic matter content (%)	6.53 ± 0.02
Dissolved organic C (mg kg DW^{-1})	52.5 ± 2.96
Total organic N (mg kg DW^{-1})	32.0 ± 3.20
Extractable NH_4^+ (mg kg DW^{-1})	0.02 ± 0.01
Extractable NO_3^- (mg kg DW^{-1})	26.1 ± 1.40
Extractable Ca (mg kg DW^{-1})	12.7 ± 1.06
Extractable K (mg kg DW^{-1})	12.5 ± 0.09
Extractable Mg (mg kg DW^{-1})	2.26 ± 0.20
Extractable Na (mg kg DW^{-1})	19.0 ± 1.46
Extractable P (mg kg DW^{-1})	1.06 ± 0.07

EC, electrical conductivity.

4.2.2 Experimental design

Four replicates of field moist soil (700 g) were placed into polypropylene containers (11 × 8 cm base, 27 cm high; Lock & Lock Ltd., Seoul, Republic of Korea). All containers were transferred to a climate controlled (CMP6010, CONVIRON, Canada) plant growth cabinet maintained with a 8 h photoperiod, light intensity of 180 μmol m^{-2} s^{-1}, and relative humidity of 75 % during the experiment. Soil moisture was maintained at 20-25 % of field capacity by adding distilled water every 2-3 days during the experiments.

To determine the effects of freeze-thaw and dry-wet events on plants, 6 wheat (*Triticum aestivum* L.) seeds (48 h pre-germinated) were planted in each of the 16 containers. Plants were grown in the plant growth cabinet at 15 °C until the plants were 15 cm tall and had 3 fully expanded leaves. At this point the temperature was reduced to 10 °C to simulate winter conditions.

The experiment consisted of two main treatments 1) a single freeze-thaw event, or 2) a single dry-wet event. For the freeze-thaw treatments, after a 5 weeks pre-treatment period, all samples were transferred to incubators at either -5 °C or -10 °C for 24 h to simulate a freezing period. They were then placed back into a growth cabinet at 10 °C and warmed naturally to induce thawing. The temperature of -5 °C was considered to be realistic for the winter condition in the UK. An extreme freezing treatment of -10 °C was used because winter wheat plants have freezing tolerance and are able to survive at freezing temperature ranging from -4 to -15 °C (Gusta *et al*., 1997). For the dry-wet treatment, after a week-long pre-treatment period (10 °C), the samples were maintained in a winter dry condition by natural-drying (not adding water) in open containers placed in the growth cabinet (10 °C) for 5 weeks. Water loss was determined by weighing. Dry-period was assumed to start when soil water content reached 50 - 60 % of field capacity

(during natural-drying). For rewetting of soil, the amount of water lost was calculated (at the end of drought period) and distilled water (same amount of water loss determined by weighing) was added to the containers to bring the water content back to the original water content. Overall, the experiment had 8 treatments: 1) Control with plants (+10 °C, constant soil moisture level), 2) Dry-wet with plants (+10 °C), 3) Freeze -thaw (-5 °C / +10 °C) with plants, 4) Freeze-thaw (-10 °C / +10 °C) with plants, 5) Control without plants (+10 °C, constant soil moisture level), 6) Dry-wet without plants (+10 °C), 7) Freeze-thaw (-5 °C / +10 °C)) without plants, 8) Freeze-thaw (-10 °C / +10 °C) without plants.

4.2.3 Soil greenhouse gas emissions

GHG samples were taken from the experimental containers (including entire plants, roots and soil). At each sampling time, gas-tight lids containing a Suba-seal® gas sampling port (Sigma-Aldrich Ltd, Poole, UK) were placed on the polypropylene containers. 20 ml of gas samples were then taken from the container's headspace through the suba-seal septum by a syringe at 0 min (T0) and at 60 min (T60). The gas sample was transferred from the syringe to a 20 ml evacuated glass vial. On each occasion, gas sampling took place twice in the same day at daytime (light condition) and night (dark condition). CO_2, N_2O, and CH_4 concentrations in the vials were determined by gas chromatography using a Clarus 500 GC with a Turbomatrix (HS-40) autoanalyzer (PerkinElmer Inc., Waltham, MA). CO_2 and CH_4 were detected by a flame ionization detector (FID) and N_2O by a ^{63}Ni electron capture detector (ECD). The gas flux was calculated according to Dunn *et al*. (2016), with a linear portion of a standard curve. Daily cumulative flux was calculated assuming constant flux between two measurements, multiplying hourly flux by the

number of h (8 during light / 16 during dark for planted) and number of days.

4.2.4 Soil chemistry analysis

Soil nutrient availability was estimated according to Jones and Willett (2006). Briefly, 2 g of soil was removed using a 1.5 cm diameter corer to 2 cm depth from the containers and extracted in 10 ml deionized water on a reciprocating shaker (Edmund Buhler GmbH, SM-30, Germany; 200 rev min^{-1}). After shaking for 10 min, they were centrifuged (6850 *g*, 5 min) and then filtered through PES 0.45 μm syringe filters (Triple Red, UK).

Soil C and N in soil solution were measured using a Multi N/C 2100/2100 analyser (AnalytikJena AG, Jena, Germany). Anions and cations were measured by ion chromatography analysis using an 850 Professional IC (Metrohm Ltd., Runcorn, UK).

4.2.5 Plant analysis

At the end of the experiment on day 138, the plants were removed from the soil, separated into shoots and roots, and oven-dried at 60 °C for 72 h. The shoots and the roots were then ground to a fine powder using a MM200 ball mill (Retsch, Düsseldorf, Germany). Total C and N of shoots and roots tissues were determined using a Truspec CN analyzer (Leco Corp., St Joseph, MI, USA). Major and trace elements in shoots and roots were determined using a S2 Picofox, Total Reflection X-ray Fluorescence (TXRF) spectrometer (Bruker Inc., MA, USA).

4.2.6 Soil microbial community structure analysis

At the end of the experiment, soil microbial community structure was determined

by phospholipid fatty acid (PLFA) analysis according to the method of Buyer and Sasser (2012). Briefly, samples (2 g) from each container were freeze-dried and Bligh–Dyer extractant (4.0 ml) containing internal standard was added. Tubes were sonicated in an ultrasonic cleaning bath (10 min, room temperature) before rotating end-over-end (2 h). After centrifuging (10 min) the liquid phase was transferred to clean 13 mm × 100 mm screw-cap test tubes and 1.0 ml each of chloroform and water were added. The upper phase was removed by aspiration and discarded while the lower phase, containing the extracted lipids, was evaporated (30 °C). Lipid classes were separated by solid phase extraction (SPE) using a 96-well SPE plate containing 50 mg of silica per well (Phenomenex, Torrance, CA, USA). Phospholipids were eluted with 0.5 ml of 5:5:1 methanol:chloroform:H_2O (Findlay, 2004) into 1.5 ml glass vials (Multi-Tier microplate, E&K Scientific, Santa Clara, CA, USA), and the solution was evaporated (70 °C, 30 min). Transesterification reagent (0.2 ml) was added to each vial, the vials were sealed with a PTFE/silicon cap mat (E&K Scientific, Santa Clara, CA, USA), and incubated (37 °C, 15 min). Acetic acid (0.075 M) and chloroform (0.4 ml each) were added. The chloroform was evaporated just to dryness at room temperature and samples were dissolved in hexane (75 µl). The samples were analyzed with a 6890 gas chromatograph (Agilent Technologies, Wilmington, DE, USA) equipped with autosampler, split–splitless inlet, and flame ionization detector. Fatty acid methyl esters (FAMEs) were separated on an Agilent Ultra 2 column, 25 m long × 0.2 mm internal diameter × 0.33 µm film thickness. Standard nomenclature was followed for PLFA (Frostegård *et al*., 1993).

4.2.7 Statistical analysis

Repeated measures analysis of variance (ANOVA) with a randomized block design

was carried out to evaluate CO_2 flux over time during the drying period. A one-way analysis of variance (ANOVA) was performed to determine the effect of freeze-thaw or dry-wet events on soils. To compare differences within groups, multiple comparisons were carried out by Tukey post-hoc test at significance level of $p < 0.05$. Pearson's product-moment correlation was carried out between factors to explore relationship between water contents and GHG flux. Principal component analysis (PCA) was used to explore relationship between fatty acid profiles (PLFAs), GHG fluxes, and nutrients in soil solution. All statistical analyses were performed using R Studio 0.99.486 (R Development Core Team, 2004).

4.3 Results

4.3.1 CO_2 flux for planted versus non-planted soil

Absolute and cumulative CO_2 fluxes of daytime, night-time and daily totals are shown in Figure 4.1. The daytime CO_2 flux from the planted soil was generally lower than the flux from unplanted soil, regardless of specific treatment type ($p < 0.001$). In comparison, the night-time CO_2 flux in both the planted and unplanted soil was higher than in the daytime, but the CO_2 flux from planted soil was higher than unplanted soil ($p < 0.001$). The total daily CO_2 flux in the planted soil was higher than that of the CO_2 flux from the unplanted treatments ($p < 0.001$).

4.3.2 CO_2 flux for dry-wet events

For the planted soil, during the drought period, the daytime CO_2 flux was similar to the control treatment, but the night-time CO_2 flux ($p < 0.001$; Figure 4.2A) and total daily CO_2 flux ($p < 0.001$; Figure S3A) from the planted soil decreased over time ($p < 0.001$;

Figure 4.2A). The night-time CO_2 flux (Figure 2B, $R^2 = 0.82$) and total daily CO_2 flux (Figure S3B, $R^2 = 0.79$) were negatively correlated with water loss during the drought period. At the end of the experiment on day 138, the cumulative CO_2 flux of night-time and total after the dry-wet event was lower (34.4 and 35.9 g CO_2 m^{-2}, respectively) than the control ($p < 0.01$ and $p < 0.05$, respectively), while the daytime CO_2 flux remianed similar ($p > 0.05$). On the other hand, for the unplanted soil, there was no change in CO_2 flux of daytime, night-time, and total during the drought period ($p > 0.05$) in comparison to the control. Overall, the CO_2 flux of daytime, night-time, and total were not influenced by soil dry-wet events.

4.3.3 CO_2 flux pulse during thawing period

The daytime CO_2 flux from the planted soil exposed to either -5 °C or -10 °C significantly increased on the first day after thawing (Figure 4.1, $p < 0.05$ and $p < 0.001$), compared to the flux under freezing conditions. The daytime CO_2 flux for the planted treatment after freeze-thaw (-10 °C) events continuously increased throughout the thawing period. The CO_2 flux of night-time and total daily flux also increased rapidly on the first day of transition from freezing (-5 °C and -10 °C) to thaw conditions ($p < 0.001$). On the other hand, for the unplanted soil, the CO_2 flux of daytime, night-time, and total daily fluxes from the unplanted soil increased after freeze-thaw (-10 °C) events ($p < 0.001$, $p < 0.001$, and $p < 0.05$, respectively). The CO_2 flux of daytime and total were similar to control ($p > 0.05$), whilst, CO_2 flux of night-time from unplanted soil increased ($p < 0.05$).

4.3.4 CO_2 flux after freeze-thaw events

At the end of the experiment (day 138) in the planted soil, the cumulative daytime

CO_2 flux after the freeze-thaw (-10 °C) event was higher compared with the control ($p <$ 0.01), but the night-time and total daily CO_2 flux was lower (115.6 and 78.6 g CO_2 m^{-2}, respectively) compared with the unfrozen control treatment ($p < 0.001$ and $p <0.01$, respectively). On the other hand, there was no statistically difference in the cumulative of daytime, night-time, and total CO_2 flux from the freeze-thaw and control unplanted soil treatments ($p > 0.05$).

4.3.5 CH_4 flux

There was no significant difference in CH_4 flux between the planted and unplanted soil treatments before or after a freeze-thaw or dry-wet event (Figure S4).

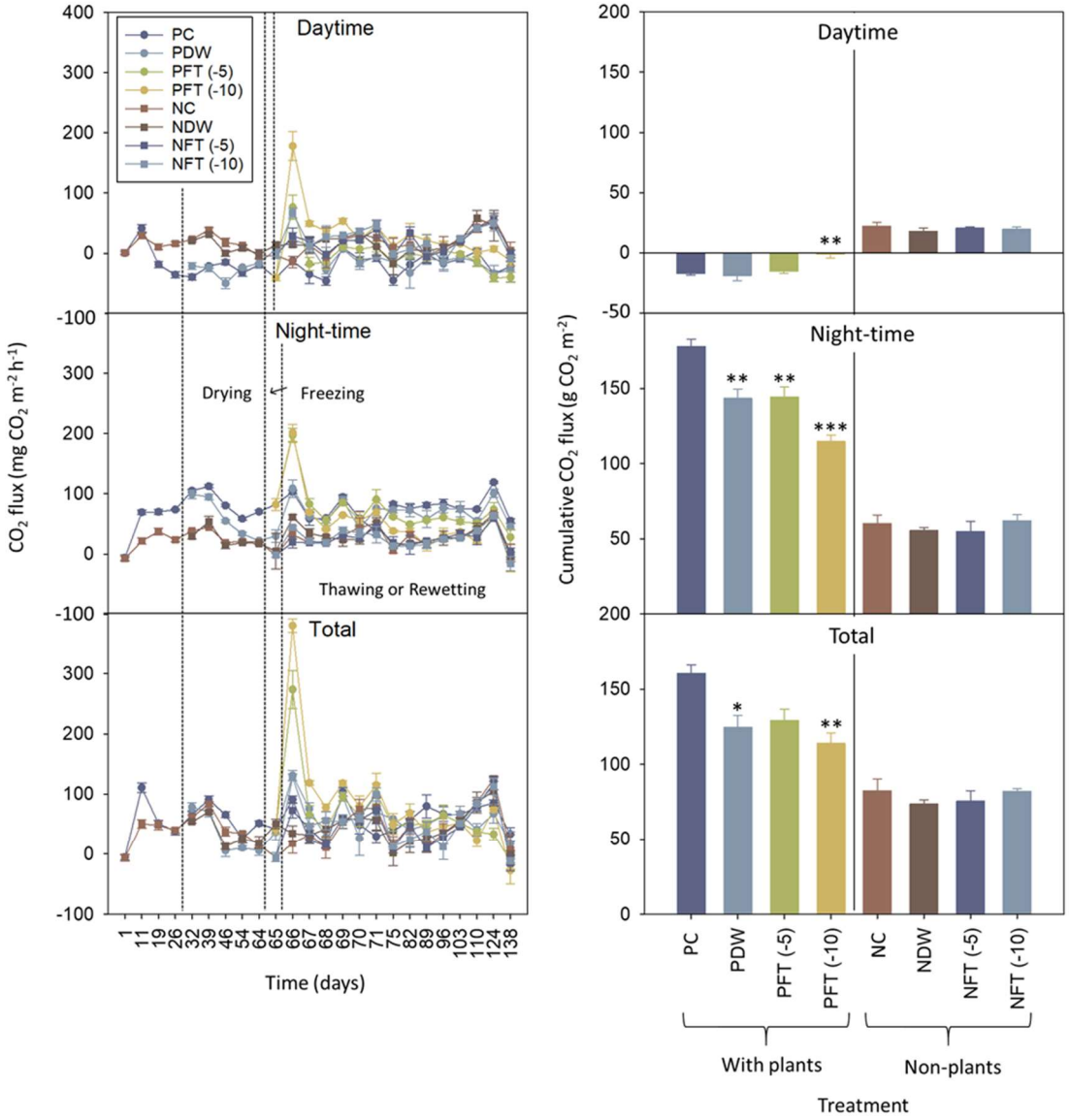

Figure 4.1. CO_2 flux (left) and cumulative CO_2 flux (right) from soil expressed as daytime (8 h light condition), night-time (16 h dark condition), and total (24 h combined) CO_2 flux before and after a single freeze-thaw (-5°C or -10°C) or dry-wet events. Stars above the plots denote significant differences from the control where *, ** and *** denote $p \leq 0.05$, $p \leq 0.01$, and $p \leq 0.001$, respectively. Values represent means ± SEM (n = 16 for pre-treatments, n = 12 for pre-freeze-thaw treatments and n = 4 during drying and after thawing or rewetting). PC = Control with plants (+10°C), PDW = Dry-wet with plants, PFT (-5) = Freeze-thaw (-5°C/+10°C) with plants, PFT (-10) = Freeze-thaw (-10°C/+10°C) with plants, NC = Control without plants (+10°C), NDW = Dry-wet without plants (+10°C), NFT (-5) = Freeze-thaw (-5°C/+10°C) without plants, NFT (-10) = Freeze-thaw (-10°C/+10°C) without plants.

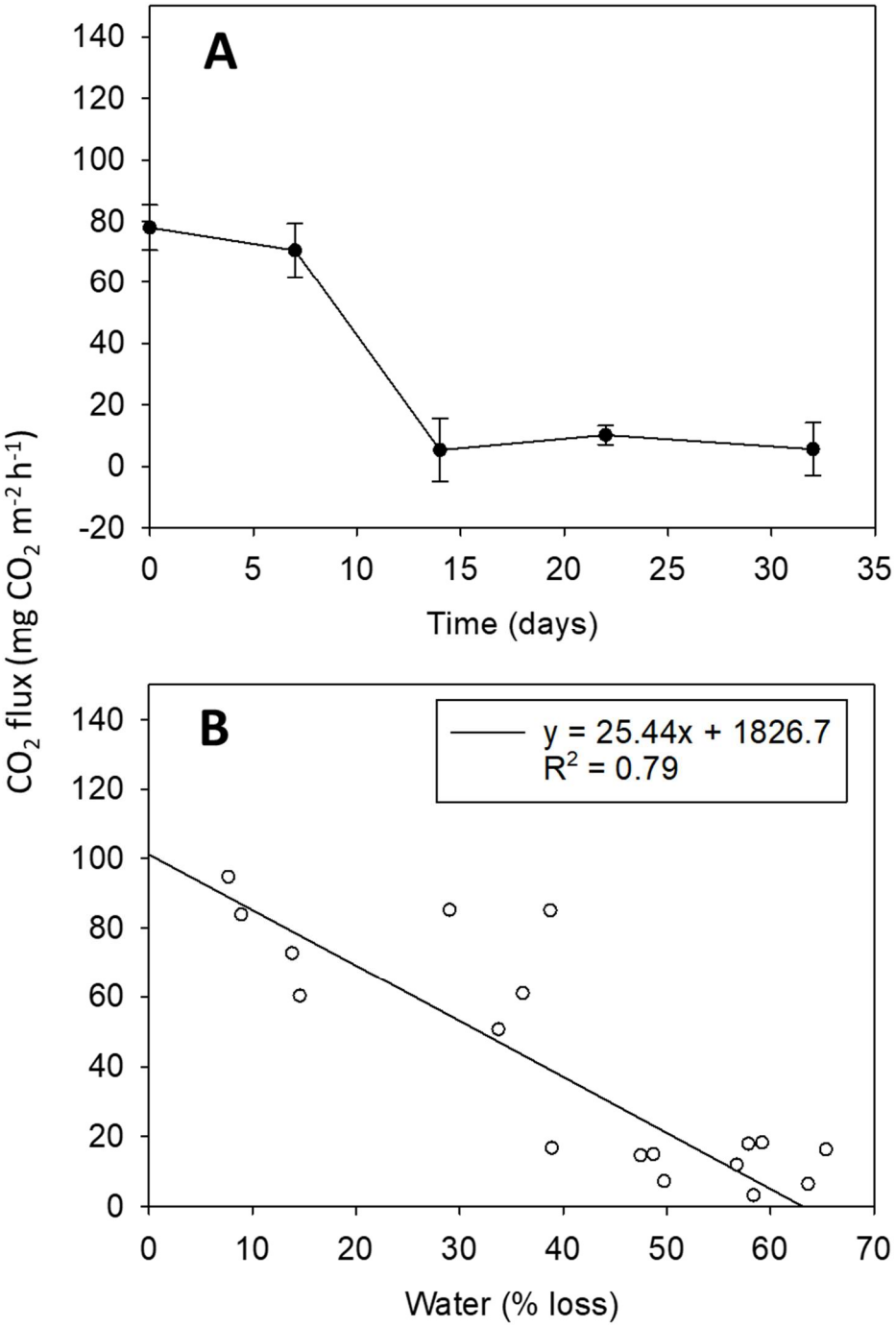

Figure 4.2. Effect of drought on total CO_2 flux. (A) total CO_2 flux from planted soil during drought period, (B) Relationship between total CO_2 flux and water loss in planted soil (Pearson's product-moment correlation. In Panel A, values represent means ± SEM (n = 4).

4.3.6 N_2O flux pulse after freeze-thaw events

There was a significant increase in N_2O flux from the unplanted soil after thawing from -10 °C ($p < 0.001$; Figure 4.3). N_2O peaked on the third day (after thawing) and then decreased for the duration of the experiment. Apart from this, there was no change in N_2O emission from both planted and unplanted soil after either thawing or rewetting events.

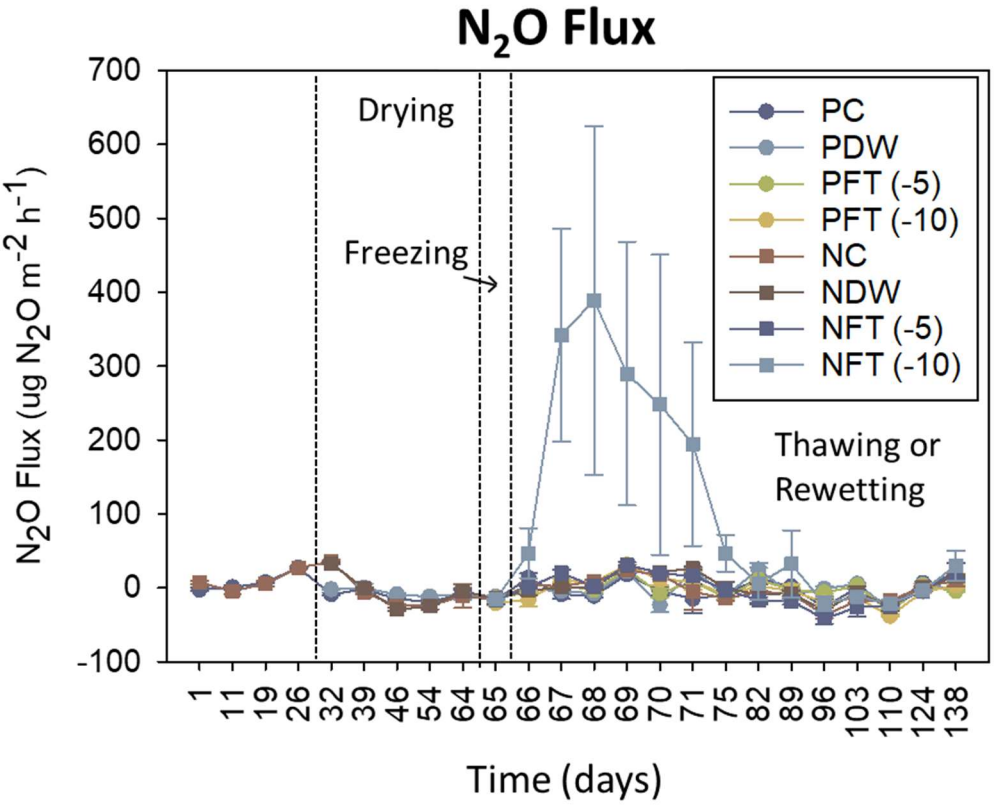

Figure 4.3. N_2O flux from soil before and after applying a single freeze-thaw (-5°C or -10°C) or dry-wet treatment in either planted or unplanted soil. Values represent means ± SEM (n = 16 for pre-treatments, n = 12 for pre-freeze-thaw treatments and n = 4 during drying and after thawing or rewetting). PC = Control with plants (+10°C), PDW = Dry-wet with plants, PFT (-5) = Freeze-thaw (-5°C/+10°C) with plants, PFT (-10) = Freeze-thaw (-10°C/+10°C) with plants, NC = Control without plants (+10°C), NDW = Dry-wet without plants (+10°C), NFT (-5) = Freeze-thaw (-5°C/+10°C) without plants, NFT (-10) = Freeze-thaw (-10°C/+10°C) without plants.

4.3.7 Nutrient dynamics in soil solution

Soil solution nutrient dynamics in response to freeze-thaw or dry-wet events is shown in Figure 4.4 and S5. No clear trend in DOC concentration was apparent with freeze-thaw or dry-wet events in either planted or non-planted soils. During the growing period, roots took up inorganic N (NH_4^+ and NO_3^-) and ions (PO_4^{3-}, Ca^+, K^+, and Mg^{2+}) from the soil resulting in lower concentrations in the planted mesocosms. After freeze-thaw events (-10 °C), TDN, NO_3^-, Ca^+ and Mg^{2+} in soil gradually increased, while PO_4^{3-} decreased over time. K^+ and Na^+ were similar to control ($p > 0.05$). There was little or no change in nutrient availability in soil after dry-wet or freeze-thaw events (-5 °C). On the other hand, for unplanted soil, TDN, NO_3^-, Ca^+ and Mg^{2+} in soil increased over time during this experiment.

4.3.8 Nutrient content of plants

C and N content in shoots and roots in response to a dry-wet and freeze-thaw cycle are shown in Figure S6. The C content in both shoots and roots was significantly lower in comparison to other treatments after freeze-thaw (-10 °C) events ($p < 0.001$). In contrast, N content in roots significantly was significantly increased after freeze-thaw (-10 °C) events ($p < 0.001$), however N content in shoots was similar between treatments. Overall, macro and micro nutrients in roots tended to be greater after freeze-thaw (-10 °C) events than other treatments including the control ($p < 0.001$, Figure S7), but this trend was not seen in the shoots (Figure S8)

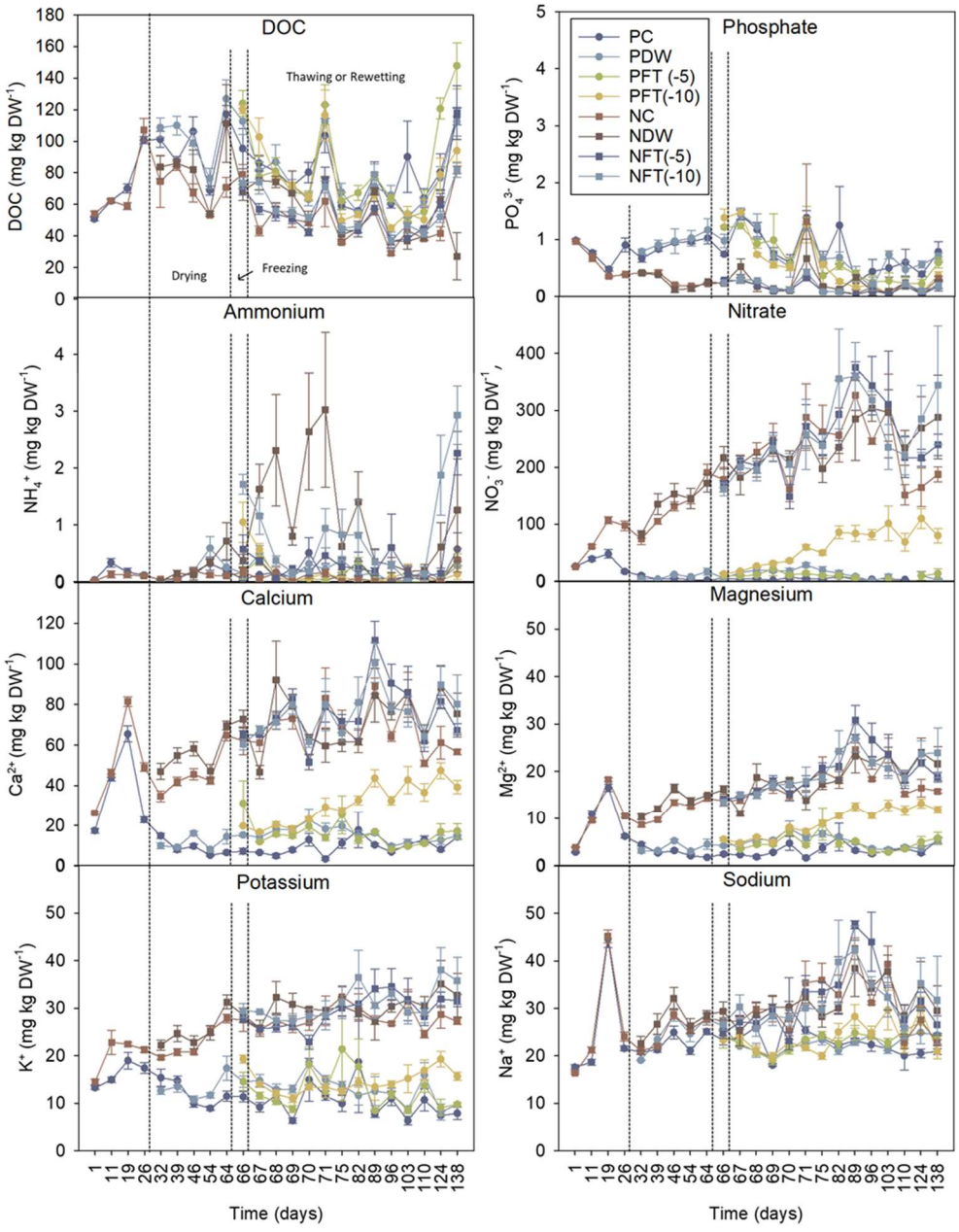

Figure 4.4. Soil solution chemistry before and after applying a single freeze-thaw (-5°C or -10°C) or dry-wet treatment in either planted or unplanted soil. Values represent means ± SEM (n = 16 for pre-treatments, n = 12 for pre-freeze-thaw treatments and n = 4 during drying and after thawing or rewetting). PC = Control with plants (+10°C), PDW = Dry-wet with plants, PFT (-5) = Freeze-thaw (-5°C/+10°C) with plants, PFT (-10) = Freeze-thaw (-10°C/+10°C) with plants, NC = Control without plants (+10°C), NDW = Dry-wet without plants (+10°C), NFT (-5) = Freeze-thaw (-5°C/+10°C) without plants, NFT (-10) = Freeze-thaw (-10°C/+10°C) without plants.

4.3.9 Microbial community structure

The amount of saprophytic fungi and Gram-negative bacteria in the planted soil treatments were significantly greater than in the unplanted soil ($p < 0.001$) while AM fungi, Gram-positive bacteria, actinomycetes and eukaryotes were relatively similar between planted and unplanted soil (Table 4.2 and 4.3). Principal component analysis of the PLFA data indicated a clear separation between planted and unplanted soil (PC1 = 95.7 %, PC2 = 2.8 %, Figure S9).

The amount of fungi and Gram-negative bacteria in planted soil was greater after freeze-thaw (-10 °C) events than other treatments or the control ($p < 0.01$, Table 4.2). Principal component analysis of the PLFA data from planted soil after freeze-thaw events (-10 °C) (PC1 = 51.4 %, PC2 = 27.2 %, Figure 4.5) separated the PFT-10 group from all other groups due to an increase in CO_2 flux, TDN, and NO_3^- concentration.

Table 4.2. Soil microbial community structure in planted soil at the end of either a single dry-wet cycle or freeze-thaw cycle (-5°C or -10°C). Values represent means ± SEM (n = 4). Stars and letters denote significant differences (*, ** and *** indicate $p \leq 0.05$, $p \leq 0.01$ and $p \leq 0.001$, respectively, while *ns* indicates non-significant, $p > 0.05$) when compared with the control treatment.

PLFA marker	Control	Dry-wet	Freeze-thaw -5°C	Freeze-thaw -10°C	ANOVA
			(nmol g^{-1})		
AM fungi	6.74 ± 0.12	6.51 ± 0.20	6.60 ± 0.09	6.21 ± 0.06	*ns*
Saprophytic fungi	1.82 ± 0.04a	1.62 ± 0.06a	1.65 ± 0.08a	2.16 ± 0.09b	**
Gram- bacteria	60.9 ± 0.8a	61.1 ± 1.0ab	61.4 ± 0.8ab	63.8 ± 0.4b	*
Gram+ bacteria	50.1 ± 1.0	51.1 ± 0.4	50.05 ± 0.8	51.3 ± 0.4	*ns*
Eukaryotes	4.57 ± 0.29	4.06 ± 0.31	3.74 ± 0.19	3.81 ± 0.04	*ns*
Actinomycetes	19.2 ± 0.3	19.4 ± 0.2	19.6 ± 0.2	19.7 ± 0.1	*ns*

Table 4.3.

Soil microbial community structure in unplanted soil at the end of either a single dry-wet cycle or freeze-thaw cycle (-5°C or -10°C). Values represent means ± SEM (n = 4). *ns* indicates non-significant difference ($p > 0.05$) when compared with the control treatment.

PLFA marker	Control	Dry-wet	Freeze-thaw -5°C	Freeze-thaw -10°C	ANOVA
			(nmol g^{-1})		
AM fungi	4.75 ± 0.15	4.92 ± 0.08	4.66 ± 0.17	4.48 ± 0.23	*ns*
Saprophytic fungi	1.70 ± 0.15	1.56 ± 010	1.47 ± 0.04	1.41 ± 0.10	*ns*
Gram- bacteria	54.3 ± 1.3	56.0 ± 0.5	53.8 ± 1.1	52.4 ± 1.8	*ns*
Gram+ bacteria	47.78 ± 1.0	49.0 ± 0.4	46.9 ± 0.9	45.6 ± 1.4	*ns*
Eukaryotes	3.42 ± 0.21	3.67 ± 0.19	3.37 ± 0.31	2.97 ± 0.24	*ns*
Actinomycetes	18.4 ± 0.3	18.8 ± 0.3	18.1 ± 0.3	17.5 ± 0.4	*ns*

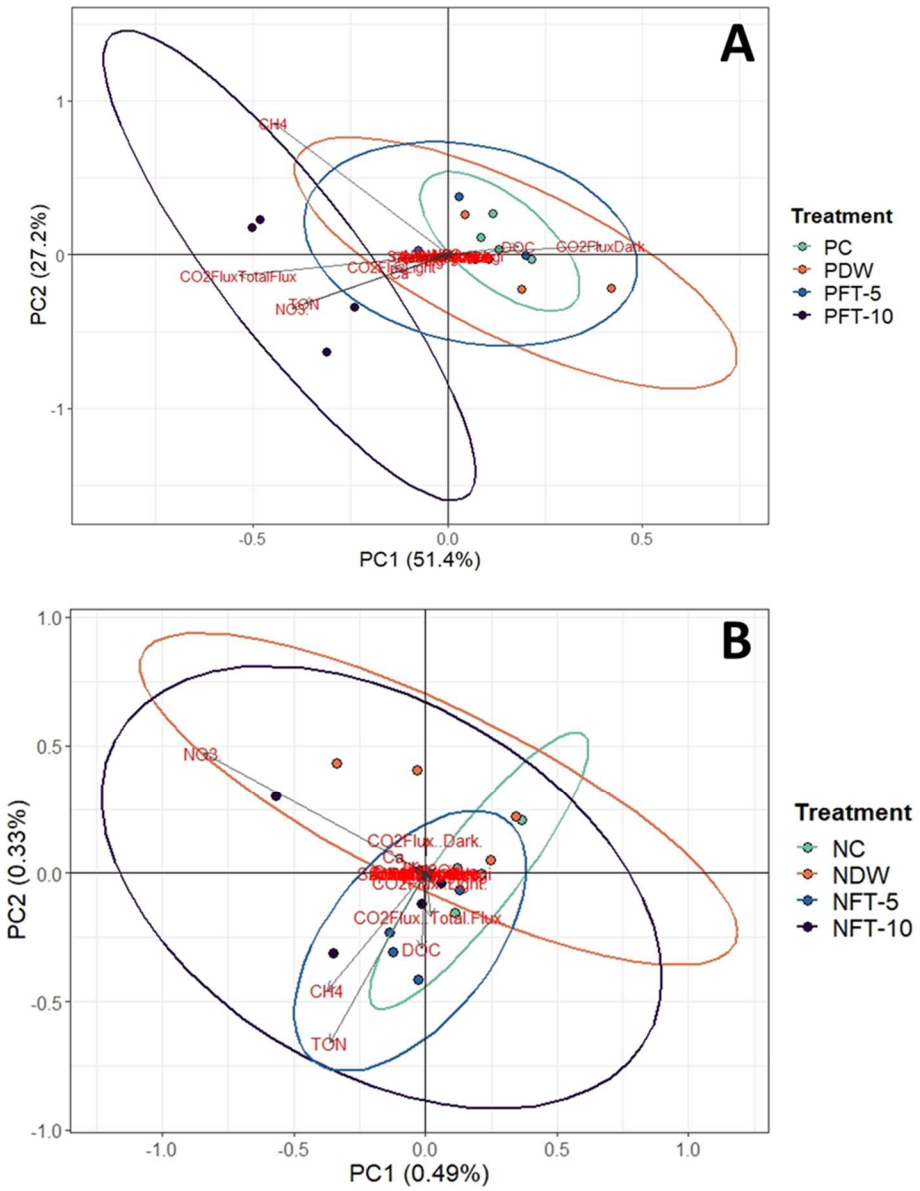

Figure 4.5. Principal component analysis of soil microbial community structure at the end of the experiment after applying a single freeze-thaw (-5°C or -10°C) or dry-wet treatment in either planted or unplanted soil. A: Planted treatments and B: non-plant treatments. Values represent means ± SEM (n = 4). PC = Control with plants (+10°C), PDW = Dry-wet with plants, PFT (-5) = Freeze-thaw (-5°C/+10°C) with plants, PFT (-10) = Freeze-thaw (-10°C/+10°C) with plants, NC = Control without plants (+10°C), NDW = Dry-wet without plants (+10°C), NFT (-5) = Freeze-thaw (-5°C/+10°C) without plants, NFT (-10) = Freeze-thaw (-10°C/+10°C) without plants. DOC (Dissolved Organic Carbon), CH_4 flux and N_2O flux are clustered around the centre point so are partially obscured from view.

4.4 Discussion

4.4.1 Response of soil CO_2 flux to drought

Soil water availability is one of the most important factors controlling temporal variation of soil CO_2 flux (Carbone *at al*., 2011). Most previous drought studies have shown that soil CO_2 fluxes decrease with declining soil water content during the onset of drought (Orchard and Cook, 1983; Zhou *et al*., 2019). This is ascribed to soil moisture being a main driver of net primary productivity and strongly affecting soil C cycling (Huxman *et al*., 2004), while other studies have not separated soil moisture / CO_2 flux dynamics into day and night. In our study, the CO_2 flux under dark conditions (night-time) decreased with declining soil water content, but not under light conditions (daytime), therefore photosynthesis is more important than water availability at regulating combined plant-soil CO_2 efflux when light is present. Reduction of night-time CO_2 flux during the drought period was likely due to reduced root exudation, which may limit microbial activity in soil (Gargallo-Garriga *et al*., 2008). The CO_2 flux of night-time was root-derived and rhizosphere respiration was accompanied with plant photosynthetic activity (Kuzyakov and Cheng, 2001).

The response of daily (total) CO_2 flux to drought depended strongly on photoperiod during the winter growing season. The observed decrease in total CO_2 flux could be due to a reduction in night-time CO_2 flux or to an increased photosynthetic C fixation in daytime, however, our results show daytime CO_2 flux did not change during the drought period, which implies that C assimilation rate did not increase during the drought period. The observed reduction in total CO_2 flux is likely to be related to a decrease in night-time respiration. The effects of drought on night-time CO_2 flux was more important than on daytime.

4.4.2 CO_2 pulse on thawing

The greater production of CO_2 during the daytime, night-time, and total from both planted and unplanted soil on the first day after thawing, suggests that a large amount of C was released into soil due to soil microbial death (Skogland *et al*., 1998) and/or accumulated osmolytes (Cushman, 2001), caused by freezing conditions. Soil aggregate disruption could also increase CO_2 after thawing (Wang *et al*., 2012). The released C were decomposed by surviving microbes, and respired CO_2 (Schimel *et al*., 2007). The size of CO_2 pulse flux after thawing in planted soil was greater than in the control, suggesting a large amount of C was released into soil via root exudates (Jones and Darrah, 1993). This released C could provide newly input labile C that was taken up by surviving microbes leading to an increase in respired CO_2. Another possible explanation is that plant cell membrane were damaged by ice crystals growth (Borochov *et al*., 1987) leading to the passive leakage of solutes out of tissue (Uemura and Steponkus, 1997). After thawing from -10 °C, the cumulative daytime CO_2 flux continuously increased, suggesting that that plants could no longer fix C (i.e. not photosynthetically active).

4.4.3 N_2O from unplanted soil following thawing from -10 °C

A pulse of N_2O release from the unplanted soil after thawing from -10 °C, suggests that an increase in substrate C was available for N_2O production due to microbial death (Christensen and Tiedje, 1990; Teepe *et al*., 2001) and/or accumulated osmolytes (Yancey, 2001). Another explanation is that freezing affected N_2O release from soil (Congreves *et al*., 2018). When soil was frozen, the soil surface was sealed by ice pores, and the ice-seals soil surface produces anaerobic conditions, preventing N_2O release (Congreves *et al*., 2018). Following thawing, the slow increase of liquid water in soil and an increase in

oxygen entry, may cause trapped N_2O to be lost in a pulse (Congreves *et al*., 2018). In addition, our NO_3^- concentration was relatively high, which could stimulate N_2O emission (Gelfand *et al*., 2015).

4.4.4 Implication of C and N cycling after plant death

Although the effect was tiny in magnitude, increased saprophytic fungi and Gram-negative bacteria in planted soil at the end of the experiment, suggests that more C was delivered to soil via root exudates or root turnover due to cell damage by freeze-thaw events. C in both plant shoot and roots significantly declined compared with control, suggesting dead plants were being decomposed by surviving soil microbes. More macronutrients and micronutrients were condensed in roots than control. Release of large amount of inorganic C and organic C from dead roots could shift microbial community composition. Dramatic changes in nutrient availability and microbial community composition in rhizosphere strongly alter ecosystem C cycling. Plant death following freeze-thaw (particularly following severe freeze event -10 °C) may therefore play an important role in regulating CO_2 flux.

We observed soluble nitrogen (TON) had continuously increased following a freeze-thaw event (-10 °C), suggesting greater microbial activity and more rhizodeposition. We speculate that the increase in TON was readily mineralized to NH_4^+ in soil. We further observed NO_3^- continuously increasing in soil after freeze-thaw event (-10 °C), suggesting that a large active community of nitrifiers could transform this NH_4^+ to NO_3^- (Jones *et al*., 2018) and potentially N could be lost to the atmosphere as N_2O or N_2. However, our results showed N_2O did not increase from planted soil after freeze-thaw events (-10 °C). We observed increased N_2O flux from unplanted, but not from planted

soil, suggesting perhaps roots prevent the flow of water, oxygen, and gas solubility (Congreves *et al*., 2018).

4.4.5 Influence of freeze-thaw or dry-wet events on planted soil

This study involved the manipulation of freeze-thaw and dry-wet events on planted and unplanted soil, with photosynthetic substrate supply an important factor in regulating soil respiration in both daily and long-term scale in wheat planted soil. The presence of roots led to a greater C input into rhizosphere via roots exudates, which in turn increased microbial activity, microbial growth, C storage and nutrient uptake by roots as in Lange *et al*. (2015). Mycorrhizal fungi contribute to C flow in the rhizosphere by releasing a range of exudates into the mycorrhizosphere and the exudates can be used as energy by microbes resulting in increase of CO_2 (Jones *et al*., 2009). In our study, AM fungi was much greater in planted soil, and likely more root exudates released into the rhizosphere, which had greater ecosystem respiration. These results suggest that microbial activity in planted soil primarily depends on photosynthesis rate and the labile C supply. Hence, plants activity could have a significant influence on the C balance. When plants die, microbial community composition changes due to new C input. Change in microbial community composition and newly input C alter CO_2 releasing from soil.

It should be noted that estimated daily CO_2 flux in this study potentially overestimated the degree of soil respiration in wheat plants as CO_2 was captured during peak flux (mid-afternoon). In addition, we measured CO_2 flux under full sunlight condition during the photoperiod. In the field, CO_2 flux in rhizosphere is highly dependent on climate condition (i.e., temperature, sunlight and cloud cover), which is controlled by photosynthesis rate during the light period (Nannipieri *et al*., 2008).

4.5 Conclusions

Total CO_2 flux (photosynthesis and respiration combined) from the experimental grassland system was greater following a freeze-thaw or dry-wet event in planted than non-planted soils with implications for C budgets. Drought reduced CO_2 flux in planted soils, and after re-wetting, CO_2 flux increased but did not exceed control. Wheat was tolerant to freezing at -5 °C, but not at -10 °C. Freeze-thaw (-10 °C) events damaged plants and lead to a greater CO_2 flux than the control. Change in C source in soil after plants die are important to soil microbial community composition and alter C cycle. In addition, freeze-thaw (-10 °C) events altered N_2O flux from unplanted soil by transportation process. However, -10 °C is unlikely happened in the UK grasslands whilst dry-wet events may occur more frequent in the future due to climate change.

4.6 Acknowledgements

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4.7 References

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Chapter 5

Freeze-thaw and dry-wet events reduce microbial extracellular enzyme activity, but not organic matter turnover in an agricultural grassland soil

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Abstract

Soils in temperate agroecosystems are frequently exposed to extremes of moisture and temperature during which time soil functioning may be negatively affected. The aim of this study was to directly compare the effects of a single dry-wet or freeze-thaw (-5°C or -20°C) cycle on extracellular enzyme activity and soil organic matter turnover. We measured the activity of six enzymes before and after imposing the freeze-thaw or dry-rewet events. Our results showed that drying had a much greater impact on total enzyme activity than a -20°C freezing event (38 vs. 10% reduction, respectively), while freezing at -5°C had no appreciable effect. Enzyme activity recovered back to control levels relatively quickly which we ascribe to *de novo* exoenzyme production (within 3 d for the -20°C freeze-thaw treatment and 14 d for the dry-wet treatment). We added ^{14}C-labelled plant residues to the soil prior to imposing the same thermal or moisture stress events. Monitoring residue decomposition before and after imposing the treatments indicated that none of the stress regimes greatly affected organic matter turnover rates. Our results did reveal, however, a pulse of $^{14}CO_2$ which was produced during the drying and freezing events themselves. We ascribe this to a shift in microbial metabolism and the production of stress avoidance metabolites (e.g. osmo- and cryo-protectants, membrane lipids). Our work highlights that extreme weather events may affect exoenzyme activity, however, these responses are transitory and are unlikely to greatly affect soil organic matter cycling unless they occur at high frequency.

Keywords: Agricultural grassland, Carbon dynamics, Enzyme production, Microbial function, Phosphatase

5.1 Introduction

Microbial degradation of organic matter plays an important role in biogeochemical cycling within agroecosystems. The primary stages of soil organic matter (SOM) turnover involves the production of extracellular enzymes by soil microbes and, to a lesser extent plant roots (Burns *et al*., 2013). These exoenzymes catalyze the breakdown of high molecular weight (MW) plant and microbial derived-polymers into soluble products which can then be rapidly assimilated by soil microbes and/or plants. They also facilitate the release of inorganic nutrients (e.g. N, P; Allison *et al*., 2006). As soil exoenzyme activities often represent the rate-limiting step in SOM turnover, they can provide a good indicator of biochemical processes operating within the soil ecosystem, SOM characteristics, and nutrient limitation (Allison *et al*., 2006).

Freeze-thaw or dry-wet events are known to frequently occur in many agricultural soils. When temperatures fall below 0°C, ice crystals can entrap and/or denature extracellular enzymes retarding their activity. Similarly, drying of soil causes enzymes to become confined in thin water films, inducing denaturation or sorption to the solid phase. A number of studies have reported that freeze-thaw or dry-wet events can affect enzyme activities, however, these reports are often contradictory showing either increases (Tabatabai and Bremmer, 1970; Sistla and Schimel, 2013), decreases (Lee *et al*., 2007; Turner and Romeo, 2010), or no net change in activity (Bandick and Dick, 1999).

Freeze-thaw or dry-wet events have often been shown to induce a significant increase in soil CO_2 production. This response has been ascribed to a range of factors including: (i) physical disruption of soil aggregates allowing access to previously trapped C (Denef *et al*., 2001); (ii) death and lysis of microbial cells and mesofauna followed by the subsequent breakdown of this necromass when the moisture or temperature regime

returns to normal; (iii) increased microbial metabolism and the use of energy during the production of stress compounds (e.g. cryo- and osmo-protectants), and (iv) changes in pH and ionic strength which increases SOM solubilization (Edwards and Cresser, 1992; Fierer and Schimel, 2002; Schimel *et al*., 2007).

The aim of this study was to directly compare the exoenzyme response of an agricultural soil to either a dry-wet or freeze-thaw event. In addition, we aimed to test whether any changes in the exoenzymes profile affected the turnover of plant residues present in soil. As both freeze-thaw and dry-wet events may affect the microbial biomass in similar ways (inducing the production of stress compounds or causing cell lysis), we hypothesized that they would both reduce exoenzyme production. We predict that this will affect *de novo* enzyme production. As most exoenzymes are quite persistent in soil, we further hypothesized that the intrinsic enzymes would be denatured to a greater extent under drying than freezing (due to the enzymes becoming sorbed to the solid phase or precipitating with humic substances concentrated in the thin water films). We predict that this will negatively affect the turnover of native soil organic matter.

5.2 Materials and methods

5.2.1 Sample collection

Soil samples (5-10 cm depth, Ah horizon) were collected from a sandy clay loam textured Eutric Cambisol in a sheep-grazed, *Lolium perenne* L. dominated grassland located at the Henfaes Experimental Station, Abergwyngregyn, UK (53°14’22”N, 4°00’60”W). The mean annual air temperature is 10.6 °C (max 28.6 °C, min -7.6 °C) and the mean annual rainfall is 1055 mm y^{-1}. The lowest temperature ever recorded in the region was -23 °C in 1940. The mean winter air temperature is 5 °C (Fig. S1). The

grassland receives regular fertilizer at an annual rate of 50 kg N ha^{-1}, 10 kg P ha^{-1} and 10 kg K ha^{-1}.

To characterize the soil, four independent replicates of soil, located 10 m apart, were collected from the field and sieved to pass 2 mm, removing stones, roots and earthworms. The pH (5.47) and electrical conductivity (121 µS cm^{-1}) were determined in a 1:5 (w/v) soil-distilled water extracts. Soil moisture (36.4 %) and organic matter content (6.53 %) were determined gravimetrically by oven-drying the soil for 24 h at 105 °C and 450 °C, respectively. Dissolved organic C (30.9 mg L^{-1}) in soil solution (1:1 w/v soil-distilled water extract) was determined using a multi N/C 2100 analyzer (Analytik-Jena AG, Jena, Germany).

5.2.2 Experimental design

The experiments were set up to reflect winter conditions and therefore the soil was maintained at 5°C until the start of the experimental treatments. Either a single freeze-thaw (-5 °C or -20 °C) or dry-wet event was applied to the soil. For the freeze-thaw treatments, the samples were placed in an incubator at either -5 °C or -20 °C for 24 h. After freezing, the samples were allowed to thaw by placing them back at +5 °C. For the dry-wet treatment, air was passed over the samples at a rate of 0.86 m s^{-1} until they had reached an air-dry state (ca. 3 h; Fig. S2). After drying (24 h), distilled water was added back to the soil to reach the pre-drying water content (5 °C). Control samples were maintained at 5 °C throughout the experiment. Each treatment had 4 independent replicates.

5.2.3 Enzyme assay

The activity of six hydrolytic soil extracellular enzymes (α-glucosidase, β-glucosidase, cellobiohydrolase, β-xylosidase, N-acetyl-β-glucosaminidase, acid phosphatase; Table S1) were measured following the 96-wellplate fluorometric method described in Freeman et al. (1995) and Dunn et al. (2014). Enzyme activity was measured both immediately before imposing the freezing or drying treatments and then 1, 3, 7 and 14 d after thawing or rewetting, respectively. Briefly, the fluorogenic 4-methylumbelliferyl (MUF)-labelled substrates were dissolved in methyl cellosolve® (ethylene glycol monomethyl ether) before being diluted in ultrapure water. No pH buffer was used in the assays. Soil (1 g) was placed in a stomacher® bag (Seward Ltd., West Sussex, UK), 7 ml of the relevant MUF substrate added and the sample mixed in a laboratory paddle blender (Stomacher® circulator, Seward Ltd.) for 30 s. The samples were then incubated (5 °C, 60 min), after which they were centrifuged (18,000 *g*, 5 min) and the supernatants placed into 96 well plates and their fluorescence quantified using a SpectraMax M2e fluorimeter (Ex. 350 nm, Em. 465 nm; Molecular Devices Inc., San Jose, CA).

5.2.4 Mineralization of plant derived high molecular weight C

To generate the ^{14}C-labelled plant residues we pulse-labelled *Lolium perenne* L. (12.3 kBq g^{-1}) with $^{14}CO_2$ as described in Hill et al. (2007). Subsequently, we isolated the ^{14}C-labelled high molucular (HMW) insoluble fraction from the shoots (e.g., cellulose, hemicellulose, protein, lignin) as described in Glanville et al. (2012). Briefly, the soluble ^{14}C component (33 ± 2% of the total ^{14}C) was removed from the plant material by extracting the shoots twice with hot water (80 °C). The remaining plant residues were

oven-dried (80 °C, 24 h) prior to use in the experiments.

To determine the mineralization of the HMW plant residues, field-moist soil (1 g) was placed in a 50 cm^3 polypropylene tube. Subsequently, 20 mg of the ^{14}C-labelled plant residues were mixed with the soil. A vial containing NaOH (5 M, 200 µl) was then suspended above the soil to capture any evolved $^{14}CO_2$. After sealing, the tubes were placed in an incubator in the dark at 5 °C and the NaOH traps replaced daily. After 14 d, the soils were exposed to a freezing or drying event for 24 h as described above. After thawing or rewetting, the samples were further incubated at 5 °C for 14 d during which time the NaOH traps were replaced daily. After dilution (1:5 v/v), the amount of ^{14}C in the NaOH traps was determined by liquid scintillation counting using a Wallac 1404 liquid scintillation counter (Wallac EG&G, Milton Keyes, UK) and HiSafe 3 scintillation fluid (PerkinElmer Inc., Waltham, MA).

5.2.5 Statistical analysis

A one-way analysis of variance (ANOVA) was performed to determine the effect of freeze-thaw or dry-wet treatments on enzyme activity and $^{14}CO_2$ evolution in soils at each time point. To compare differences within groups, multiple comparisons were carried out by Tukey post-hoc testing at a significance level of $p < 0.05$. Repeated measures ANOVA was undertaken to evaluate differences in cumulative $^{14}CO_2$ evolution between treatments. Statistical analysis was undertaken in R Studio 0.99.486 (R Development Core Team, 2004).

5.3 Results

5.3.1 Extracellular enzyme activity

Overall, a dry-wet cycle had a greater effect on soil exoenzyme activity than a freeze-thaw cycle ($p < 0.001$; Figure 5.1). In the case of drying, the activity of all six exoenzymes were reduced, with the biggest response seen directly after the soil was rewetted (i.e. within 24 h). In some cases, the negative impact of drying persisted for up to 14 d after rewetting (e.g. β-xylosidase, β-glucosidase). When all six enzymes were considered together, a dry-wet cycle caused an initial reduction in enzyme activity of 38 %, however, these activities progressively recovered over time but were still lower at day 14 in comparison to the control (ca. 20 %; Figure 5.2).

In the case of a freeze-thaw event, the reduction in enzyme activity was generally more severe at -20 °C in comparison to soils exposed to -5 °C (Figure 5.1). In contrast to the drying treatment, some enzymes were unaffected by freezing (e.g. acid phosphatase, cellobiohydrolase), while others were affected but to a lesser extent (e.g. α- and β-glucosidase). In most cases, levels of enzyme activity recovered quickly after freezing with few differences detected after 3 d of thawing in comparison to the unfrozen control. When total enzyme activity was considered, no significant differences to the control were observed for the -5 °C treatment, while only a 10% reduction was observed for the -20 °C treatment (Figure 5.3).

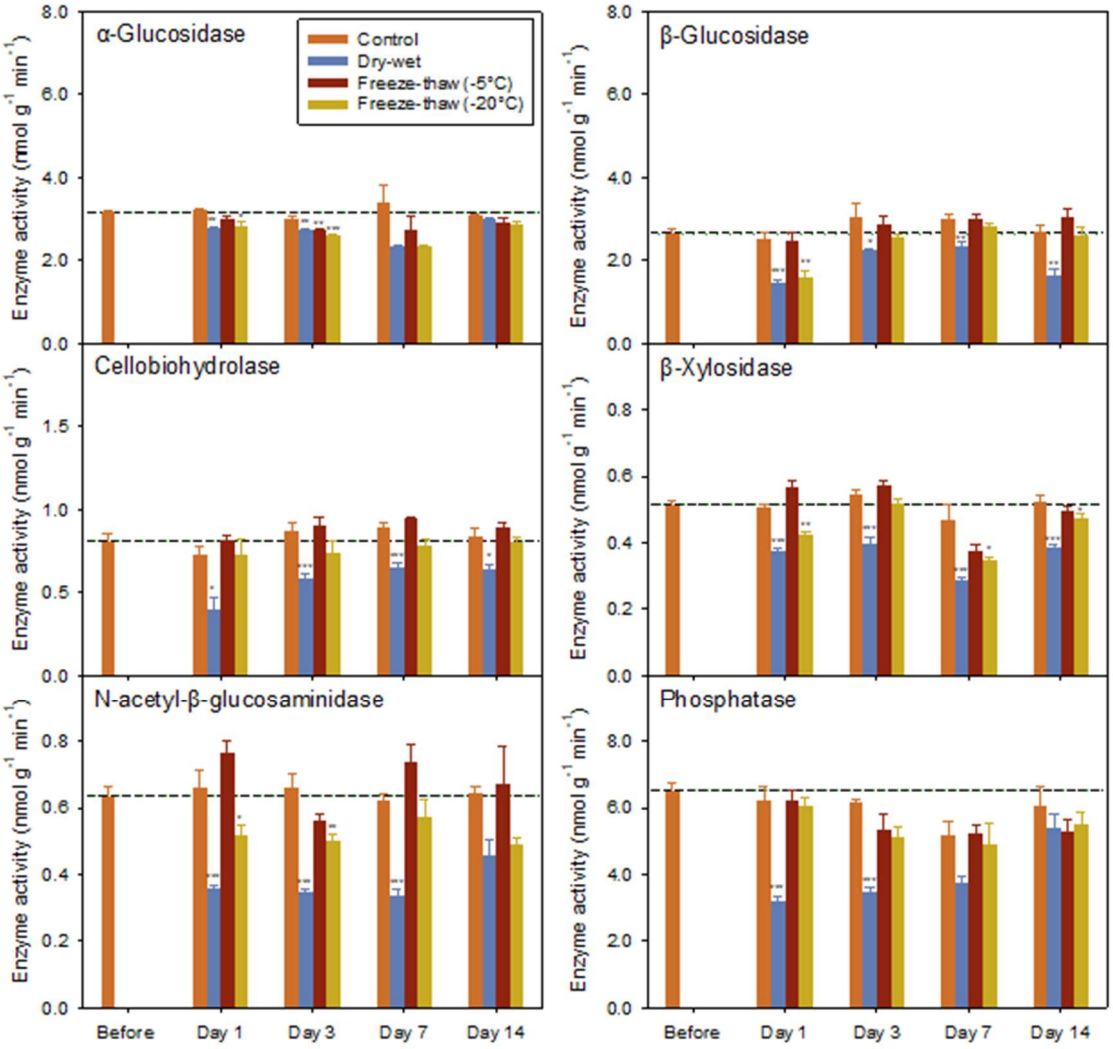

Figure 5.1. Activity of six extracellular enzymes in soil before and after the application of a freeze-thaw (-5 °C or -20 °C) or dry-wet event. The legend is the same for all panels. The dotted line represents the enzyme activity before applying the treatment. Stars above the plots denote significant differences from the control where *, ** and *** denote $p \leq 0.05$, $p \leq 0.01$, and $p \leq 0.001$ respectively. Values represent means ± SEM ($n = 4$).

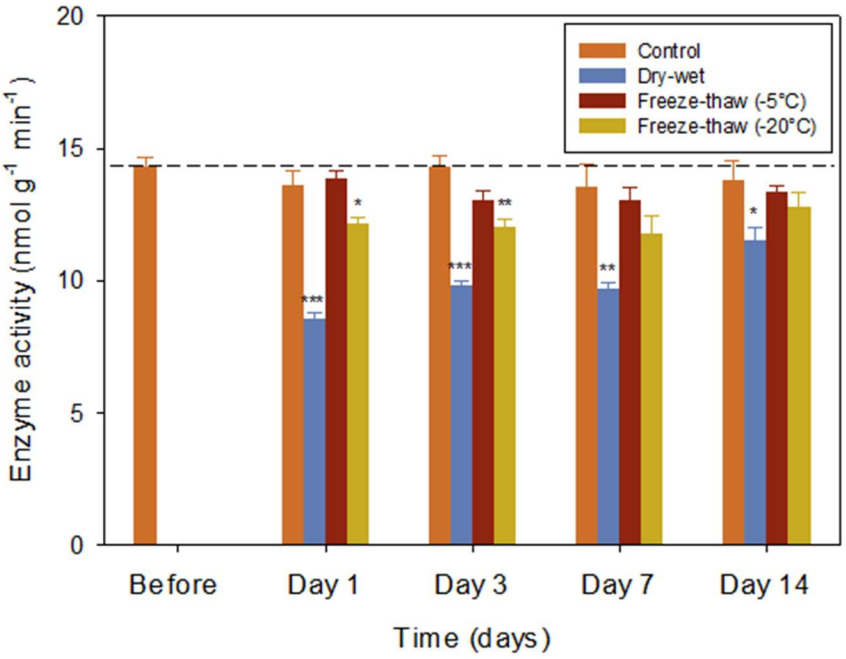

Figure 5.2. Total hydrolytic extracellular enzyme activity in soil before and after the application of a freeze-thaw (-5 °C or -20 °C) or dry-wet event. The dotted line represents the enzyme activity before applying the treatment. Stars above the plots denote significant differences where *, ** and *** denote $p \leq 0.05$, $p \leq 0.01$, and $p \leq 0.001$ respectively. Values represent means ± SEM ($n = 4$).

5.3.2 Decomposition of high molecular weight compounds

Overall, the rate of plant residue mineralization was slow with only 1.4 % of the plant material being mineralized in the control treatment over the 30 d incubation period (Figure 5.3). The rate of mineralization was not affected by freezing the soil at -5 °C. In contrast, the amount of $^{14}CO_2$ recovered in both the dry-wet and -20 °C freezing treatments were higher than observed in the control ($p < 0.001$). Most of this additional $^{14}CO_2$, however, was produced during the stress event itself, with no major difference seen after this point (Figure 5.3).

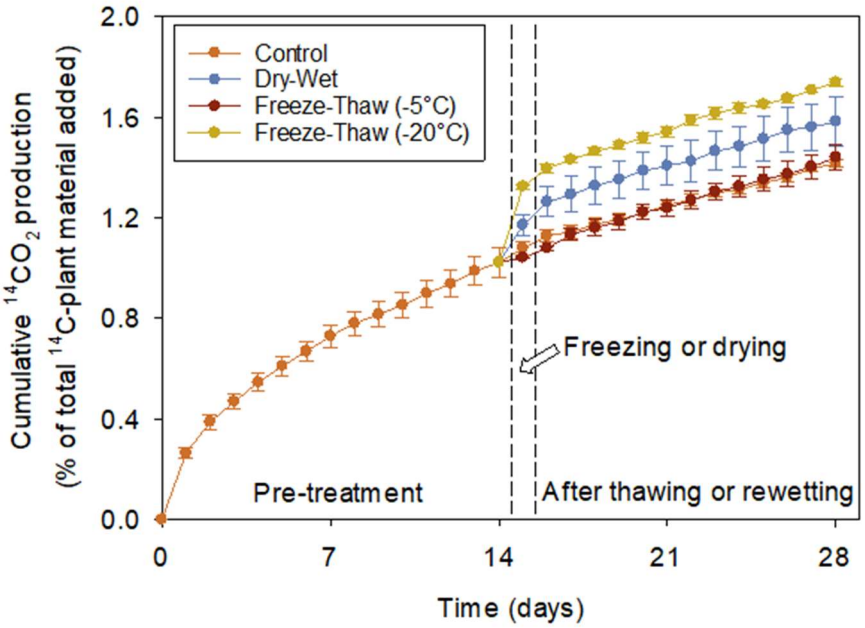

Figure 5.3. Cumulative $^{14}CO_2$ production following addition of ^{14}C-labelled plant material to soil before and after the application of a freeze-thaw (-5°C or -20°C) or dry-wet event. Values represent means ± SEM (n = 16 for pre-treatments and n = 4 after thawing or rewetting). The dotted lines denote the times at which the conditions were changed.

5.4 Discussion

Relative to the conditions at the study site, our results show that exposure to mild freezing (-5 °C) had no appreciable effect on enzyme activity, while exposure to extreme freezing (-20 °C) caused a transient reduction in enzyme activity. These results are consistent with other studies assaying different exoenzymes in contrasting ecosystem and soil types (Li *et al*., 2012; Mannisto *et al*., 2018; Sorensen *et al*., 2018). The initial reduction in enzyme activity at -20 °C are probably related to protein aggregation, binding to humic substances, sorption to the solid-phase, and ice-induced conformational changes, all of which are known to affect catalytic rate (Champion *et al*., 2000; Cao *et al*., 2003; Terefe *et al*., 2004; Nardid *et al*., 2014). The lack of effect at -5°C could be due to the

protection of exoenzymes and microbial cells in unfrozen liquid water (Edwards and Cresser, 1992; Brooks *et al*., 1997). Our results also show that upon thawing, enzyme activity recovered quickly to those seen in the unfrozen control soil. Interestingly, at no time did their levels exceed those in the controls, suggesting that the freezing event did not greatly stimulate microbial activity or make C substrates more bioavailable. This is also consistent with the lack of effect seen on the mineralization of the ^{14}C-labelled plant residues which showed no change in mineralization rate after thawing. The apparent increase in $^{14}CO_2$ production during the freezing event itself we ascribe to plant-derived ^{14}C that was now immobilized in the microbial biomass and which is rapidly respired due to changes in metabolic activity in an effort to protect against extreme temperatures (e.g. changes in membrane lipid composition and production of cryoprotectants; Feng *et al*., 2007; Marx *et al*., 2009).

In contrast to freeze-thaw, a dry-wet cycle had a much greater impact on enzyme activity. In our experience, these drying events are much more frequent than freezing at our field site, especially at the soil surface (0-1 cm). Our finding that drying reduces enzyme activity and that recovery occurs within 2 weeks are consistent with studies in other ecosystems (Pohlon *et al*., 2013; Frossard *et al*., 2015). They do, however, contrast with others which have shown an increase in enzyme activity following rewetting, a response that has been ascribed to endoenzyme release during cell lysis (Tabatabai and Bremmer, 1970; Zhao *et al*., 2010; Burns *et al*., 2013). The slow recovery of enzyme activity in comparison to freeze thaw may suggest that the microbial community was affected to a greater extent (i.e. greater cell lysis). However, the observation that the mineralization of the ^{14}C-plant residues was not affected by drying strongly suggests that this may not be the case. Rather, the peak in $^{14}CO_2$ during the drying event we ascribe to

changes in microbial metabolism upon imposition of the moisture stress (e.g. osmoprotectant production). This is also consistent with Magid *et al*. (1999) who showed no effect on SOM turnover. We therefore hypothesize that although exoenzyme rates were reduced by drying that this is not the rate limiting step in SOM breakdown, at least for the enzymes measured here. As many hundreds of enzymes are involved in SOM turnover, further work is needed to ascertain if our findings are consistent across other functional enzyme groups (e.g. those involved in protein, lipid and lignin degradation). The recovery of exoenzyme activity back to control levels within 14 d also indicates that the *de novo* production of exoenzymes by the microbial community is relatively rapid (Burns, 1982).

5.5 Conclusions

In conclusion, this study shows that extracellular enzymes were relatively insensitive to a single freeze-thaw event but were greatly affected by a single dry-wet event. In both cases, enzyme activity recovered quickly after removal of the stress. The fact that enzyme activities never increased above the control levels, and that the decomposition of plant residues was unaffected by dry-wet and freeze-thaw, suggest that these extreme weather events have minimal effect on SOM turnover. Our evidence, however, does suggest that the microbial community rapidly responds to the stress event itself (drying/freezing) by increasing metabolism, however, further work examining the metabolic responses of the microbial community are required to confirm this.

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Chapter 6

Impact of a single freeze-thaw and dry-wet event on soil metabolic profiling

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Abstract

Soil freeze-thaw and dry-wet cycles are common phenomena in temperate regions. Such events may have a significant influence on the structure and functioning of the soil microbial community, with potentially important implications for soil carbon (C) and nitrogen (N) cycling. The aim of this study was therefore to compare the effects of a single freeze-thaw or dry-wet event on microbial functioning and metabolism in an agricultural soil (Eutric Cambisol) under simulated winter conditions. In a laboratory-based mesocosm experiment, planted (*Brassica napus* L.) or unplanted topsoil was exposed to a single freezing (-5°C, 24 h) or rapid drying event (60.4 to 68.5 % of moisture lost, 24 h). Soil nutrient concentrations (e.g. DOC, PO_4^{3-}, NO_3^-, NH_4^+, K^+) were measured before each event and then immediately after either thawing or rewetting. In addition, a GC-MS analytical platform was used to perform untargeted metabolomics to determine changes in the low molecular weight metabolite profile of the soil during these events. Overall, our results showed that a dry-wet cycle had a much greater impact on nutrient and metabolite concentrations in the unplanted soil than a freeze-thaw cycle. Furthermore, the changes in dissolved C and N after removal of the stresses were associated with specific compounds. Of the 125 individual compounds measured in our samples, only 20 % showed significant concentration differences in comparison to the untreated control. Of these, drying and freezing caused increases in sugars, polyols and amino acids, particularly in the unplanted soil. This is consistent with their enhanced microbial production to alleviate extreme temperature or moisture stress (i.e. cryoprotectants, osmolytes). Our results also showed an increase in nucleobase concentration in the unplanted soil after a dry-wet cycle, suggesting a breakdown of microbial DNA released from damaged cells. In conclusion, our results suggest that the microbial community

quickly responds to freezing or drying events by altering cellular metabolism, but that a freeze-thaw event causes less disruption to C and N cycling in comparison to dry-rewetting events.

Keywords: Biogeochemical cycling; Birch effect; Climate stress response; Metabolic profiling; Osmoregulation.

6.1 Introduction

Soils in many agroecosystems frequently experience temperature and moisture extremes (e.g. freeze-thaw and dry-wet cycles; Brabson *et al*., 2005). These can negatively affect soil biogeochemical cycling, leading to reduced plant productivity and a decline in the delivery of a range of ecosystem services (Sanghera *et al*. 2011; Fahad *et al*., 2017). One of the most common responses to soil freezing and desiccation is the release of large amounts of DOC and TON into soil solution (Yu *et al*., 2011; Schimel *et al*., 2007). This increase has been ascribed to the abiotic release of solutes physically trapped in soil organic matter (SOM) as well as biotic release from damaged microbial and root cells (Soulides and Allison, 1961; Steponkus, 1984; Swindell *et al*., 2007). Cellular damage can be induced by a range of mechanisms including: loss of membrane integrity, protein denaturation, cytoskeletal collapse, excess accumulation of free radicles, etc (Goldberg, 2003; Westman *et al*., 2019). In the case of freezing, damage can also occur due to ice crystal growth both inside and outside the cell (Steponkus, 1984). During these extreme stress events, intracellular enzymes may be released upon cell lysis and death which can promote native SOM turnover and a further release of DOC (Miura *et al.*, 2019).

Although many plants and soil microbes are sensitive to cold and desiccation stress, there is strong evidence suggesting that many soil microorganisms can adapt to mild freeze-thaw or dry-wet events (Gusta *et al*., 1997; Craine *et al*., 2012; Schimel *et al*., 2007). Typically, this response is underpinned by the synthesis and bioaccumulation of common low molecular weight (MW) metabolites inside the cell (e.g. amino acids, sugars and polyols) (Yancey *et al*., 1982; Cushman, 2001; Yancey, 2001). In addition, the onset of stress may induce the synthesis of protective molecules (e.g. antifreeze proteins), a

change in membrane lipid composition and fluidity and the release of extracellular polymeric substances (Koide *et al*., 2000; Bae *et al*., 2004; Lin *et al*., 2005). Studies in pure cultures have shown these mechanisms to be highly effective at reducing cell damage upon exposure to a range of abiotic stresses, however, the relative expression of these different traits in soil microbial communities remains unclear (Yancey, 2001; Rabbani and Choi, 2018).

Cellular increases in osmolyte concentration in response to stress may be due to *de novo* production within the cytoplasm or to a lesser extent due to uptake of solutes from the surrounding environment (Rabbani and Choi, 2018). Consequently, these low MW solutes often become highly concentrated inside the cell (50-350 mM; Yancey *et al*., 1982; Welsh, 2000). These solutes, however, must be rapidly disposed of upon stress removal (i.e. thawing, rewetting) to prevent osmotic shock and cell rupture (Schimel *et al*., 2007). This can occur by rapid conversion to other more benign compounds, partitioning into respiratory processes and excretion into the surrounding soil. The release of large concentrations of osmolytes into the soil may alter intrinsic C and N cycling, leading to both a negative and positive priming of SOM turnover depending upon the type, quality and amount of SOM present (Kuzyakov *et al*., 2000; Rousk *et al*., 2015).

The aim of this study was to compare the relative effects of a single freeze-thaw or dry-wet event on soil microbial activity, with specific emphasis on shifts in the microbial community's metabolite profile. We hypothesized that the microbial response to these two common abiotic stresses, freezing and drying, would be similar given that both induce osmotic stress and would be characterized by an accumulation of specific solutes. We also hypothesized that this response would be exacerbated in the presence of plant roots due to the increase in soil microbial activity in the rhizosphere and the release

of solutes from damaged root cells.

6.2 Materials and methods

6.2.1 Sample collection

Soil samples (5-10 cm depth, Ah horizon) were collected from a sandy clay loam textured Eutric Cambisol in a sheep-grazed, *Lolium perenne* L. dominated grassland located at the Henfaes Experimental Station, Abergwyngregyn, UK (53°14'22"N, 4°00'60"W). The mean annual air temperature at the site is 10.6 °C (max 28.6 °C, min -7.6 °C) and the mean annual rainfall is 1055 mm y^{-1}. The lowest temperature ever recorded in the region was -23 °C in 1940. The mean winter air temperature is 5 °C. The grassland receives regular fertilizer at an annual rate of 50 kg N ha^{-1}, 10 kg P ha^{-1} and 10 kg K ha^{-1}.

To characterize the soil, four independent replicates of soil, located 10 m apart, were collected from the field and sieved to pass 2 mm, removing stones, roots and earthworms. The pH (5.47) and electrical conductivity (121 μS cm^{-1}) were determined in a 1:5 (w/v) soil-distilled water extracts. Soil moisture (36.4 %) and organic matter content (6.53 %) were determined gravimetrically by oven-drying the soil for 24 h at 105 °C and 450 °C, respectively. DOC (30.9 mg l^{-1}) in soil solution (1:1 w/v soil-distilled water extract) was determined using a multi N/C 2100 analyzer (Analytik-Jena AG, Jena, Germany).

6.2.2 Experimental design

Microcosms were set up by placing replicate samples of field-moist soil (1 g) into 5 ml polypropylene tubes. For the planted microcosms, a single pre-germinated oil

seed rape (*Brassica napus* L.) seedling was planted into individual microcosms, while the unplanted microcosms contained no plants. The microcosms were initially placed in a CMP6010 climate-controlled plant growth cabinet (Controlled Environments Ltd, Winnipeg, Canada) at 15 °C, 75 % relative humidity, with 8 h photoperiod and light intensity of 180 μmol m^{-2} s until the shoots were 5 cm in length (2 weeks). The temperature was then reduced to 10 °C (3 days) to simulate the start of a winter cold spell prior to the start of the experiments. The soil moisture was maintained at field-conditions by regular addition of distilled water to the soil surface.

The experiment consisted of six treatments with 5 replicates per treatment: 1) Control with plant (+10 °C); 2) Dry-wet with plant (+10 °C); 3) Freeze (-5 °C) - thaw with plant (+10 °C); 4) Control without plant (+10 °C); 5) Dry-wet without plant (+10 °C), and 6) Freeze (-5 °C) - thaw without plant (+10 °C). For the freezing treatments, the microcosms were placed at -5 °C for 24 h, and the samples naturally thawed by placing them back at +10 °C. For the dry-wet treatment, the soil in the microcosms was dried by passing air over the samples at a rate of 0.85 m s^{-1} until they had reached a quasi-stable air-dry state (ca. 3 h when 60.4 to 68.5 % of the water was lost; +10 °C). After being maintained in a dry state for 24 h, distilled water was added back to the soil to reach the initial water content. Plants survived during both the freezing and the drying periods. After thawing or rewetting for 3 h, the microcosms were harvested and the plants were removed before the soil extraction.

6.2.3 Soil chemical analysis

Soil nutrient availability was estimated according to Jones and Willett (2006). Briefly, 1 g of soil was removed from the microcosms and extracted with 5 ml of either

distilled water (1:5 w/v) or 0.5 M K_2SO_4 (1:5 w/v) on a reciprocating shaker (Edmund Buhler GmbH, SM-30, Germany; 200 rev min^{-1}). After shaking (10 min), the samples were centrifuged (6850 *g*, 5 min), filtered through PES 0.45 µm syringe filters (Triple Red Ltd, Long Crendon, UK) and the samples stored at -5 °C to await analysis. DOC and TON in the soil extracts with both water and K_2SO_4 was measured using a Multi N/C 2100/2100 analyser (AnalytikJena AG, Jena, Germany). Anions and cations (water extract) were measured by ion chromatography analysis using an 850 Professional IC (Metrohm, Runcorn, UK).

6.2.4 Metabolomic analysis of soils

Untargeted metabolite analysis was carried out according to Fiehn *et al*. (2008). Prior to analysis, all samples were snap-frozen in liquid nitrogen, freeze-dried, and kept at -20 °C before shipping to the West Coast Metabolomics Center at University of California, Davis. The samples were extracted using a mixture of 3:3:2 (v/v/v) isopropanol/acetonitrile/water. Non-targeted primary metabolism analysis was performed using an Automated Linear EXchange-Cold Injection System (ALEX-CIS) GC Time Of Flight (TOF) MS using the methods of Fiehn *et al*. (2008). Briefly, 0.5 µl of each sample was injected onto a Rtx-5Sil MS capillary column (30 m length × 0.25 m i.d; 0.25 µm 95% dimethylsiloxane/5% diphenylpolysiloxane coating; Restek Corp., Bellefonte, PA). Using a He mobile phase, the GC thermal programme was 50 °C for 1 min, ramped to 330 °C at 20 °C min^{-1} and finally held at 330 °C for 5 min. Upon elution, samples were injected into a Pegasus IV GC-time of flight mass spectrometer (Leco Corp., St Joseph, MI), using mass resolution of 17 spectra s^{-1}, from 80-500 Da, at -70 eV ionization energy and 1800 V detector voltage with a 230 °C transfer line and 250 °C ion source.

6.2.5 Statistical analysis

R Studio 0.99.486 (R Development Core Team, 2004) was used to test the effects of freeze-thaw or dry-wet events on soil nutrients in either plant or unplanted soil by performing one-way analysis of variance (ANOVA) comparing the control with the treatments. To compare differences within the groups, multiple comparisons were carried out by Tukey post-hoc test at a significance level of $p < 0.05$.

The metabolomics data were pre-processed using ChromaTOF (v2.34; Leco Corp.). Briefly, subtraction of the baseline was applied just above the noise level and automatic mass spectral deconvolution and peak detection applied at a 5:1 signal-to-noise ratio throughout the chromatogram. A BinBase algorithm (rtx5) was applied, spectra were cut to 5% base peak abundance and matched to database entries. Unmatched peaks were entered as new database entries where the signal-to-noise ratio was >25 and purity <1.0. All subsequent analyses were completed using MetaboAnalyst v4.0 (Chong *et al*., 2018). Briefly, missing and zero values were replaced with a low value (half the minimum positive value) as an assumed detection limit to prevent complications in downstream analysis. The data were then normalized by $\log_{10}$ transformation and pareto scaling for multivariate analysis. Principal Component Analysis (PCA) was applied as an unsupervised method of determining variance within and between soil classes. A pairwise score plot was generated to determine the most appropriate combination of Principal Components (PC) to include in the 2D score plot. Agglomerative hierarchical clustering analysis was applied to metabolite concentration data and soil classes using two separate methods. Firstly, similarity was determined by Euclidean distance for analysis of the differences in metabolite concentrations, and clustering was performed using Ward's

linkage. A one-way ANOVA coupled with Tukey comparisons was used to identify significant differences between metabolite concentrations within treatments using a P <0.05 cut-off value to denote statistical significance. Prior to analysis, the data were normalized by $\log_{10}$ transformation.

6.3 Results

6.3.1 Changes in soil nutrient concentrations in response to a freeze-thaw or dry-wet cycle

Overall, DOC and TDN concentration in planted and unplanted soils after freeze-thaw or dry-wet event were greater than the control ($p < 0.05$). A similar trend was also observed in the soil with K_2SO_4 extract (Figure S1). The effects of dry-wet on DOC and TDN in the unplanted soil were greater than freeze-thaw ($p < 0.05$, Figure 6.1). On the other hand, for planted soil, the effects of freeze-thaw on DOC was greater than dry-wet ($p < 0.05$). There was no change in soil NH_4^+ concentration in either the planted or unplanted soils (Figure S2).

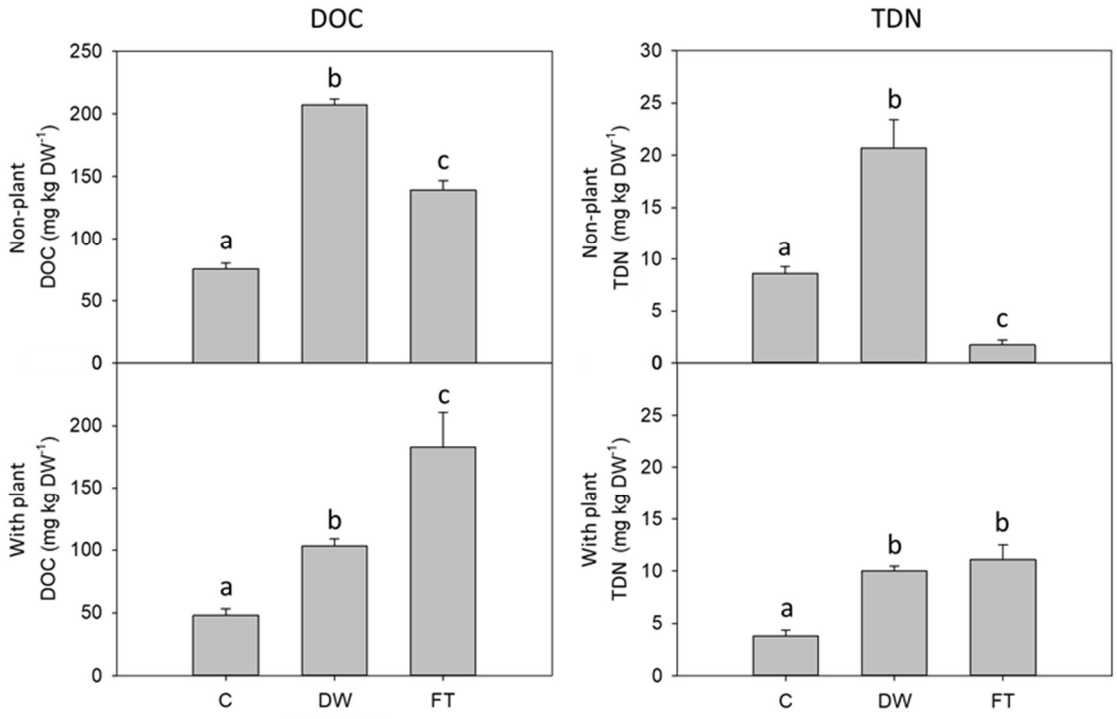

Figure 6.1. DOC and TDN concentrations in the planted and non-planted soil (water extract) exposed to either a single freeze-thaw or wet-dry cycle. Values represent means ± SEM (n = 4). C = Control; DW = Dry-wet; FT = Freeze-thaw. Different letters indicate significant differences between treatments at the $p \leq 0.05$ level.

6.3.2 Soil metabolomics

Metabolite analysis detected 352 individual compounds, of which approximately 44 % were known compounds including sugars, polyols, amino acids nucleosides (Table S1). Eighteen acknowledged osmotic compounds were identified in the soil. It should be noted, however, that some other major osmoregulation compounds (i.e. mannitol, sorbitol, trehalose) were not detected in any of the samples.

Overall, a dry-wet event had a greater effect on metabolite concentrations in soil in comparison to a freeze-thaw event. Compared with the controls in the unplanted soil, sugars and polyols (maltotriitol maltotriose, myo-inositol and sophorose) and fatty acids

(isoheptadecanoic acid NIST, linolenic acid and palmitoleic acid) were greater in the unplanted dried soil ($p < 0.05$, Figure 6.2). These compounds were also higher in the dry-wet treated soil ($p < 0.05$) than in the controls. Various amino acids in unplanted soil increased after the freeze-thaw or dry-wet event. All of the nucleosides detected in the dry-wet treated soil were significantly higher than in the controls and other treatments and the PCA of nucleosides (97.9 % variance) showed a clear separation of dry-wet treated soil (Figure S3).

Following the dry-wet event a greater number of metabolites were found in unplanted soil than in planted soil. Sugar and polyols such as lyxitol (arabinitol) and sophorose in the planted soil increased during the drought period.

In contrast to the dry-wet event, following the freeze-thaw more metabolites were found in planted soil than in unplanted soil. Various sugars (fructose, erythrose, ketohexose, sophorose, and tagatose) in planted soil increased after freeze-thaw events.

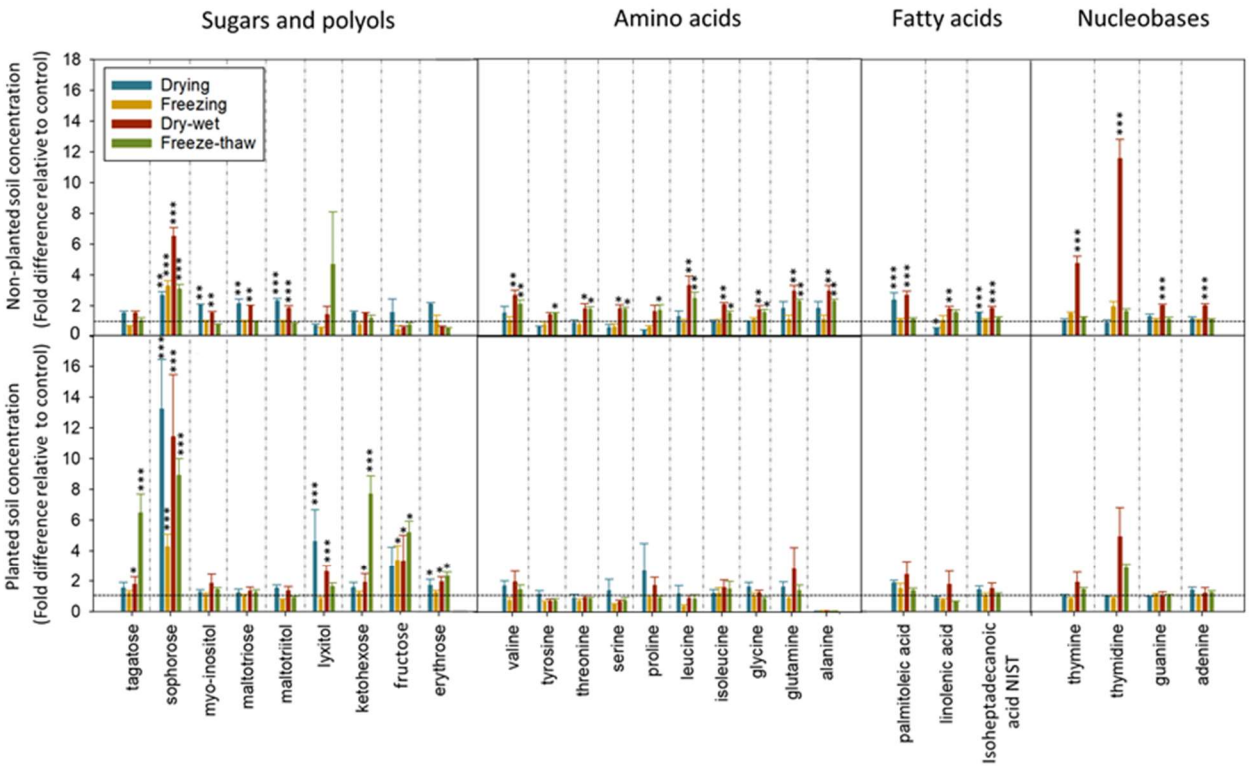

Figure 6.2. Concentration of sugars, polyols, fatty acids, and nucleobases identified in the planted and non-planted soil both during (drying, freezing) and immediately after exposure to a single freeze-thaw or wet-dry cycle relative to concentration in the un-stressed control treatment. The dotted horizontal line represents the metabolite concentration before applying drying or freezing. Stars above the plots denote significant differences from the control where *, ** and *** denote $p \leq 0.05$, $p \leq 0.01$, and $p \leq 0.001$ respectively. Values represent means ± SEM ($n = 5$).

6.4 Discussion

6.4.1 Response to a single dry-wet event

Our results indicated that microbial accumulation of solutes (osmolytes) probably occurred in response to moisture (drying) stress, increasing multiple metabolites (i.e. sugars, polyols) in the microbial biomass. This finding is consistent with Bouskill *et al.* (2016) who showed increases in multiple sugar compounds in different soil types undergoing prolonged drought. As expected, a range of compounds in dry-wet treated soil were also enriched, This may suggest that after rewetting, some of the accumulated osmotic compounds were released into the soil by microbes to prevent cell rupture, while others may have been used as C sources to support maintenance and/or growth (Schimel *et al.*, 2007). For example, myo-inositol could be used to build phospholipids (Michell, 2008).

It is also known that lyxitol and myo-inositol promote osmoregulation in bacteria and/or fungi (Beever and Laracy, 1986; Trüper and Galinski, 1986; Majee *et al*., 2004; Burg and Ferraris, 2008) and myo-inositol is known to promote osmoregulation in plants (Burg and Ferraris, 2008). The accumulation of maltotrilitol, maltotriose, and sophorose under moisture stress conditions also suggests that these were also osmolytes of soil microbes, which have not yet been documented in previous studies.

Accumulation of synthesized osmotic solutes under extreme drought conditions is an energetically expensive processes for microbes (Schimel *et al*., 2007). Here, we found that fatty acids (palmitic acids) increased in unplanted soil in response to drying stress. This is consistent with other stress response studies on different fatty acids in contrasting ecosystem and soil types (Pádrová *et al*., 2016; Ding *et al*., 2019). The increase in fatty acids may suggest that microbes accumulate lipids as a reserve energy source (Welte and

Gould, 2017), which can be used for maintenance under drying conditions.

Drying is known to damage soil microbes due to denaturation of proteins or nucleic acids (Steger, 1994). Here we found that free nucleobases increased in concentration after a dry-wet event, suggesting disruption of DNA (Rubbi and Milner, 2003) and/or transcriptional inhibition resulting in major changes in nuclear structures (Boulon *et al*., 2010; Bensaude, 2011). We also found that free amino acids (and soluble N) increased in concentration following dry-wet events, suggesting an increase in proteolysis. The lack of an effect due to drying alone, suggests that amino acids were not accumulated as osmolytes. It may also indicate that re-wetting gave rise to a rise in enzymatic cleavage of proteins and nucleic acids, which was not present or functional during the drying event, perhaps due to a lack of water.

In our study, plants proved tolerant to the drought stress. Compared with soil without plants, drying and re-wetting of planted soil had smaller impacts on DOC, soluble N and most of the measured metabolites, indicating that soil microbes adapted better to extreme drought when roots were present. The increase in sugars during drying and re-wetting probably indicates a plant response to drought or microbial accumulation of sugars where C availability is increased due to the presence of living roots. This suggests that the presence of roots may play an important role in the mitigation of the effects of drying stress on soil microbial communities and their function (Comas *et al*., 2013).

6.4.2 Response to a single freeze-thaw event

In contrast to dry-wet, a freeze-thaw event had smaller impact on DOC, soluble N and microbial metabolites, especially in unplanted soils. The smaller effect of freeze-thaw may suggest that soil microbes can be active at -5°C (Clein and Schimel, 1995) and

that significant unfrozen liquid water is available in the soil at this temperature (Brooks *et al*., 1997). Amino acid concentrations were increased following freeze-thaw. However, again, the lack of an effect of freezing alone suggests that they were not accumulated by microbes as osmolytes or cryoprotectants, but perhaps generated due to an increase in proteolysis following thawing. As in the drying treatment, proteases may have been inactive during freezing due to a lack of liquid water.

Only sophorose seems likely to have been accumulated in response to freezing where plant roots were absent. We observed increased DOC and various sugars in planted soil after the freeze-thaw event, but the sugars were mostly unlikely to be osmolytes from either plants or soil microbes because there was little or no effect on freezing alone. It seems more likely that increases in sugars resulted from damage to plant membranes, but why this should happen during thawing and not during freezing is unclear (Steponkus, 1984).

6.5 Conclusions

This study has demonstrated that a single dry-wet event greatly affected the microbial community in the soil by increasing metabolite concentrations, whereas freeze-thaw had less impact on the soil. Plants proved sensitive to freeze-thaw, but tolerant to dry-wet. The mechanisms involved in microbial osmolyte and lipid synthesis in soil require further investigation.

6.6 Acknowledgements

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Chapter 7

Life in the dark: Impact of future winter warming scenarios on C and N cycling in Arctic soils

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Abstract

It is well known that terrestrial Arctic ecosystems store large amounts of C. Climate change is likely to have a profound influence on this C store by altering primary productivity and soil organic C and N biogeochemical cycling. Specifically, increasing temperatures may shorten the period of winter freezing and extend the plant growing season (e.g. by year 2050). In extreme scenarios, it has been predicted that Arctic soils may remain unfrozen during the winter, when there is permanent darkness (e.g. by year 2100). However, there is little information about how these winter warming scenarios will affect C and N cycling in high Arctic tundra ecosystems. In this study, we assessed the effects of a simulated warmer winter on soil C and N dynamics. We collected intact soils cores from two contrasting biomes from Svalbard, namely a moss-dominated peat mire and purple saxifrage-dominated dry heath. In the laboratory, we used an Arctic light regime and three contrasting winter temperature regimes to simulate a range of climate scenarios: (i) current (present-day) climate in which the soils were frozen in winter and thawed in the summer, (ii) a year 2050 regime in which the soils were both frozen and thawed in winter), and (iii) a 2100 regime in which the soils remained unfrozen during winter. We monitored GHG emissions and nutrients in soil solution throughout a year. Our results revealed that a simulated warmer winter increased CO_2 efflux from the peat soil. This suggests that microbial decomposition of C was greater than photosynthetic C fixation. We also found that warmer winter increased N available to roots and mycorrhiza in soil in winter. This was probably due to increased decomposition of organic N. Future warmer winters will have important implications for C and N cycling, particularly in Arctic regions.

Keywords: CO_2, Carbon, Nitrogen, Arctic, Climate change

7.1 Introduction

Over the past 30 years, the Arctic region has warmed faster than any other region on earth with a resultant decrease in the extent of snow cover, sea ice, permafrost and mountain glaciers (Serreze and Barry, 2011). The snow cover in the Arctic terrestrial area in early summer has reduced by 18% since 1966 and permafrost temperature has risen up to 2 °C over the past two to three decades (AMAP, 2011).

Temperatures in the Arctic regions are expected to increase further during the 21st century, with autumn-winter temperatures projected to increase by between 3 and 6 °C by 2080 and duration of average snow cover predicted to decline up to 20% by 2050 (AMAP, 2011). As a result of these rising temperatures, a large area of permafrost is predicted to disappear by the year 2100 (Stendel and Christensen, 2002; Overland *et al*., 2013). The effect of snow cover and permafrost decline in the Arctic region, on soils, plants and animals is largely unknown (Schütte *et al*., 2019).

The warming of the climate system is altering the movement of C between the land and the atmosphere in the Arctic region with implications for global C stores (Hartley *et al*., 2012). The northern permafrost region contains approximately 1672 Pg of organic C (1466 Pg in perennially frozen soils) and accounts for approximately 50% of the estimated global belowground organic C pool (Tarnocai *et al*., 2009). A warming climate is predicted to lead to permafrost thaw with accelerated decomposition of organic matter and increased emissions of greenhouse gases (CO_2 and CH_4 in particular) to the atmosphere (Schuur *et al*., 2015).

A warming climate can lead to changes in productivity and distribution of vegetation in Arctic ecosystems that in turn could influence climate feedback under future warming scenarios (Pearson *et al*., 2013). For example, Bjorkman *et al*. (2018) found that

plant community height has increased across the Arctic over the past three decades. In addition, climate modelling studies predicted that shrubs and trees in the Arctic region will expand to cover over half the total land area in the next 30-70 years (Lawrence and Swenson, 2011; Bonfils *et al*., 2012; Pearson *et al*., 2013). These plant community changes cause multiple feedbacks between the biosphere and atmosphere, which are highly likely to have important implications for C and N cycling (Chapin *et al.*, 1994).

The Arctic region has 24 h of daylight in summer and 24 h of darkness in winter (Burn, 2015). In the summer, the Arctic receives constant sunlight which drives C fixation by plants via photosynthesis. At present, Artic region soils are often frozen in winter (Fig. S1) with winter CO_2 emissions minimal due to an inactive microbial community (Björkman *et al*., 2010; Lupascu *et al*., 2018). Future climate modelling suggests that soil will remain frozen for shorter periods over winter as a result of rising winter temperatures (Hollesen *et al*., 2011; Overland *et al*., 2013) with soils remaining above 0 ˚C for the entire winter period by 2100. If soils are not frozen over the winter period, it is possible that enhanced microbial activity due to increased soil temperature will increase soil respiration and thus winter soil CO_2 and CH_4 emissions (Hursh *et al*., 2017). However, there are substantial uncertainties as to the change in magnitude of soil GHG emissions if the Arctic becomes warmer in winter as predicted, indeed whether Arctic soils would act as a net source or sink of C. In addition to C dynamics, the impact of a warming climate on N cycling and N_2O emissions is also largely unknown. An understanding of the impact of warming Arctic soils on biochemical process such as C and N cycling are important to understand the implications of climate change in this region, and also potential feedbacks. If the ground is not frozen in winter, soil microbes are likely to increase rates of organic N mineralization and nitrification which can provide nutrients

to plant roots and associated mycorrhizas (Read and Perez-Moreno, 2003; Hill *et al*, 2011), which in turn could lead to a shift in Arctic vegetation communities (Chapin *et al.*, 1994).

Here we investigated the impact of predicted future climate change scenarios on C and N dynamics in intact plant-soil mesocosms from two common tundra vegetation types (peat mire and dry heath). We hypothesize that: i) simulated warmer winters will increase CO_2 and CH_4 emissions from both vegetation types due to increased decomposition of C by soil microbes without a concurrent increase in photosynthetic C fixation; ii) peatland will emit more CO_2 and CH_4 in warmer winter than the dry heath; iii) simulated warmer winters will increase the mineralization of soil organic N leading to an increase in inorganic N availability and a shift in vegetation cover.

7.2 Materials and Methods

7.2.1 Sample collection

Intact monoliths (10 cm diameter, 10 cm depth) containing soil and above-ground vegetation were collected from Svalbard in the high Arctic in June, 2017. In total, 20 mesocosms were collected from a moss-dominated (*Calliergon spp Campyliadelphus stellatus, Scorpidium revolvens, Tomenthypnum nitens,* and *Warnstorfia exannulata*; Nakatsubo *et al*., 2015) peat mire ecosystem located at Stuphallet (N78.96040, E011.64769). A further 20 mesocosms were collected from dry tundra heath dominated by Purple Saxifrage (*Saxifraga oppositifolia* L.) located at Knudsenheia (N78.93815, E011.84340). All samples were transported to the UK and placed in a climate-controlled room for experimentation. The saxiflowers were wilted during the transportation.

The peat mire sampled here represents one of the largest areas of moss carpet

formed on Svalbard. In the dry heath, *S. oppositifolia* represents one of the most widely distributed plants in Svalbard (Brysting *et al*., 1996), is colonized by arbuscular mycorrhizas (Oehl and Koerner, 2014) and represents a sentinel for early spring flowering (Panchen and Gorelick, 2015). The soil characteristics for each site are shown in Table 7.1. Mean monthly temperature and incident radiation from a weather station located in Ny-Ålesund, Svalbard (N78.92359, E011.92560), close to the field sampling sites, is shown in Figure. S1-S2.

Table 7.1. Characteristics of the two soils used in the experiments. Values represent means ± SEM (n = 5).

	Peat	**Tundra**
Temperature in the field (°C)	4.0 ± 0	6.0 ± 0
pH	7.0 ± 0	6.5 ± 0
Dissolved C (mg/L)	29.4 ± 5.6	9.39 ± 0.82
Total dissolved N (mg/L)	7.95 ± 1.03	13.3 ± 4.6
Ammonium (mg N/L)	0.20 ± 0.02	< 0.01
Nitrate (mg N/L)	0.01 ± 0	0.39 ± 0.11
Phosphate (mg/L)	0.26 ± 0.01	0.02 ± 0.01

7.2.2 Experimental design

In the high arctic, the sun does not set between 18 April and 24 August (24 hour daylight), and does not rise between 24 October and 18 February (24 hour darkness). The experiment was therefore designed to simulate a 12 month Arctic seasonal pattern with different winter freezing conditions. Firstly, all the mesocosms were exposed to summer conditions (24 h light for 9 weeks at +7 °C), followed by an autumn (12 h of light/12 h of darkness for 3 weeks at +7 °C). Prior to imposing winter conditions, the cores of each

vegetation type were randomly assigned to one of three groups (n = 5) reflecting three potential winter scenarios (Figure 7.1):

Scenario 1 (present-day) in which the mesocosms were frozen over the dark winter and spring period (-10 °C; 29 weeks) and then naturally thawed (+7 °C) in the summer during the 24 h light period (15 weeks).

Scenario 2 (year 2050) in which the mesocosms were frozen over the majority of the simulated winter (-10 °C; 18 weeks) and thawed (+7 °C) 14 days before the end of this dark winter period. They then experienced spring (+7 °C; 3 weeks) and summer conditions as detailed above;

Scenario 3 (year 2100) in which soils were not frozen during the entire winter and spring period and remained at +7 °C throughout the experiment.

The photosynthetically active radiation (PAR) during the light periods was 600 µmol m^{-2} s^{-1} while during the dark period it was 0 µmol m^{-2} s^{-1} (PAR-FAR Sensor; Skye Instruments Ltd, Powys, UK). When the cores were not frozen, artificial rainwater (NaCl = 15.48 mg/L, KCl = 1.45 mg/L, $MgSO_4$ = 4.77 mg/L, $CaCl_2$ = 18.87 mg/L) (Krawczyk *et al.*, 2008) was added back to the soil surface to maintain moisture contents reflecting those at the time of collection. Gas and soil solution were measured weekly once the experiment had commenced.

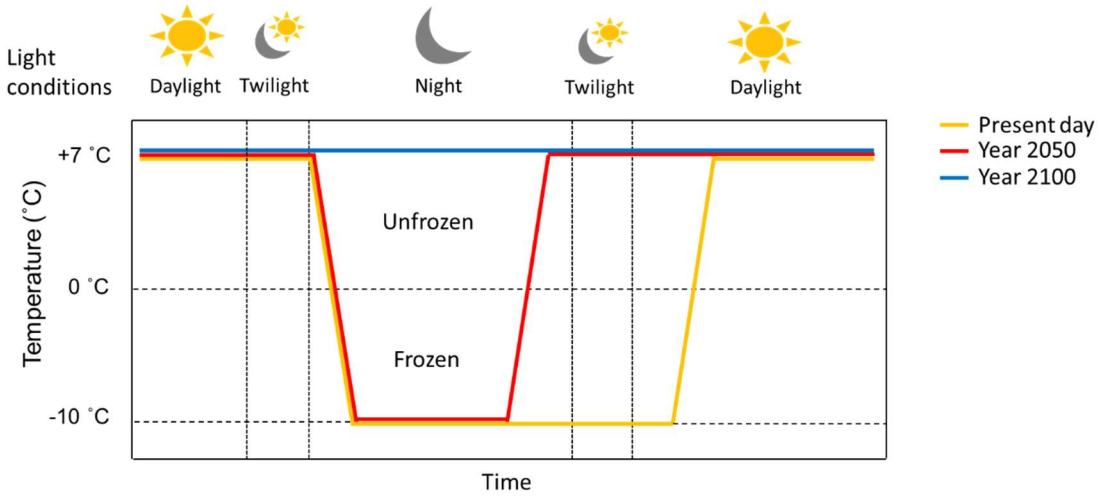

Figure. 7.1. Schematic of experimental design. Present day = frozen during the dark and thawed under light condition (-10 °C / +7 °C). Year 2050 = Frozen and thawed under dark conditions (-10 °C / +7 °C). Year 2100 = Unfrozen during both the dark and light period (+7 °C).

7.2.3 Soil greenhouse gas emissions

GHG samples were taken weekly throughout the sampling year. At each sampling time, a 1.8 L gas chamber was placed on the top of each soil core and 20 mL gas samples collected from the container's headspace through a rubber septum using a syringe and placed into pre-evacuated 12 mL vials, at time 0 and after 1 h. Samples were taken under both light and dark conditions, where appropriate. The light condition is a measurement of photosynthesis and plant-soil respiration combined. The dark condition measures soil-plant respiration only. CO_2, CH_4 and N_2O concentrations in the vials were determined by gas chromatography using a Varian 450 GC (Bruker Ltd., Coventry, UK) with a CombiPAL autosampler (PerkinElmer Inc., Waltham, MA). CO_2 and CH_4 were detected by a flame ionization detector (FID) and N_2O by a ^{63}Ni electron capture detector (ECD). The gas flux was calculated according to Dunn *et al*. (2016), using the linear

portion of the standard curve. Daily cumulative flux for each gas was calculated assuming a constant flux rate between each measurement, multiplying the hourly flux rate by 24 h for constant dark or light treatment or 12 h each for dark + light conditions. The sum of each daily flux estimate was expressed cumulatively as the sum of all previous days in g CO_2 m^{-2}. To estimate total cumulative CO_2 flux, cumulative light CO_2 flux was added to cumulative dark CO_2 flux (Total cumulative CO_2 flux = cumulative light CO_2 flux + cumulative dark CO_2 flux). Global warming potential (GWP) of CO_2, CH_4, and N_2O was estimated in CO_2 equivalents (g CO_2e m^{-2} y^{-1}) by multiplying the cumulative fluxes of one year for 100 year GPW (CO_2 = 1; CH_4 = 34; N_2O = 298) (IPCC, 2013).

7.2.4 Soil chemical analysis

Soil solution was collected weekly using a syringe and Rhizon-MOM samplers (Rhizosphere Research, Wageningen, Netherlands) from each soil core. DOC and total TDN in soil solution were determined using a multi N/C 2100 (Analytik Jena AG, Jena, Germany). NH_4^+, NO_3^- and PO_4^{3-} were measured by ion chromatography using an 850 Professional IC (Metrohm UK Ltd., Runcorn, UK).

7.2.5 Plant biodiversity and ground cover

At the end of the experiment, the relative abundance of plant species was estimated using a quadrat with 1 cm^2 square grid intersections.

7.2.6 Statistical analysis

A one-way analysis of variance (ANOVA) was performed to determine the effect of warming winter on nutrient concentrations (DOC, TDN, NO_3^- and PO_4^{3-}), GHG

emissions (CO_2, CH_4 and N_2O) between the three climate scenarios. To compare differences between pairs of climate scenarios, multiple comparisons were carried out by Tukey post-hoc test at significance level of 0.05. Repeated measure analysis of variance (ANOVA) was undertaken to evaluate differences in nutrient concentrations over time. All statistical analyses were performed using RStudio (R Development Core Team, 2004).

7.3 Results

7.3.1 Greenhouse gas emissions from the peat-mire mesocosms

Cumulative CO_2 fluxes from the peat soil decreased (i.e. net C fixation) during simulated summer-autumn conditions over 23 weeks prior to the start of the climate scenario treatments (Figure 7.2).

Cumulative CO_2 flux during the simulated winter period was greater for the 2050 and 2100 climate scenarios than the present-day climate scenario ($p < 0.001$). For the present-day simulations, after the peat cores were thawed in the summer (24 h light) season cumulative light CO_2 flux was significantly greater than in the other two climate scenarios (Figure 7.2, $p < 0.001$), however, this relationship was not apparent for total cumulative flux, where no significant differences are apparent between the three climate scenarios over the 12 month experimental period.

There was no significant difference in N_2O flux between all treatments over the course of the experiment ($p = 0.37$; Figure S3). There were some peaks in CH_4 flux in peat soil during the experiment, however, theses fluxes were small and did not differ between treatments ($p = 0.48$; Figure S3).

7.3.2 Greenhouse gas emission from the dry heath soil mesocosms

Cumulative CO_2 fluxes in the light were greater for the 2050 climate scenario treatment than either the 2100 or present-day climate scenarios at the end of the experiment ($p < 0.01$, Figure 7.2). This response was largely driven by fluxes in the light following thawing. However, cumulative total CO_2 flux during the simulated winter period did not differ between climate scenarios ($p = 0.16$).

For both the 2050 and 2100 climate scenarios, there were significant increases in N_2O during thawing ($p < 0.001$ and $p < 0.05$, respectively, in comparison to the present-day treatment; Figure S3). There was no significant difference in CH_4 flux between all treatments over the experiment ($p = 0.12$; Figure S3). There was no significant difference in GPW between treatments over the 12 month duration of the experiment ($p = 0.07$; Figure S4)

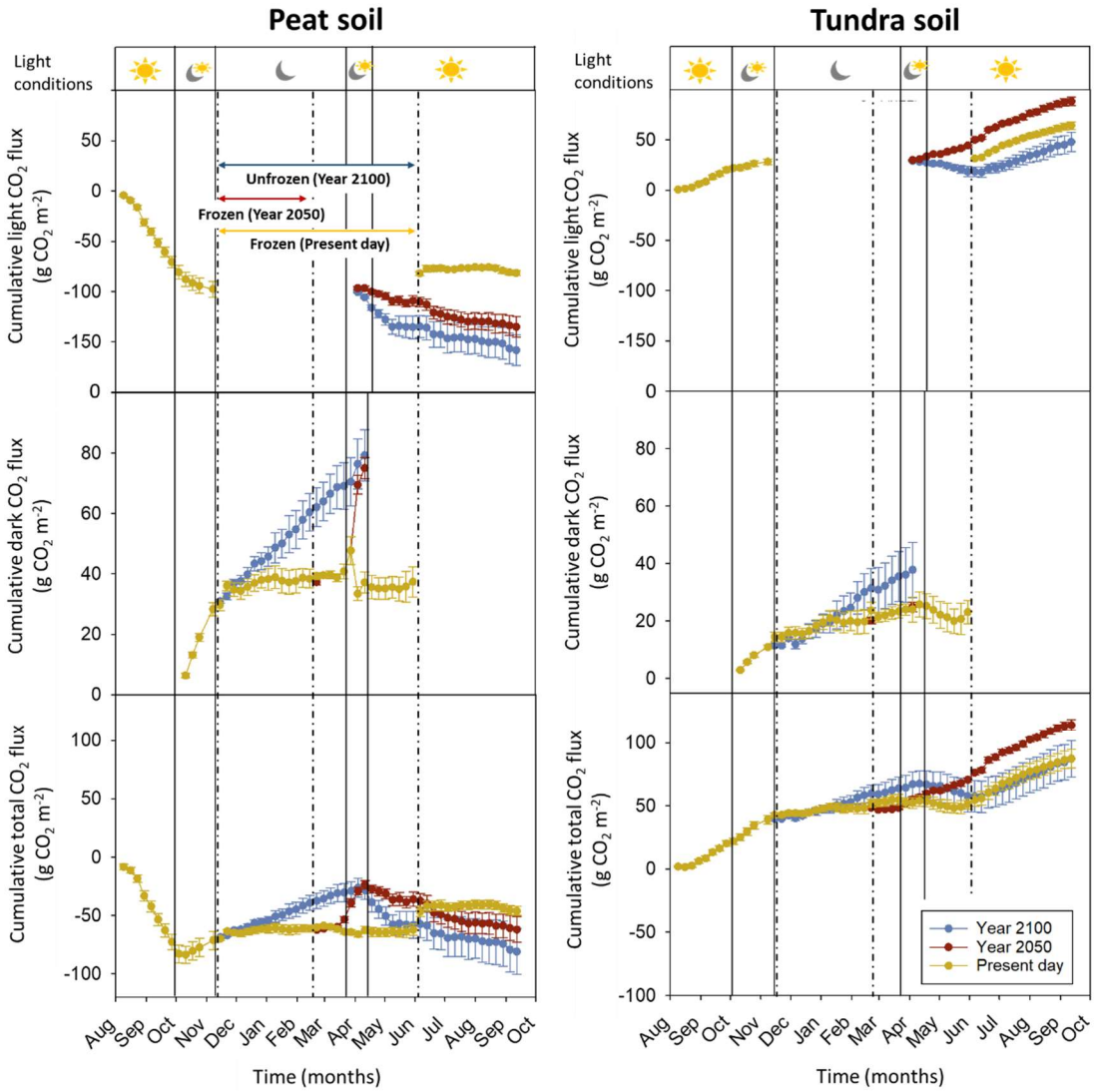

Figure 7.2. Cumulative CO_2 flux of daytime, night-time, and total under three different simulated winter climate regimes (present day, year 2050 and year 2100) in a wetland peat soil and a mineral tundra soil. Values represent means ± SEM (n = 5). Present day = frozen during the dark and thawed under light condition (-10 °C / +7 °C). Year 2050 = Frozen and thawed under dark conditions (-10 °C / +7 °C). Year 2100 = Unfrozen during both the dark and light period (+7 °C). The solid lines denote the times at which the light conditions were changed, and the dotted lines denote the times at which the temperature conditions were changed (Figure 7.1.).

7.3.3 Nutrient dynamics in the peat-mire mesocosms

Soil DOC concentrations differed between all three simulated climate scenarios ($p < 0.001$, Figure 7.3). At the end of the experimental period, the greatest DOC concentrations were in the present-day climate treatment, lowest in the 2100 scenario and intermediate in the 2050 climate scenario. We did not measure any nutrient concentration in soil solution during the period when the cores were frozen. After thawing of samples both in the winter (2050 scenario) and the summer (present-day scenario) conditions, DOC concentration was higher than immediately before freezing ($p < 0.01$ and $p < 0.05$, respectively). The increased DOC concentration then declined gradually over time ($p < 0.01$ and $p < 0.05$, respectively).

Soil phosphate concentrations appeared to peak on thawing in the present-day and 2050 climate scenario treatments followed by a slow gradual decline ($p < 0.001$), mirroring the DOC results (Figure 7.3). Soil phosphate concentrations differed between all three climate scenarios with greatest soil phosphate evident in the present-day climate treatment, lowest in the 2100 scenario and intermediate in the 2050 scenario ($p < 0.001$, Figure 7.3), again following the DOC results. TDN concentration in soil solution also followed a very similar pattern ($p < 0.01$, Figure 7.3).

7.3.4 Nutrient dynamics in the dry heath mesocosms

DOC, PO_4^{3-} and NH_4^+ concentrations in soil did not differ between climate scenarios in the tundra soils throughout the study period ($p > 0.05$; Figure 7.3 and 7.4). In contrast, soil NO_3^- concentrations increased ($p < 0.001$) over the winter period in the unfrozen mesocosms (2100 scenario), NO_3^- then decreased to below the concentrations

recorded in the present-day and 2050 scenarios ($p < 0.01$). After thawing, NO_3^- in both the present-day and 2050 scenarios was greater than the day before freezing ($p < 0.05$ and $p < 0.01$, respectively). TDN concentrations in soil solution followed the same pattern as observed for NO_3^- ($p < 0.001$, Figure 7.4).

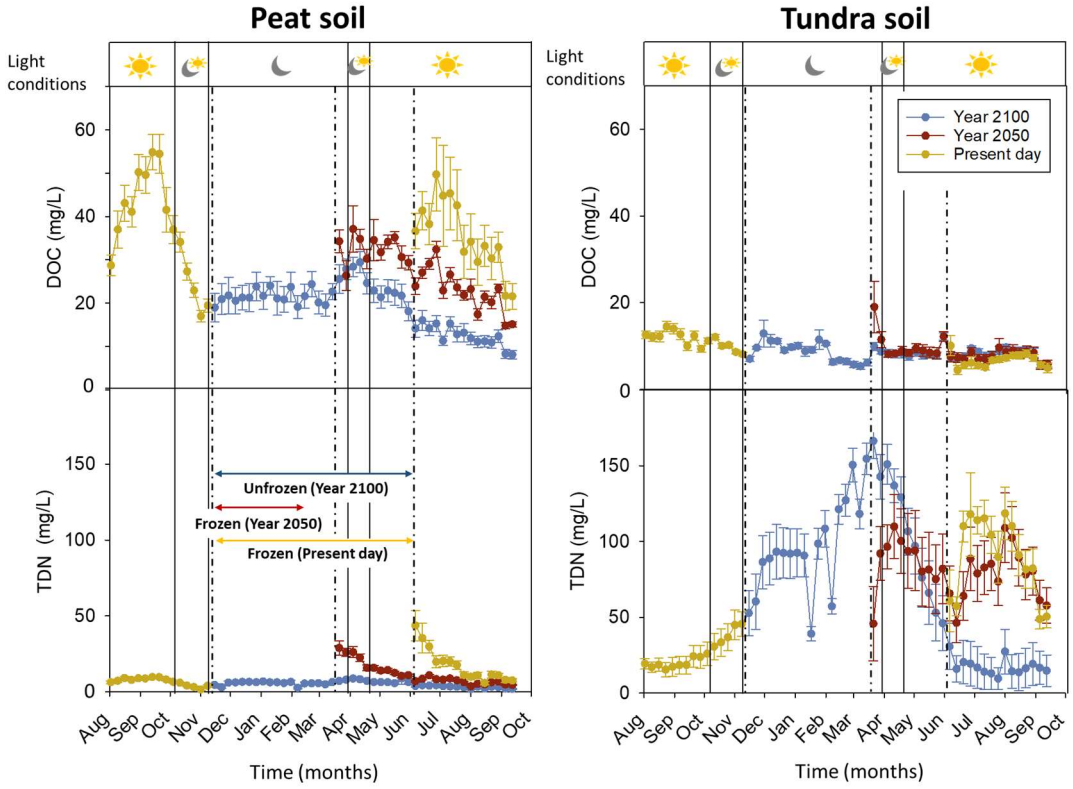

Figure 7.3. DOC and TDN in soil solution under three different simulated winter climate regimes (present day, year 2050 and year 2100) in a wetland peat soil and a mineral tundra soil. Values represent means ± SEM (n = 5). Present day = frozen during the dark and thawed under light condition (-10 °C / +7 °C). Year 2050 = Frozen and thawed under dark conditions (-10 °C / +7 °C). Year 2100 = Unfrozen during both the dark and light period (+7 °C). The solid lines denote the times at which the light conditions were changed, and the dotted lines denote the times at which the temperature conditions were changed (Figure 7.1.).

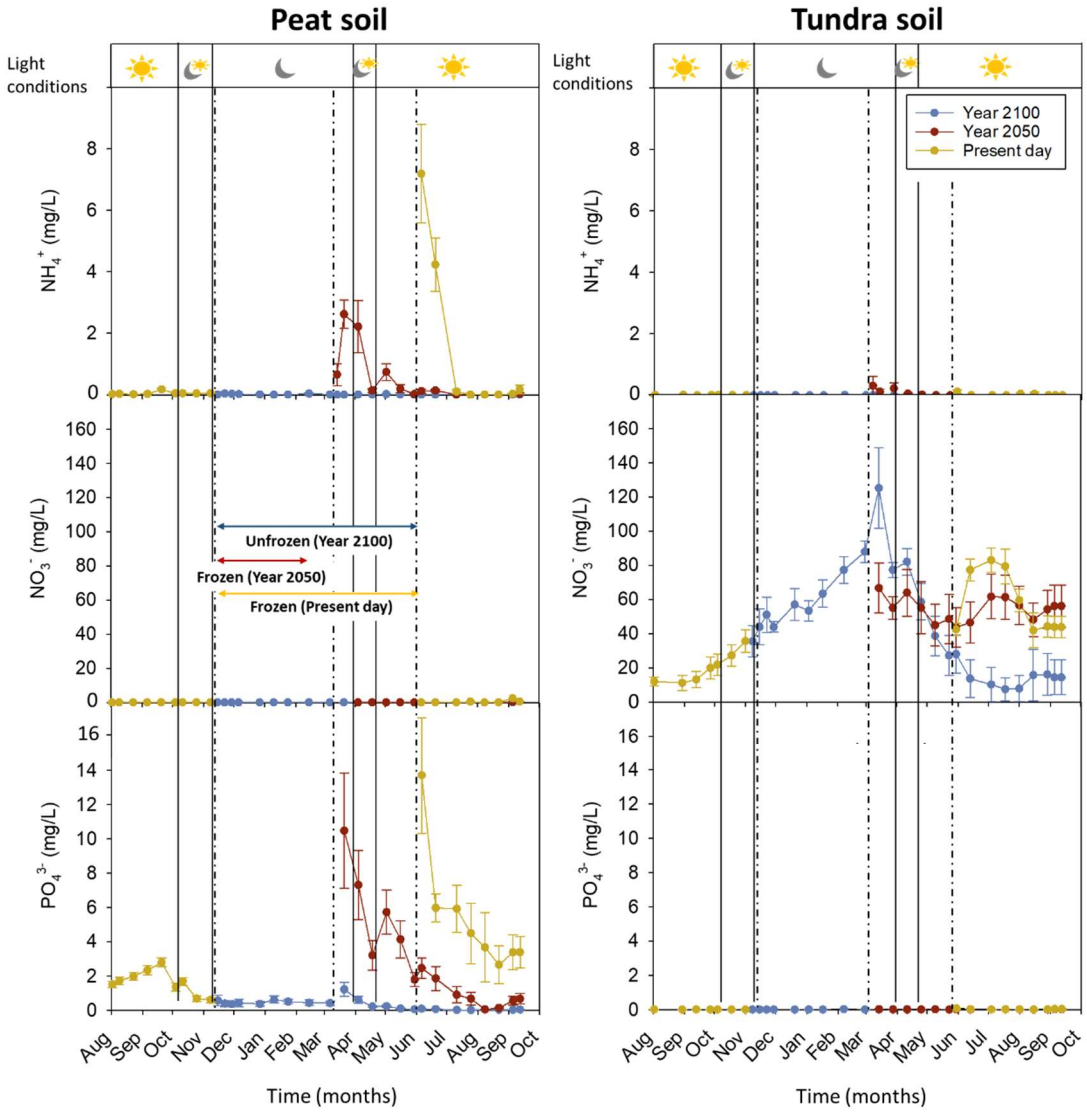

Figure 7.4. Ammonium (NH_4^+), nitrate 'NO_3^-), and phosphate (PO_4^{3-}) in soil solution under three different simulated winter climate regimes (present day, year 2050 and year 2100) in a wetland peat soil and a mineral tundra soil. Values represent means ± SEM (*n* = 5). Present day = frozen during the dark and thawed under light condition (-10 °C / +7 °C). Year 2050 = Frozen and thawed under dark conditions (-10 °C / +7 °C). Year 2100 = Unfrozen during both the dark and light period (+7 °C). The solid lines denote the times at which the light conditions were changed, and the dotted lines denote the times at which the temperature conditions were changed (Figure 7.1.).

7.3.5 Above-ground vegetation

After 2-3 weeks of being in complete winter darkness, the above-ground moss vegetation in the peat cores under the 2100 climate scenario changed from yellow to green. *Carex spp.* also appeared in certain cores during the experimental period (less than 20 % of cover) but did not appear to be affected by treatment (Figure S5).

In the dry heath cores, most of the flowers of *S. oppositifolia*. senesced before the experiment started because of its short growing season (Pietiläinen and Korpelainen, 2013). Lots of dead flowers remained on the surface of the dry heath soil cores throughout the experiment.

Under the 2100 treatment (where soils remained at +7 °C over the dark winter period) more flower buds of *S. oppositifolia* appeared during the simulated spring than in the other two treatments. They also started to flower earlier in the season. Under the 2100 climate scenario other plant species (e.g. *Draba lactea*, *Saxifrage nivalis*, *Cerastium arcticum*) began to replace *S. oppositifolia* over time (Figures S6 and S7).

7.4 Discussion

7.4.1 CO_2 emissions

In our study, the net mean summer CO_2 flux from peat-mire soil was -58.3 ± 2.7 mg CO_2 m^{-2} h^{-1}, which indicates more CO_2 uptake in peat moss by photosynthesis than CO_2 release to the atmosphere by microbial activity. A similar negative flux was also observed in peatland in Ny-Ålesund by Nakatsubo *et al*. (2015). In contrast, the net mean summer CO_2 flux from the dry heath tundra soil was (+14.8 ± 1.2 mg CO_2 m^{-2} h^{-1}), in which C in tundra soil was being lost into the atmosphere. This is probably due to lack of

vegetation cover and relatively high soil temperatures (Li *et al*., 2017).

As freezing soils at -10 °C largely inhibits microbial respiration and growth (Segura *et al*., 2017), cumulative winter CO_2 fluxes during the frozen period tended to be constant (i.e. net zero emissions) due to limited biological activity (Schimel *et al*., 2007). We observed that large fluxes of CO_2 from soils were observed following thawing (Figure 7.2). This is consistent with previous studies which observed CO_2 pulses from different types of soil following spring-thaw (Raz-Yaseef *et al.*, 2017).

As we expected, cumulative winter CO_2 flux from unfrozen soil increased during the polar night (Figure 7.2). This suggests that the winter dark-period limits CO_2 uptake by photosynthesis and restricts the energy supply for essential metabolic processes (Wilhelm and Selmar, 2011), which may affect C substrate supply regulating rhizosphere processes such as rhizosphere respiration and root exudation (Jones *et al*., 2004). Our results revealed that total winter CO_2 emissions from the peat-mire soil (74.5 ± 8.2 g CO_2 m^{-2}) were greater than from the tundra dry heath soil (35.0 ± 9.3 g CO_2 m^{-2}). This may reflect the greater soil C content, water content, and vegetation cover in the peat-mire soils.

7.4.2 C cycling and nutrient dynamics in peatland

Freezing can damage root cells and microbes (Soulides and Allison, 1961; Pearce, 2001) and/or increase accumulated osmolytes in roots and/or microbes to adapt to the extreme environment (Burg and Ferraris, 2008). Our data revealed that DOC, TDN, and PO_4^{3-} concentration in peat soil solution (Figure 7.3 and 7.4) increased following thawing both in the dark winter and the summer daylight conditions, suggesting that released C, N and P mainly originated from the disruption of microbial and/or root cellular

components which underwent lysis upon thawing (Schimel *et al*., 2007). The concentration of DOC, TDN and PO_4^{3-} decreased probably due to immobilization by surviving soil microbes and uptake by plant roots and associated mycorrhizas.

In the 2100 climate scenario, more C was lost to the atmosphere from unfrozen soil during dark winter than in other scenarios. This suggests that DOC concentration in peat-mire soil solution was reduced over the winter as the available C was decomposed by soil microbes and respired as CO_2. In addition, roots may have taken up DOC and organic N from soil under the unfrozen conditions. Although, as peat moss lack roots (Weston *et al*., 2015) C transportation in the peat moss may be limited.

7.4.3 CH_4 emissions from the peat-mire mesocosms

Although the magnitude of CH_4 emissions from soil was relatively small (Figure S3), the observed pulses of CH_4 from some peat-mire cores were probably caused by the sporadic ebullition of gas bubbles which is common in these ecosystems (Bon *et al*., 2014). In addition, the growth of *Carex spp.* in the mesocosms may also have facilitated CH_4 loss (Bowes and Hornibrook, 2006). *Carex. spp* is one of the most widespread of all arctic salt marsh plants (Stenström *et al*., 2002) and its stems may provide a conduit for CH_4 release from soil (Ford *et al*., 2012). Previous studies found that higher peatland CH_4 emissions when *Carex rostrata* is present with emission positively correlated with active plant growth in waterlogged soils (Noyce *et al*., 2014). This response, however, appears not to be related to convective stem flow but may relate to the provision of increased C substrate for CH_4 production either from root exudation or root turnover (Chanton, 2005). However, contradictory results obtained by Corbett *et al.* (2013) suggest that more work is needed to establish the role of *Carex. spp* in mediating and modulating CH_4 losses from

soil.

The freeze-thaw process may release CH_4 to the atmosphere, resulting decomposing organic C under anaerobic waterlogged soils (Knoblauch *et al*., 2018). Some studies revealed that thawing permafrost increased CH_4 emission from arctic peatland and arctic tundra because of increasing water saturation and changing in methanogen communities on thawing (Johnston *et al*., 2004; Natali *et al*., 2015). However, despite the soil being waterlogged throughout the experimental period, our CH_4 results showed that thawing of peat soil did not alter CH_4 emission.

7.4.4 N cycling in arctic tundra

Future changes in soil nutrients, especially inorganic N, are likely to have important implication for ecosystem functioning. NO_3^- may be an important N source in Arctic vascular plants and is highly mobile in soil (Atkin, 1996). Increased soil NO_3^- concentrations in the dry heath during the dark winter period (Figure 7.4), suggest NO_3^- was produced by a combination of organic N mineralization, ammonification, and nitrification. As the mesocosms were hydrologically isolated this led to the accumulation of NO_3^- in soil. Irrespective of whether the soil was under light or dark condition, soil microbes take up organic N (e.g. amino acids, peptides) and NH_4^+, but generally they do not favour the uptake of NO_3^- as it energetically inefficient (Hill *et al.*, 2011). *S. oppositifolia* can acquire a variety of N forms including organic N, ammonium, and nitrate (Volder *et al*., 2000), but this occurs when daylight is present. In the absence of daylight, plants probably did not take up any N due to a lack of available energy being supplied by photosynthesis (Iivonen and Vapaavuori, 2002; Pavlovič *et al.*, 2010) and also due to a lack of demand for plant growth. The high concentration of NO_3^- in soil,

however, provides a ready source of N for plants once they regain photosynthetic activity in the spring/summer. This is supported by the decline in soil solution N after the dark treatment. Arctic plants (including *S. oppositifolia*) also have a high capacity to absorb nitrate (Atkin *et al*., 1993; Chapin *et al*., 1993), however, this did not translate into an observable increase in *S. oppositifolia* growth in our experiment. Alternatively, NO_3^- was converted into NH_4^+ or other organic N by mycorrhizae before roots, as some mycorrhizae could assimilate NO_3^- (Veresoglou *et al*., 2012; Näsholm *et al*., 2013). Ectomycorrhizal (EM) and ericoid mycorrhizal (ECM) fungal communities can contribute about 61-86% of the plant N in the Arctic tundra (Hobbie and Hobbie, 2006). *S. oppositifolia* has been reported to form both arbuscular and EM symbiosis in the high Arctic (Kytöviita, 2005).

In addition, more NO_3^- in soil could be lost as N_2O (Benckiser *et al*., 2015), however, our results showed higher N_2O emissions were not found during the NO_3^- declining period. Either the low temperature and alkane pH inhibit denitrification (Shammas, 1986; Glass and Silverstein, 1998), or the NO_3^- was fully denitrified through to N_2.

In the dry heath tundra soil, we observed an increase in NO_3^- after thawing both in the dark winter (2050 simulation) and light summer (present-day simulation), however, no such response was observed for NH_4^+. This contradicts the results from Song *et al*. (2017) who observed NH_4^+ and NO_3^- concentration significantly increased following thawing and NH_4^+ increased more than NO_3^- because nitrification was inhibited by low temperature and NH_4^+ can be retained on exchange sites. Our results showed the increased NO_3^- concentration remained high in soil solution, suggesting that NO_3^- was not taken up by either plants or soil microbes, with only small amounts lost as N_2O.

7.4.5 Implication of C and N cycling

Arctic soils are highly variable in space in response to local variations in parent material, hydrological regime, climate, pedogenic age and nutrient inputs (Lev and King, 1999; Meynier and Brun, 2018). Our study showed that the response of Arctic soils to climate change is both highly dependent on soil and associated vegetation type. Winter warming significantly promoted C cycling in peat-mire soil and N cycling in the dry heath. When daylight was present, a large proportion of CO_2 loss from tundra soils and uptake of CO_2 in the peat-mire cores occurred, suggesting that peat-mire soil had greater inputs of labile C via photosynthesis than tundra soil.

Under the 2100 climate scenario, we found early flowering of *S. oppositifolia* in the dry heath mesocosms. 8 % of the area from the Tundra core had shifted to different plant species by late summer (Figure S6). A combination of warming and longer growing seasons can allow the roots of plants to penetrate the soil and increase the rate of decomposition of dead organisms, which increase the number of nutrients introduced into the soil (Kuperman, 1999). These changes are very highly vulnerable to future vegetation shifts and can affect C and N cycling (Chapin *et al.*, 1994).

Our laboratory did not have grazing animals, however several studies showed that grazing pressure strongly affects plant growth, plant community composition, plant biomass, species richness, moss cover and lichen cover (van der Wal *et al*., 2001; Olofsson, 2009). Warmer winters are likely to increase the overall carrying capacity of the Arctic tundra for animals (e.g. reindeer). If winter to have no snow, food will be available for longer during the winter. Wild animals and migratory birds can provide more N input on soils (Hayashi *et al*., 2018). Vegetation shifts are currently occurring in arctic region due to grazing pressure and atmospheric N deposition (Olofsson, 2006). Grazing

pressure and animal derived-organic N maybe sensitive to vegetation change.

The peat depth in Stuphallet is > 100 cm (Rozema *et al*., 2006), but we only measured surface of this peat soil (10 cm in depth). This means the total amount of CO_2 released from soil could actually be much greater than the release of CO_2 from the surface layers, indicating the importance of peatland as a potential C source. As greater snow cover in the arctic provides soil thermal insulation (Männistö *et al*., 2013), soil microbes and plant cells in the arctic ecosystem could be more sensitive to snow-free freeze-thaw treatments. We only applied a single freeze-thaw treatment, but repeated freeze-thaw process may also frequently occur under both current and near-future climate conditions.

7.5 Conclusions

We found that cumulative CO_2 fluxes under future climate change scenarios (2050 and 2100) were not significantly different from the current climate model in both peat-mire and dry heath tundra Arctic soils. Warmer winters altered peatland C cycling, from a net sink to a net source of CO_2 in winter, but this is compensated for by enhanced photosynthesis in summer. We also found future climate scenarios decreased DOC in these peat-mire soils.

Warmer winters may also alter N cycling in Arctic regions, particularly in dry heath tundra soils, with the likely impact being increased nutrient supply to roots, due to increases in plant-available N from microbial mineralization of native soil organic matter. This available N may lead to a shift in Arctic vegetation due to higher soil temperatures and lack of snow cover under future climate scenarios (2100 scenario)

7.6. Acknowledgements

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Chapter 8

General discussion

8.1. Introduction

In this chapter, the main findings of the experimental studies (Chapter 3-7) presented in this thesis are summarised and general conclusions based on the findings are described. Furthermore, implications for soil C and N dynamics under environmental change scenarios are considered and suggestions for further research presented. This chapter concludes with a synthesis of the results of these studies.

8.2. Summary of key findings and experimental results

The aim of this thesis was to investigate i) the effects of a single freeze-thaw or dry-wet event on C pools, GHG emission, extracellular enzyme activity, soil organic matter, metabolic products, and ii) the effects of a warming Arctic winter on GHG emission and nutrients availability in soils.

Chapter 3 investigated the effect of freeze-thaw and dry-wet events on microbial activity in soils. Our results show a significant increase of CO_2 and DOC from different microbial pools in soils after rewetting and thawing from soils.

Chapter 4 investigated how the C budget of the combined plant-soil system responded to freeze-thaw and dry-wet events. CO_2 flux (photosynthesis and respiration combined) from the experimental grassland system was greater following a freeze-thaw event in planted than non-planted soils. CO_2 flux from planted soil reduced during the drought period and the CO_2 flux from planted soil was negatively correlated with water. Freeze-thaw events (particularly at -10 ˚C) damaged plants, increasing microbial activity and rhizodeposition, which resulted in increased CO_2 flux.

Chapter 5 investigated the effects of single freeze-thaw or dry-wet events on extracellular enzyme activity and organic matter turnover in an agricultural grassland soil.

Enzyme activities which control decomposition of plant residues (i.e. β-Glucosidase) decreased after the freeze-thaw or dry-wet event. We also showed that the decomposition of plant residues was unaffected by either a freeze-thaw or dry-wet event. However, CO_2 increased during freezing or drying of the soil.

Chapter 6 determined changes in the low molecular weight metabolite profile of the soil during freezing or drying periods by an untargeted GC-MS mass spectrometry. Drying and freezing caused increases in metabolites (i.e. sugars, polyols), particularly in the unplanted soil. Plants proved sensitive to freeze-thaw, with release of sugars and polyols due to damaged cells.

Chapter 7 investigated how future climate scenarios affect arctic climate and ecosystems. We monitored GHG emissions and nutrients in soil solution throughout a year. Our results revealed that a simulated warmer winter increased CO_2 efflux from the peat soil. We also found that warmer winter increased N available to roots and mycorrhiza in soil in winter.

These results clearly showed that the mechanisms (e.g. evidence of osmolytes) responsible for CO_2 and DOC release after a single freeze-thaw or dry-wet event, and plants are a key factor controlling soil C cycling. The studies also demonstrated that importance of modeling the impact of future climate change scenarios on soil C and N cycling. We have answered the fundamental questions listed in Figure 1.1.

8.3 Synthesis and implication for C and N under environmental changes

The potential future effects of global climate change on soils in temperate grassland and polar plant-soil systems include more frequent freeze-thaw and dry-wet cycles, with longer periods of drought, and early snow melt predicted (IPCC, 2007).

These events can have variable effects on C and N cycling.

Grassland covers 37 % of the terrestrial area (O'Mara, 2012) and contains range 96 to 190 pg C (20 cm depth) (Ojima *et al*., 1993). Plants play an important role in C cycling because they absorb CO_2 and release O_2 as part of the process of photosynthesis. Freeze-thaw or dry-wet events can damage plants, with release of C and N from plant organs into the soil (Chapter 4 and 6). When plants are killed by freeze-thaw or dry-wet events, they no longer absorb CO_2 by photosynthesis. This may change the long-term microbial respiration rates from soil and implies the change in terrestrial C cycling in which these agricultural ecosystems become net source of C from C sink. Freeze-thaw or dry-wet may change the soil C balance in grassland soils between changes in C losses from decomposition and C gains through photosynthesis. For example, drought reduces CO_2 emissions from soil due to limited soil microbial activity (Chap 4; Gargallo-Garriga *et al*., 2008). This suggests that freeze-thaw or dry-wet (especially extreme conditions) significantly affect not only soil functioning but also terrestrial ecosystems across the earth. Frequent freeze-thaw or dry-wet events may affect plant growth and the productivity of crops and livestock, including milk yields, may decline (Osakabe *et al*., 2014; Zampieri *et al*., 2017). Shifting seasonal rainfall patterns may delay planting and harvesting. In the present day, the global population has grown, in particular India and China are increasing rapidly (United Nations, 2019). However, reduced food production means that millions of people may face food poverty.

Freeze-thaw or dry-wet events may kill some soil microbes (Chapter 3 and 6; Soulides and Allison, 1961; Skogland *et al*., 1988) because soil microbes directly suffer from freezing or drying stress. Microbial community composition may change with decreasing soil microbes in response to freeze-thaw or dry-wet events (Lundquist *et al*.,

1999b; Mclean and Huhta, 2000) and this may lead to changes in C and N mineralization rate in soils. Loss of some of these microbes by freeze-thaw or drying-rewetting could reduce the mineralization rate of SOM (Chapter 3; Fierer and Schimel, 2002), which affect nutrient availability for plants.

On the other hand, soil microbes can adapt to extreme environmental conditions (i.e. freeze-thaw and dry-wet). It is known that soil microbes can accumulate LMW C (i.e. sugars, polyols and amino acids) in the microbial cell (Chapter 6; Cushman, 2001). This LMW C is released into soils on thawing or rewetting (Chap 3 and 6). Addition of LMW C into soils may stimulate SOM turnover (priming effect) and increase in C to the atmosphere (Xiang *et al*., 2008), which is important implication for soil C cycling. Studies frequently have observed an increase in soil respiration (CO_2 pulse) immediately after dry-wet or freeze-thaw events (Chapter 3 and 4). Many studies concluded that this was due to a biological disruption in response to extreme environmental changes, not SOM turn over (Chapter 5; Fierer and Schimel, 2003; Schimel *et al*., 2007). This biological adaption strategies to freezing or drying stress has crucial implications for terrestrial GHG emissions.

Extracellular enzymes in soil are sensitive to temperature and moisture (Chapter 5) and our results showed enzymes decreased after freeze-thaw or dry-wet events and these reductions are unlikely to greatly affect SOM mineralization rate. However other studies observed extracellular enzymes increased after thawing or rewetting (Burns *et al*., 2013). This provides more opportunities to degrade of SOM and may affect microbial respiration rate in soils, which implies a positive feedback to climate change.

Under future climate scenarios (Chapter 7), plants are a key factor controlling C and N cycling. Increased winter temperatures in artic plant-soil systems stimulated more

CO_2 producing microbial activity in unfrozen soils, which may important implication for global C cycling. Permafrost soils in northern latitudes contain approximately half of the global belowground organic C (~ 1700 Pg C) (Tarnocai *et al*., 2009). Future climate scenarios predict thawing of permafrost completely by 2100 (Stendel and Christensen, 2002; Overland *et al*., 2013). If this happened in the future, huge amount of C from unfrozen soils will release to the atmosphere (Chapter 7; Schuur *et al*., 2015). More GHGs raise the earth's temperature and this may lead to risk to human life all over the world.

Warming winters may also increase N mineralization rates due to enhanced microbial activity (Chapter 7), leading to a shift in plant composition and the potential for invasive species from low latitude to be introduced to the Arctic region in future (Pearson *et al*., 2013). More N in soils provides opportunities to grow bigger plants (Chapin *et al*., 1994) which will absorb more CO_2 from the atmosphere by photosynthesis. Global warming may shift agricultural land use to higher latitudes, where soil and nutrients may be suitable for producing crops. In the future, Arctic terrestrial ecosystems will be completely different from what we see currently.

8.4 Overall conclusions

This thesis explored how a single freeze-thaw or dry-wet event affects temperate and polar soils and future climate scenario in arctic soils. The studies clearly showed that i) freeze-thaw or dry-wet event increased DOC and N (organic and inorganic) in soils, greenhouse gas emission, and soil metabolites while extracellular enzymes were reduced, ii) CO_2 flux (photosynthesis and respiration combined) from the experimental grassland system was greater following a freeze-thaw event in planted than non-planted soils, iii) warming winter increased CO_2 efflux and N cycling. These changes may have important

implications for ecosystems C and N cycling. The presence of plants is key in determining how ecosystem respond to these events. Our results also suggest that a warmer winter led to enhanced microbial decomposition of soil organic matter with increased CO_2 efflux and more N becoming available to roots and associated mycorrhiza in the Arctic soils. This data may be useful in that it attempts to predict Arctic soil C and N response to future warming scenarios.

8.5 Suggestions for future research

The experimental studies presented in this thesis contributed to the existing body of knowledge by presenting a novel understanding of the effects of environmental change on soil C and N cycling. However, there are some suggestions for further research that future studies might consider:

1. Laboratory studies (Chapter 3, 4, 5, 6 and 7) controlled climatic conditions (i.e. temperature and light). However, there are some gaps in climate variables between laboratory and field study. Future studies will need to be conducted in the field to obtain more accurate results accounting for a greater number of variables and the uncertainty around natural phenomenon that could influence soil responses to freeze-that or wet-dry events.
2. We observed a CO_2 pulse from soils immediately after thawing or rewetting (Chapter 3, 4, 5 and 7). This was potentially due to biological disruption and/or surviving microbes respiring after utilizing LMW C produced from dead microbes via cell lysis (Chapter 3), however future research is needed into how consistent these mechanisms are across contrasting ecosystems.
3. We provided important information about the effects of snow-free freeze-thaw

events (Chapter 3, 4, 5, 6 and 7) on soils and plants. Snow cover plays an important role in the climate, hydrological and ecological systems of terrestrial areas, and it may influence surface energy balance, water balance (e.g. water storage and release), thermal insulation, vegetation and trace gas fluxes (Grundstein *et al.*, 2005; Callaghan *et al.*, 2011). It might be interesting to investigate how snow cover and non-snow cover treatments differ in CO_2 flux response.

4. As we mentioned in chapter 7, the Arctic soil in the future will be unfrozen during winter due to global warming. Moonlight (especially full moon), starlight, and/or planet light may affect plant growth (Raven and Cockell, 2006). It might also be interesting in future research to examine the effects of northern lights or moonlight on plants under no snow cover.

8.6 References

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Appendix 1

Supplementary material for Chapter 3

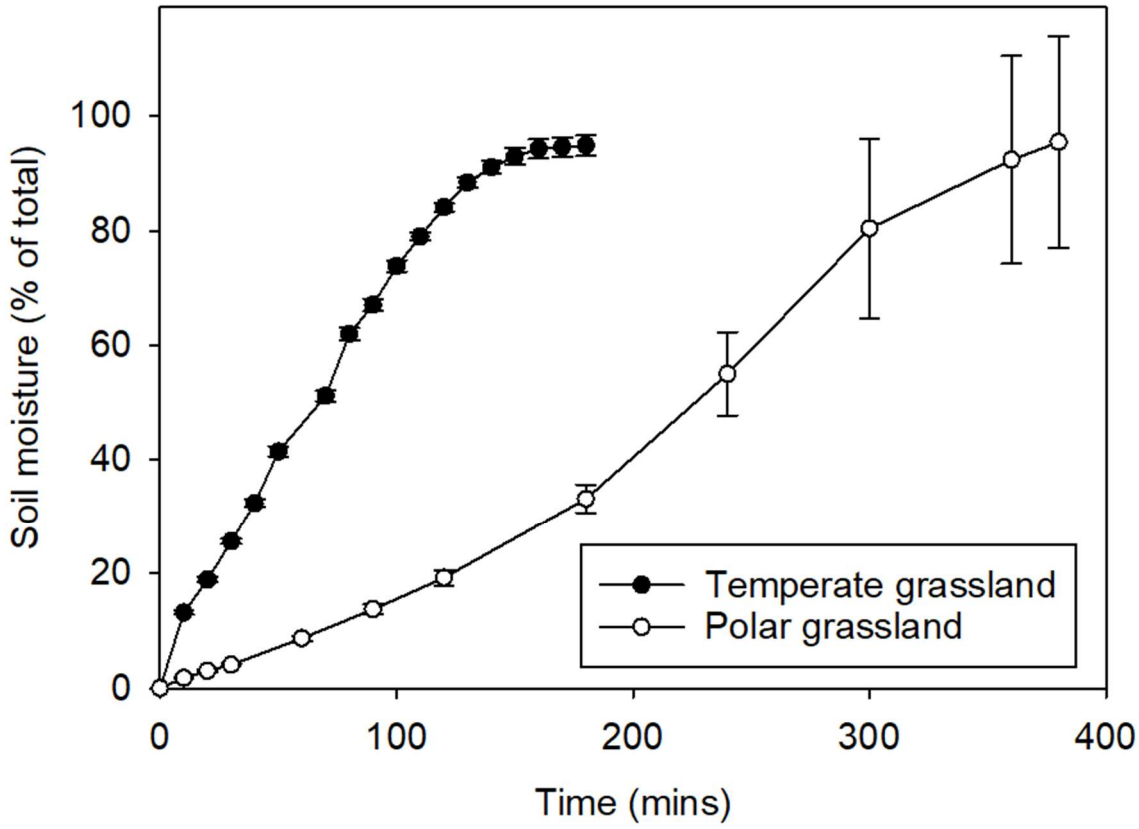

Figure S1. Loss of moisture from the temperate and polar grassland soils during the drying treatment. Values represent means ± SEM (n = 4). The soils lost 0.31 ± 0.00 g (temperate grassland) and 0.47 ± 0.04 g (polar grassland) of water per g of field moist soil over the drying period.

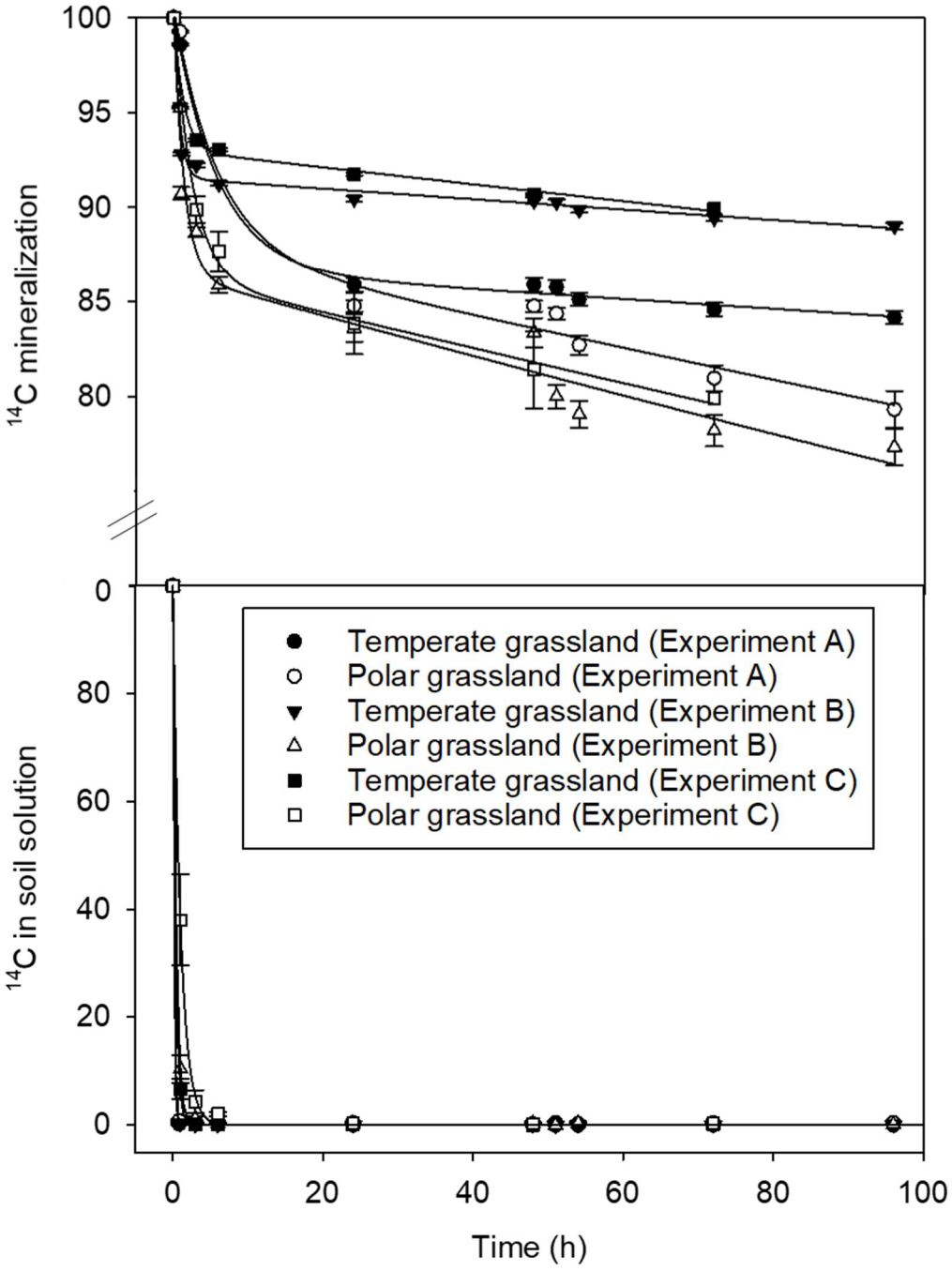

Figure S2. ^{14}C glucose mineralizarion (above) and ^{14}C glucose in soil solution (below). Lines represent model fits for decay equations. Values represent means ± SEM (n = 4).

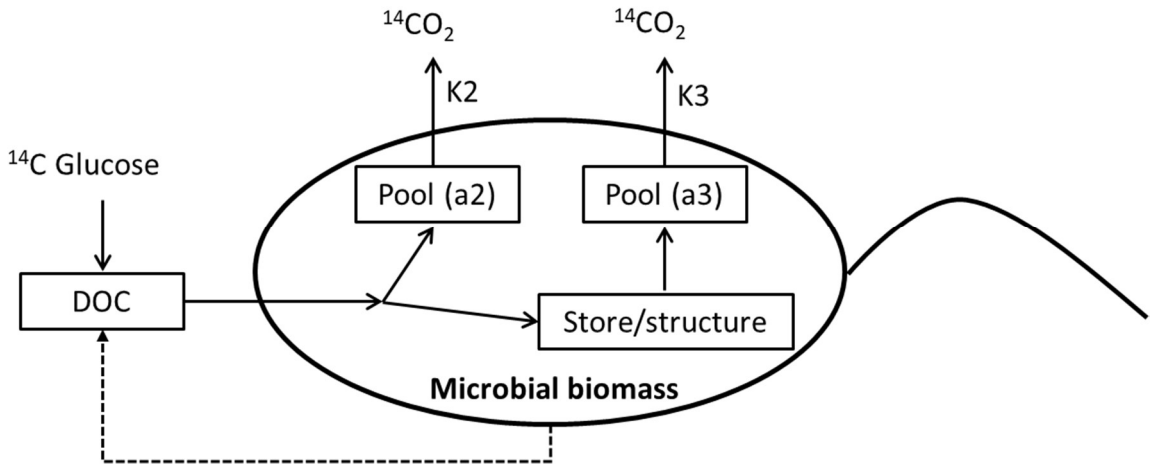

Figure S3. A schematic diagram represents ^{14}C glucose flow in soil. ^{14}C is respired from fast pool (a2) and/or slow pool (a3) from microbial biomass. K2 = respiration rate from fast pool, K3 = respiration rate from slow pool.

Appendix 2

Supplementary material for Chapter 4

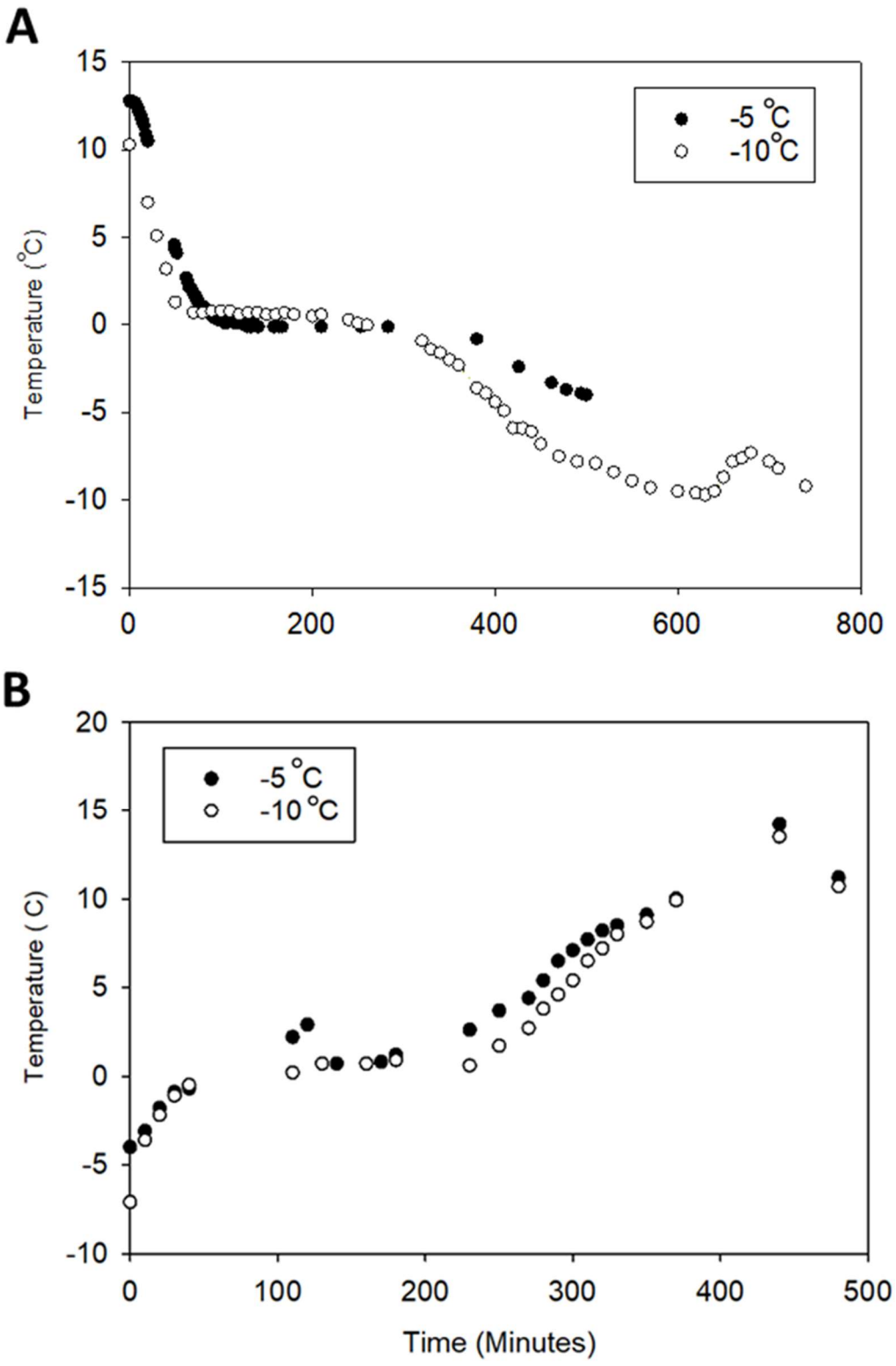

Figure S1. Soil temperature during soil freezing (A) and thawing (B).

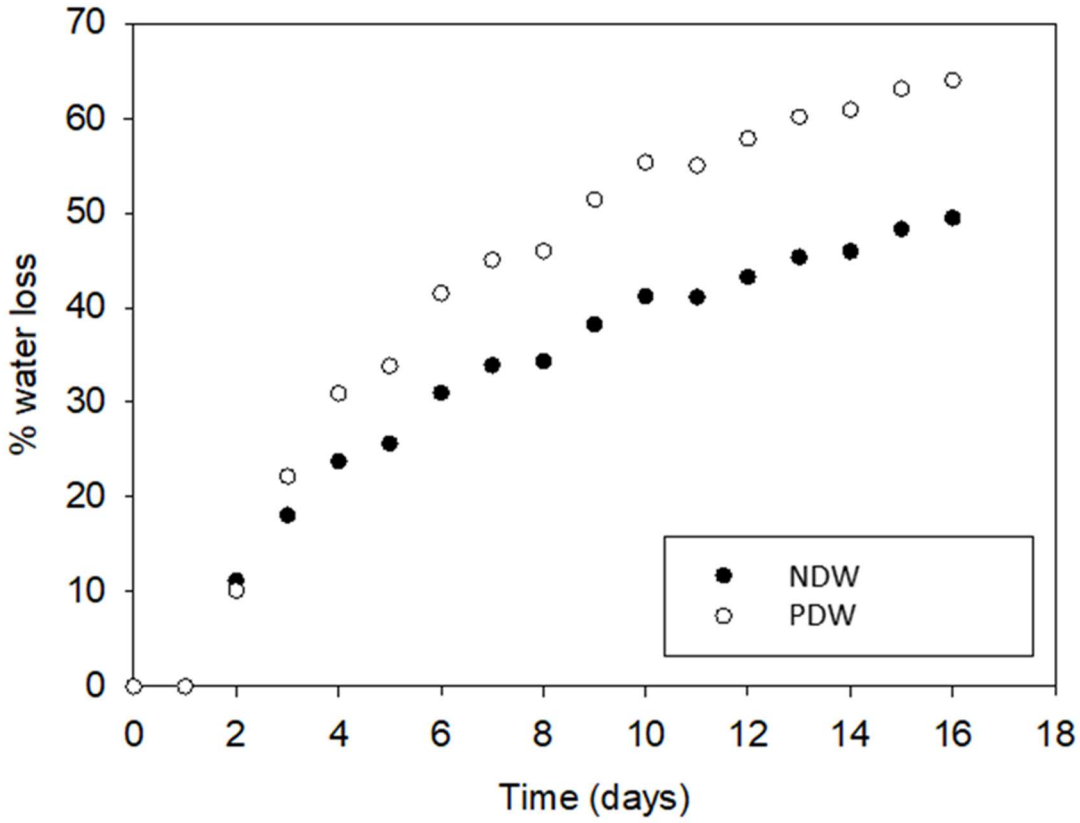

Figure S2. Soil water loss following removal of watering (carried out during pre-treatment period) for both for planted (PDW) and non-planted (NDW) soil during the initial stages of the dry treatment.

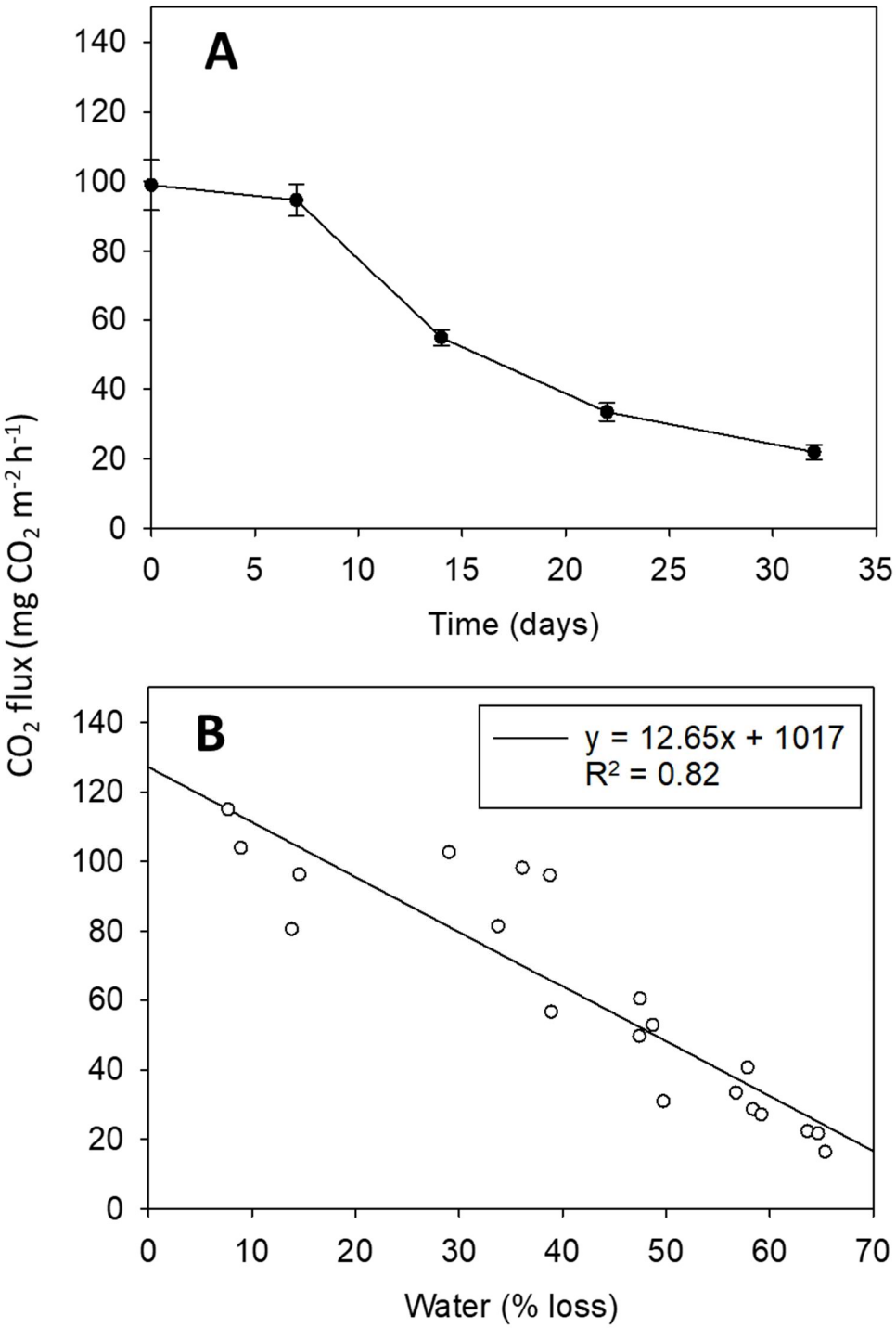

Figure S3. Effect of drought on night-time respiration. (A) CO_2 flux in night-time from planted soil during drought period, (B) Relationship between night-time CO_2 flux and water loss in planted soil (Pearson's product-moment correlation). Values represent means ± SEM (n = 4).

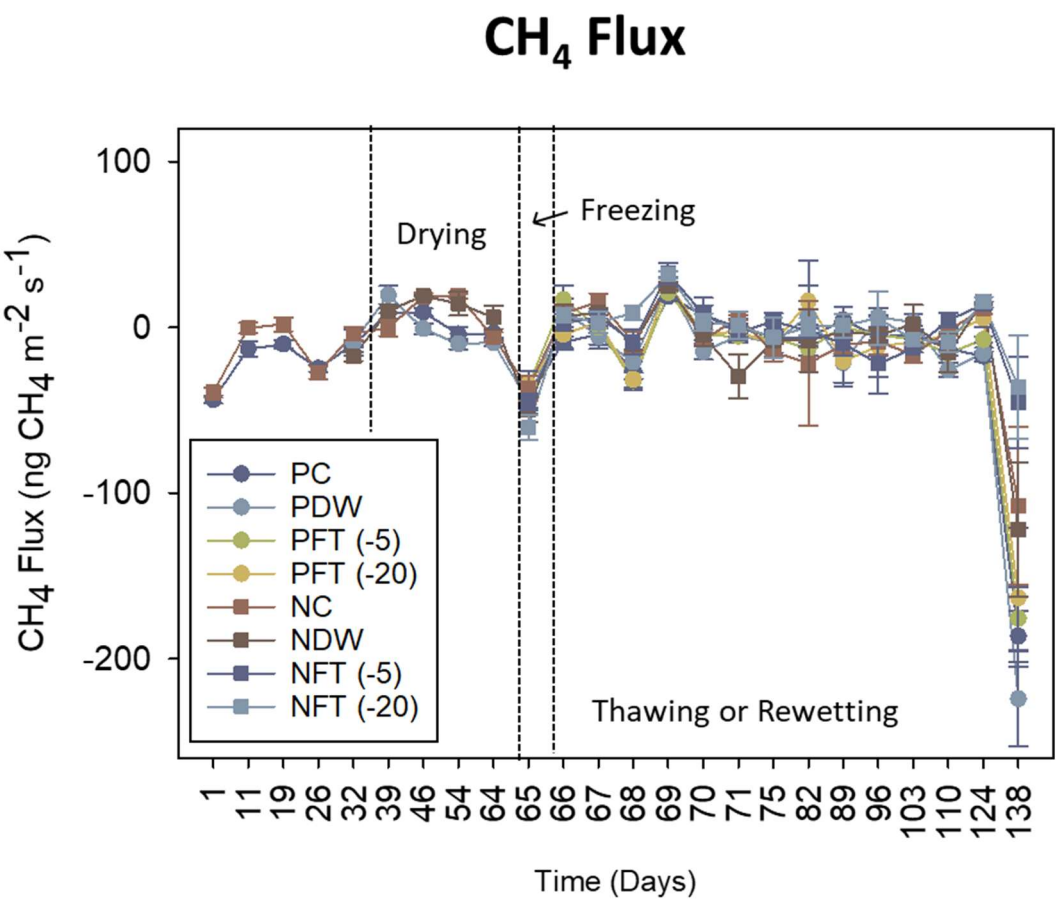

Figure S4. CH_4 flux from soil chemistry before and after applying a single freeze-thaw (-5°C or -10°C) or dry-wet treatment in either planted or unplanted soil. Values represent means ± SEM (n = 16 for pre-treatments, n = 12 for pre-freeze-thaw treatments and n = 4 during drying and after thawing or rewetting). PC = Control with plants (+10°C), PDW = Dry-wet with plants, PFT (-5) = Freeze-thaw (-5°C/+10°C) with plants, PFT (-10) = Freeze-thaw (-10°C/+10°C) with plants, NC = Control without plants (+10°C), NDW = Dry-wet without plants (+10°C), NFT (-5) = Freeze-thaw (-5°C/+10°C) without plants, NFT (-10) = Freeze-thaw (-10°C/+10°C) without plants.

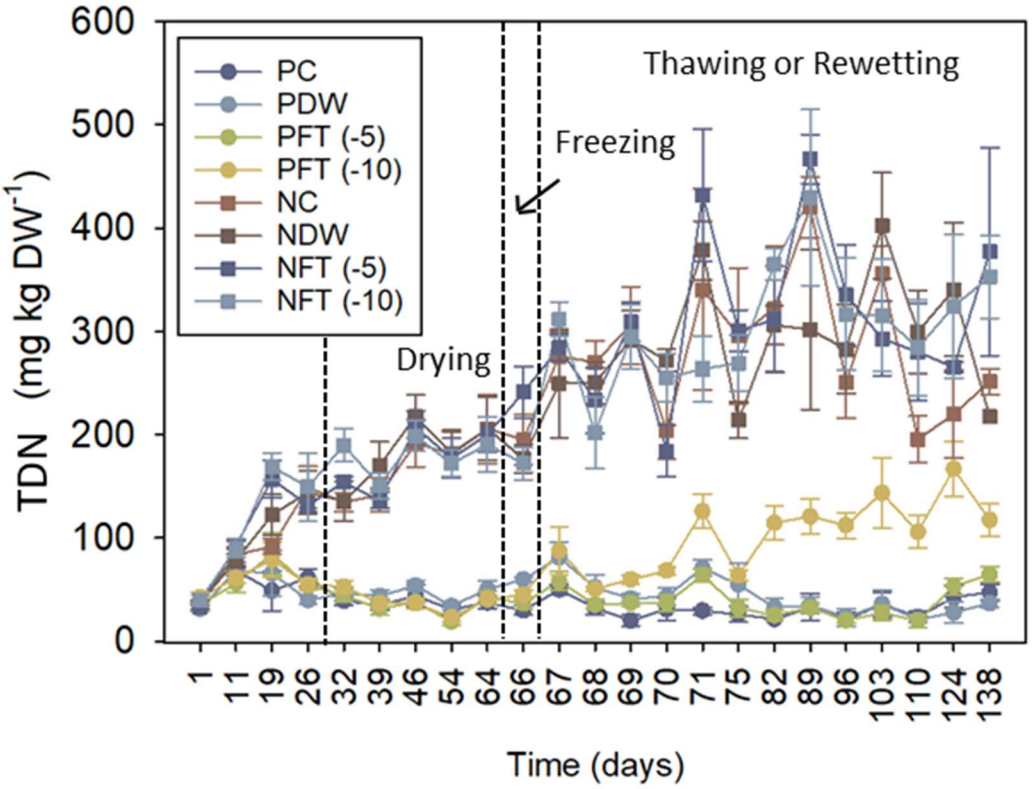

Figure S5. TDN in soil solution before and after applying a single freeze-thaw (-5°C or -10°C) or dry-wet treatment in either planted or unplanted soil. Values represent means ± SEM (n = 16 for pre-treatments, n = 12 for pre-freeze-thaw treatments and n = 4 during drying and after thawing or rewetting). PC = Control with plants (+10°C), PDW = Dry-wet with plants, PFT (-5) = Freeze-thaw (-5°C/+10°C) with plants, PFT (-10) = Freeze-thaw (-10°C/+10°C) with plants, NC = Control without plants (+10°C), NDW = Dry-wet without plants (+10°C), NFT (-5) = Freeze-thaw (-5°C/+10°C) without plants, NFT (-10) = Freeze-thaw (-10°C/+10°C) without plants.

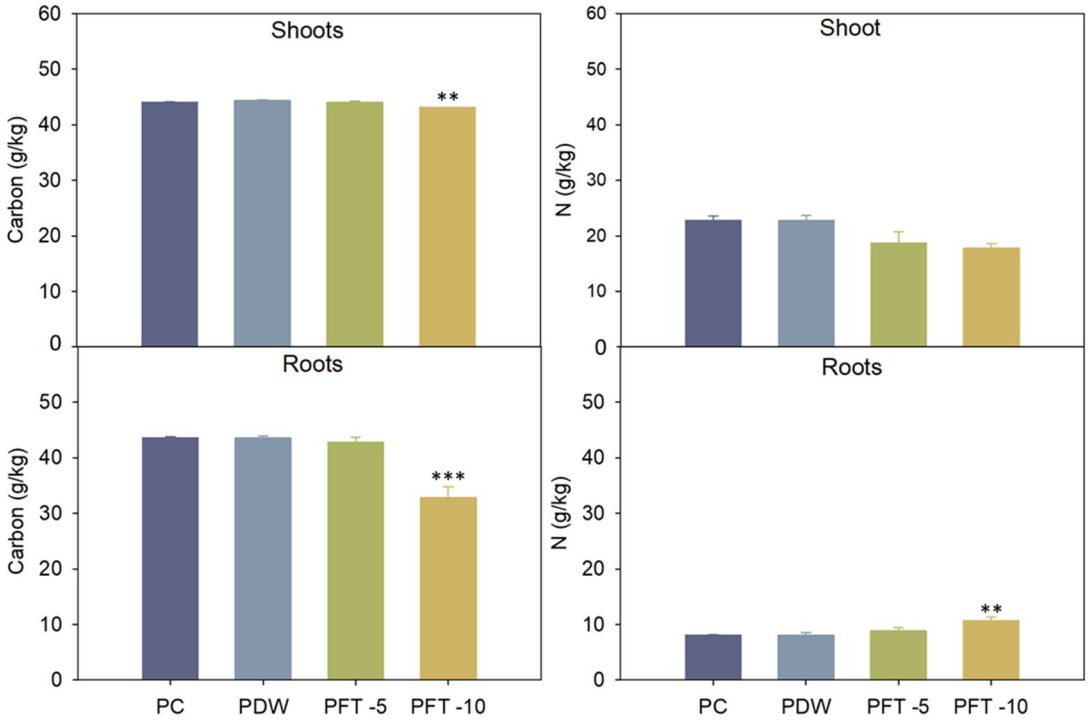

Figure S6. C and N contents in shoots and roots at the end of the experiment after applying a single freeze-thaw (-5°C or -10°C) or dry-wet treatment. Stars above the plots denote significant differences (*, ** and *** indicate $p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively) when compared with the control treatment. Values represent means ± SEM ($n = 4$). PC = Control with plants (+10°C), PDW = Dry-wet with plants, PFT (-5) = Freeze-thaw (-5°C/+10°C) with plants, PFT (-10) = Freeze-thaw (-10°C/+10°C) with plants.

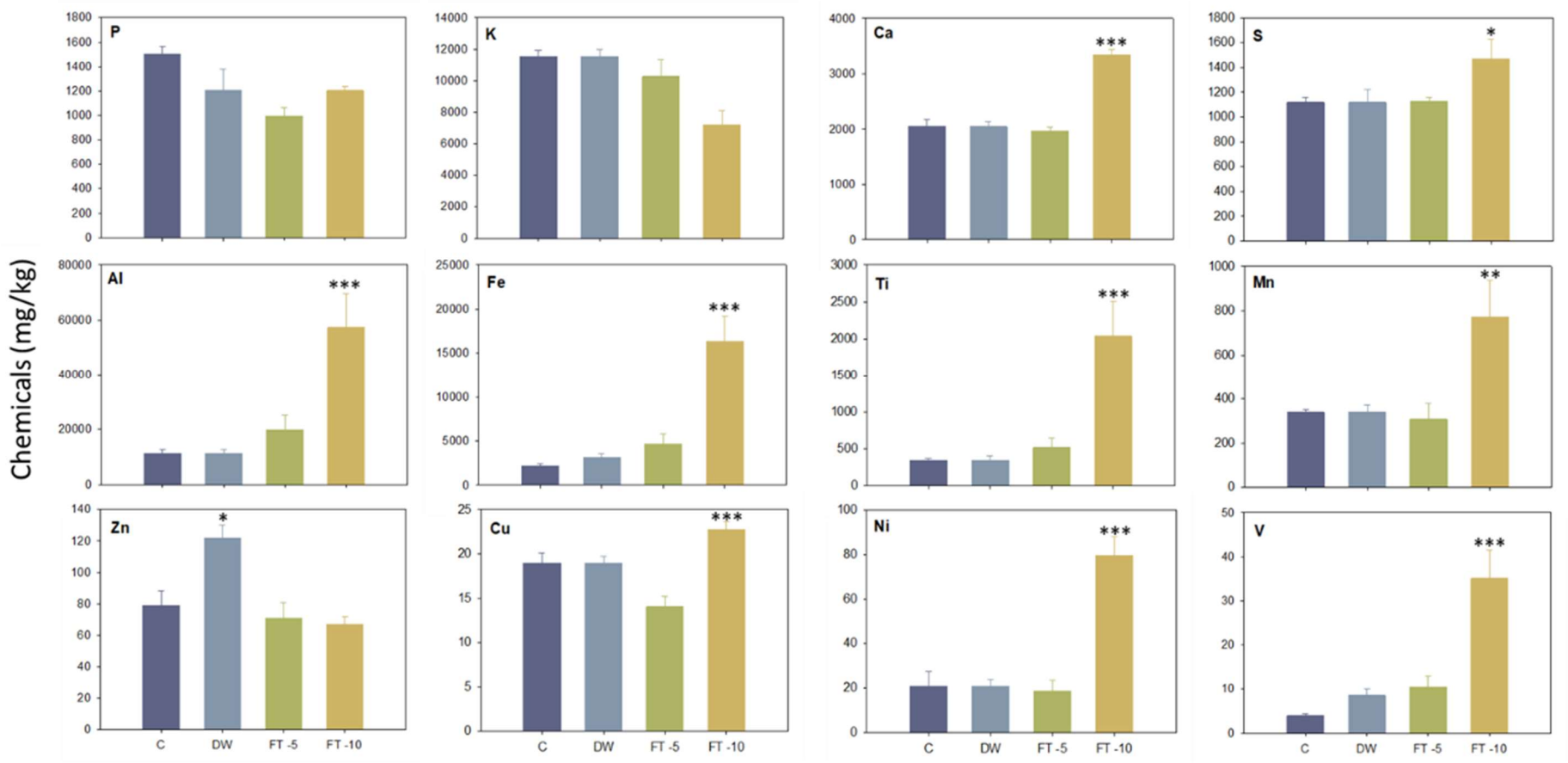

Figure S7. Macronutrient and micronutrient concentration in roots at the end of the experiment after applying a single freeze-thaw (-5°C or -10°C) or dry-wet treatment. Stars above the plots denote significant differences (*, ** and *** indicate $p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively) when compared with the control treatment. Values represent means ± SEM ($n = 4$). PC = Control with plants (+10°C), PDW = Dry-wet with plants, PFT (-5) = Freeze-thaw (-5°C/+10°C) with plants, PFT (-10) = Freeze-thaw (-10°C/+10°C) with plants.

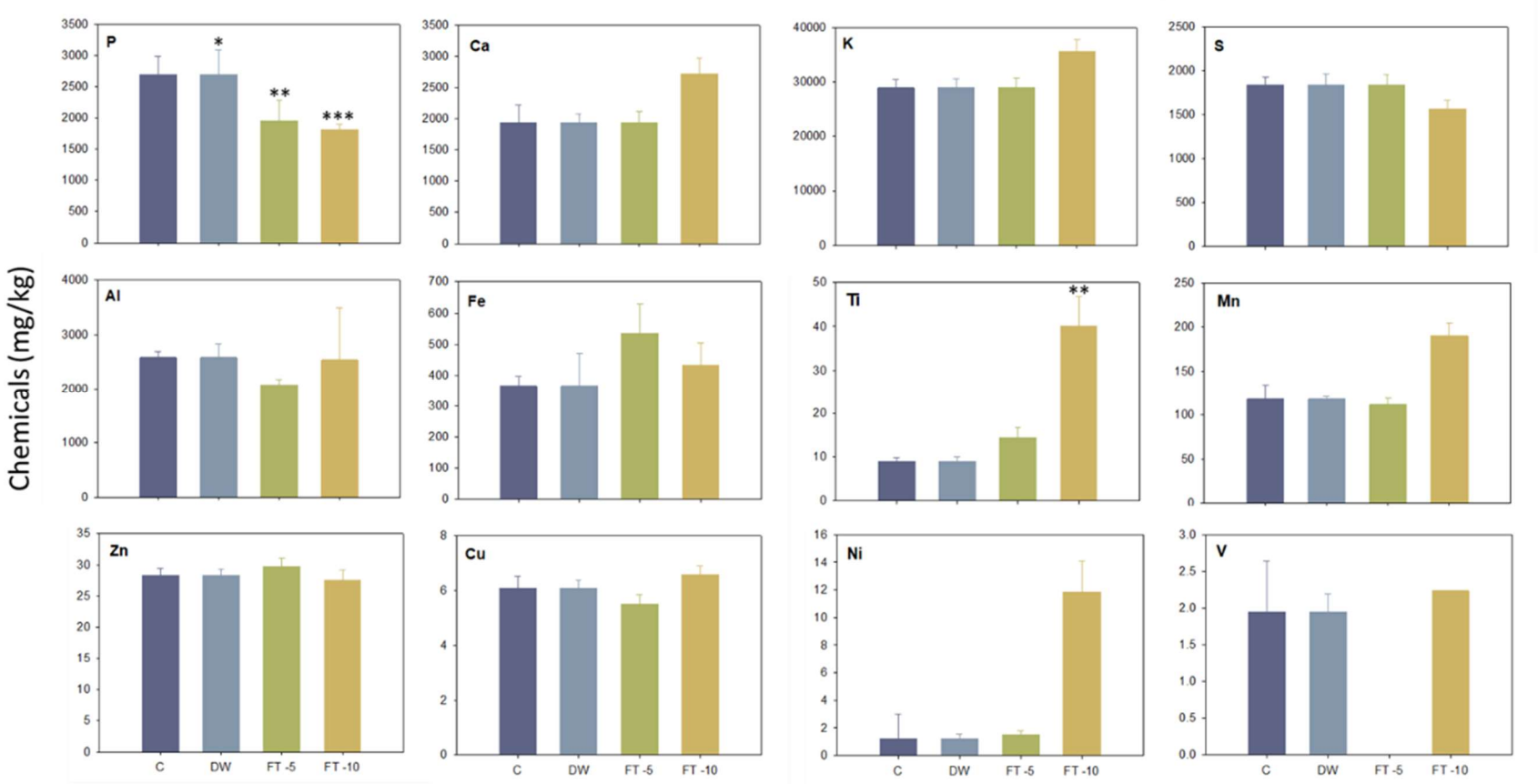

Figure S8. Macronutrient and micronutrient concentration in shoots at the end of the experiment after applying a single freeze-thaw (-5°C or -10°C) or dry-wet treatment. Stars above the plots denote significant differences (*, ** and *** indicate $p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively) when compared with the control treatment. Values represent means ± SEM ($n = 4$). PC = Control with plants (+10°C), PDW = Dry-wet with plants, PFT (-5) = Freeze-thaw (-5°C/+10°C) with plants, PFT (-10) = Freeze-thaw (-10°C/+10°C) with plants.

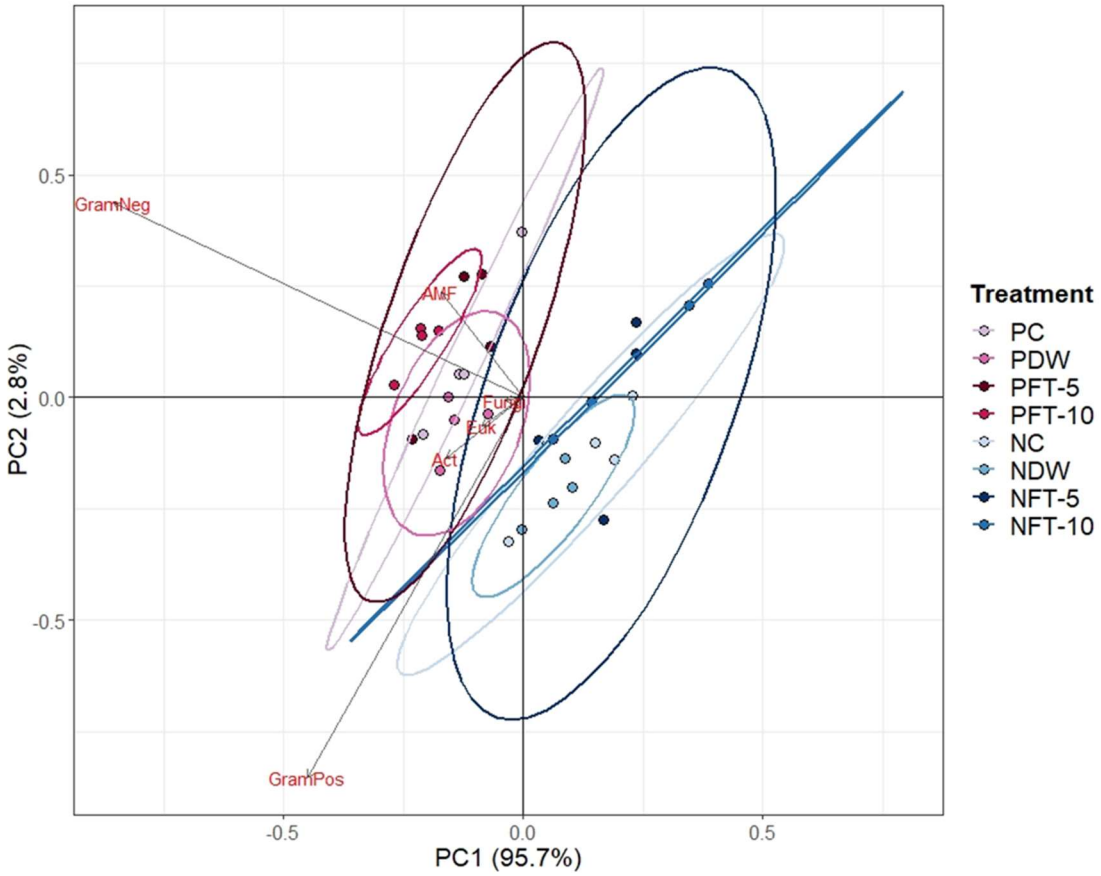

Figure S9. Principal component analysis of soil microbial community structure at the end of the experiments after applying a single freeze-thaw (-5°C or -10°C) or dry-wet treatment in either planted or unplanted soil. Red: planted soil and Blue: unplanted soil. Values represent means ± SEM (n = 4). PC = Control with plants (+10°C), PDW = Dry-wet with plants, PFT (-5) = Freeze-thaw (-5°C/+10°C) with plants, PFT (-10) = Freeze-thaw (-10°C/+10°C) with plants, NC = Control without plants (+10°C), NDW = Dry-wet without plants (+10°C), NFT (-5) = Freeze-thaw (-5°C/+10°C) without plants, NFT (-10) = Freeze-thaw (-10°C/+10°C) without plants. GramNeg = Gram-negative bacteria, GramPos = Gram-positive bacteria, Act = Actonomycetes, Euk = Eukaryotes, Fungi = Saprophytic Fungi, AMF = arbuscular mycorrhizal fungi.

Appendix 3

Supplementary material for Chapter 5

Table S1. Summary of the six extracellular enzymes assayed in this study.

Enzymes	Function	Substrate
α-Glucosidase	Produces glucose by breaking down disaccharides (i.e. starch, maltose)	4-MUF α-D-glucopyranoside
Cellobiohydrolase	Produces disaccharide (i.e. cellobiose) by breaking down cellulose	4-MUF β-D-cellobiopyranoside
β-Glucosidase	Produces glucose by breaking down cellulose or cellobiose	4-MUF β-D-glucopyranoside
β-Xylosidase	Produces xylose by breaking down hemicellulose (i.e. xylan)	4-MUF β-D-xylopyranoside
N-acetyl-β-glucosaminidase	Produces N-acetyl glucosamine by breaking down chitin	4-MUF N-acetyl-β-D-glucosaminide
Acid phosphatase	Produces PO_4^{3-} by removing a phosphate group from organic molecules	4-MUF phosphate (free acid)

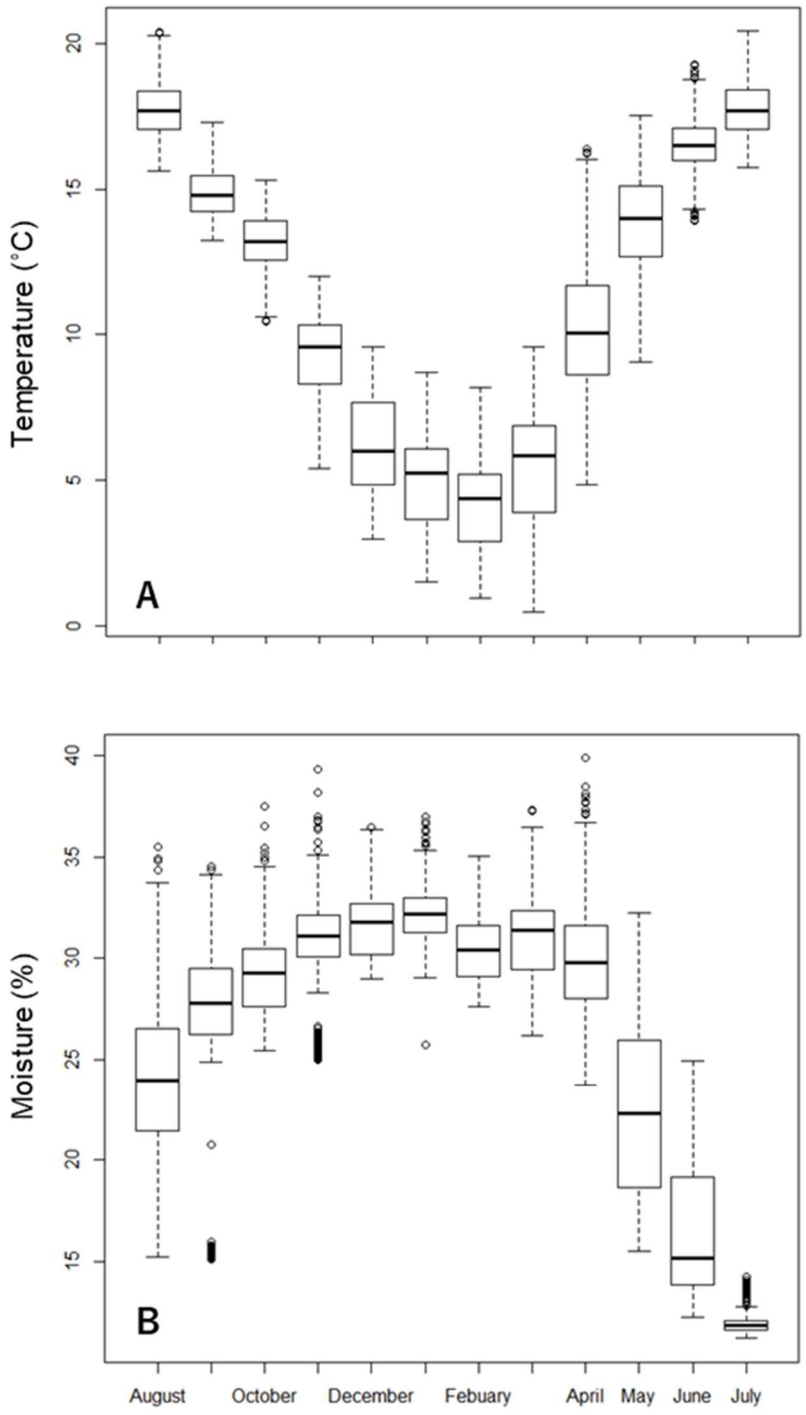

Figure S1. Range in mean daily soil temperature (A) and moisture (B) in the top soil (10 cm) at the study site on a monthly basis for the period 1st August, 2017 to 31st July, 2018. Boxes are bounded on the first and third quartiles; horizontal lines denote medians. Black dots are outliers beyond the whiskers, which denote 1.5× the interquartile range.

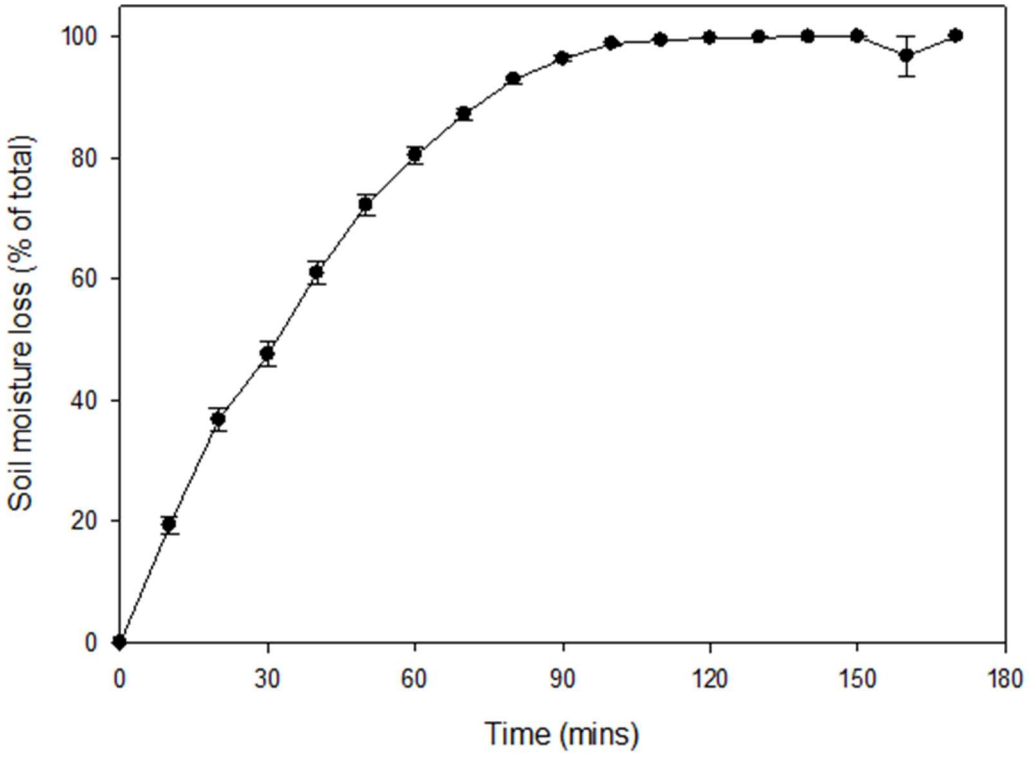

Figure S2. Progressive loss of moisture from the soil during the drying treatment. Values represent means ± SEM (n = 4). Over the monitoring period the soil lost 0.36 ± 0.01 g of water per g of field moist soil.

Appendix 4

Supplementary material for Chapter 6

Table S1. Metabolites identified in the planted and non-planted soil.

Sugars & Polyols	**Amino acids**	**Fatty acids**	**Nucleobases**
1-monostearin	alanine	1-monopalmitin	adenine
arabinose	aspartic acid	2-deoxytetronic acid	guanine
arbutin	glutamic acid	arachidic acid	thymidine
cellobiose	glutamine	behenic acid	thymine
erythritol	glycine	cerotinic acid	
erythrose	isoleucine	heptadecanoic acid	**Others**
fructose	leucine	isoheptadecanoic acid	urea
fucose	phenylalanine	NIST	organic
galactinol	proline	lignoceric acid	compounds
glucose	serine	linoleic acid	
glyceric acid	threonine	linolenic acid	
glycerol	tryptophan	montanic acid	
glycolic acid	tyrosine	myristic acid	
ketohexose	valine	octadecanol	
lyxitol		oleamide NIST	
maltotriitol		palmitic acid	
maltotriose		palmitoleic acid	
mannose		pentadecanoic acid	
myo-inositol		stearic acid	
N-acetylmannosamine			
palatinitol			
pinitol			
raffinose			
ribose			
sophorose			
sucrose			
tagatose			
threonic acid			
xylose			
xylulose NIST			

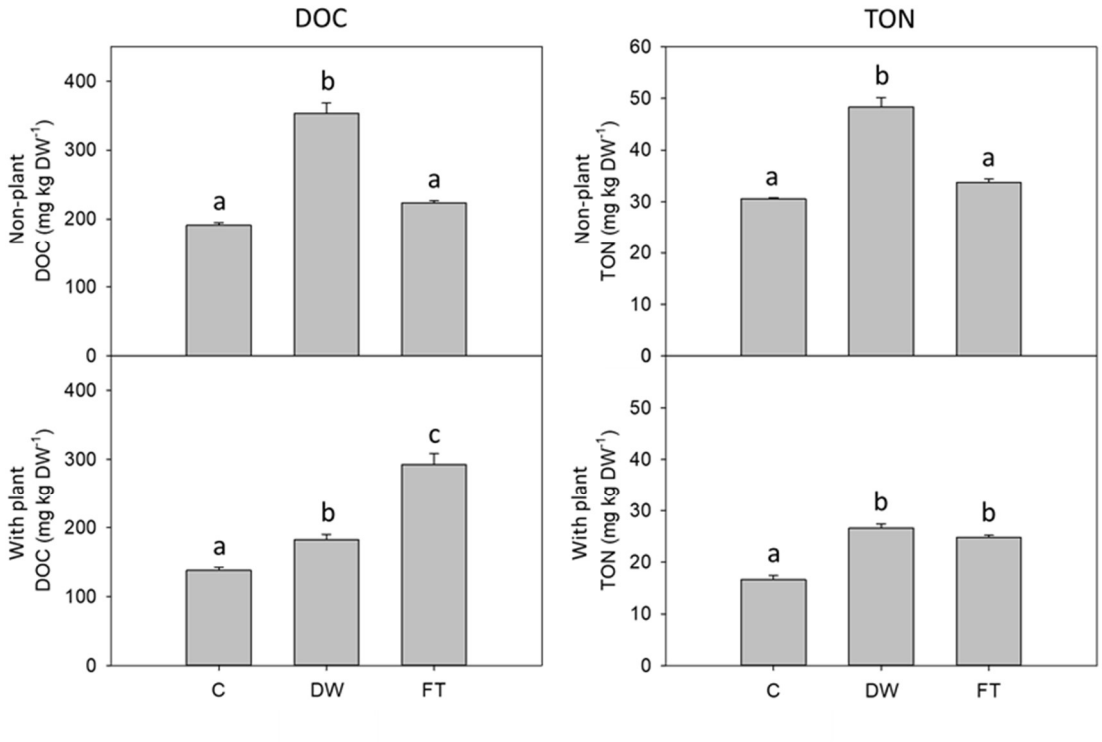

Figure S1. DOC and TON concentrations in the planted and non-planted soils (K_2SO_4 extract) exposed to either a single freeze-thaw or wet-dry cycle. Values represent means ± SEM (n = 4). C = Control; DW = Dry-wet; FT = Freeze-thaw. Different letters indicate significant differences between treatments at the $p \leq 0.05$ level.

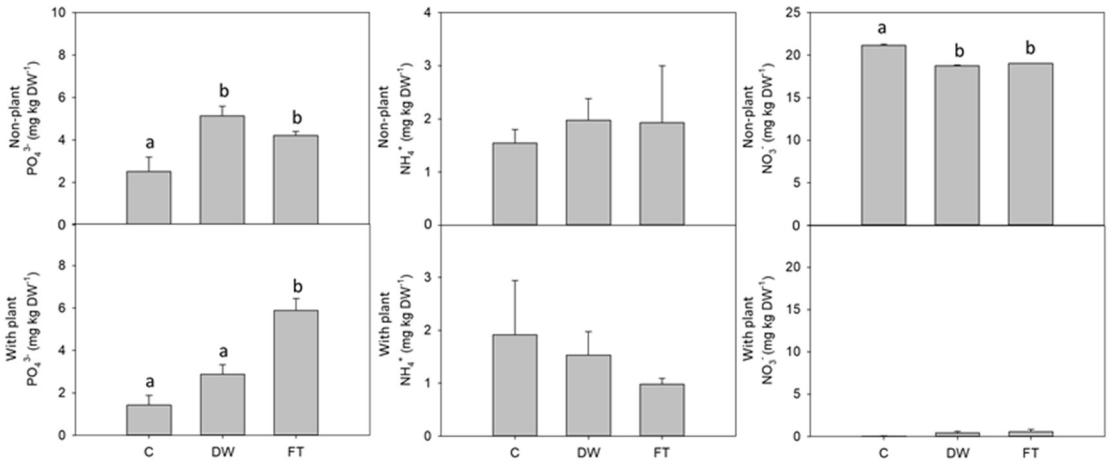

Figure S2. Phosphate, ammonium and nitrate in the planted and non-planted soils extracted with water. Values represent means ± SEM (n = 4). C = Control; DW = Dry-wet; FT = Freeze-thaw. Different letters indicate significant differences between treatments at the $p \leq 0.05$ level. If no letters are presented, then $p > 0.05$.

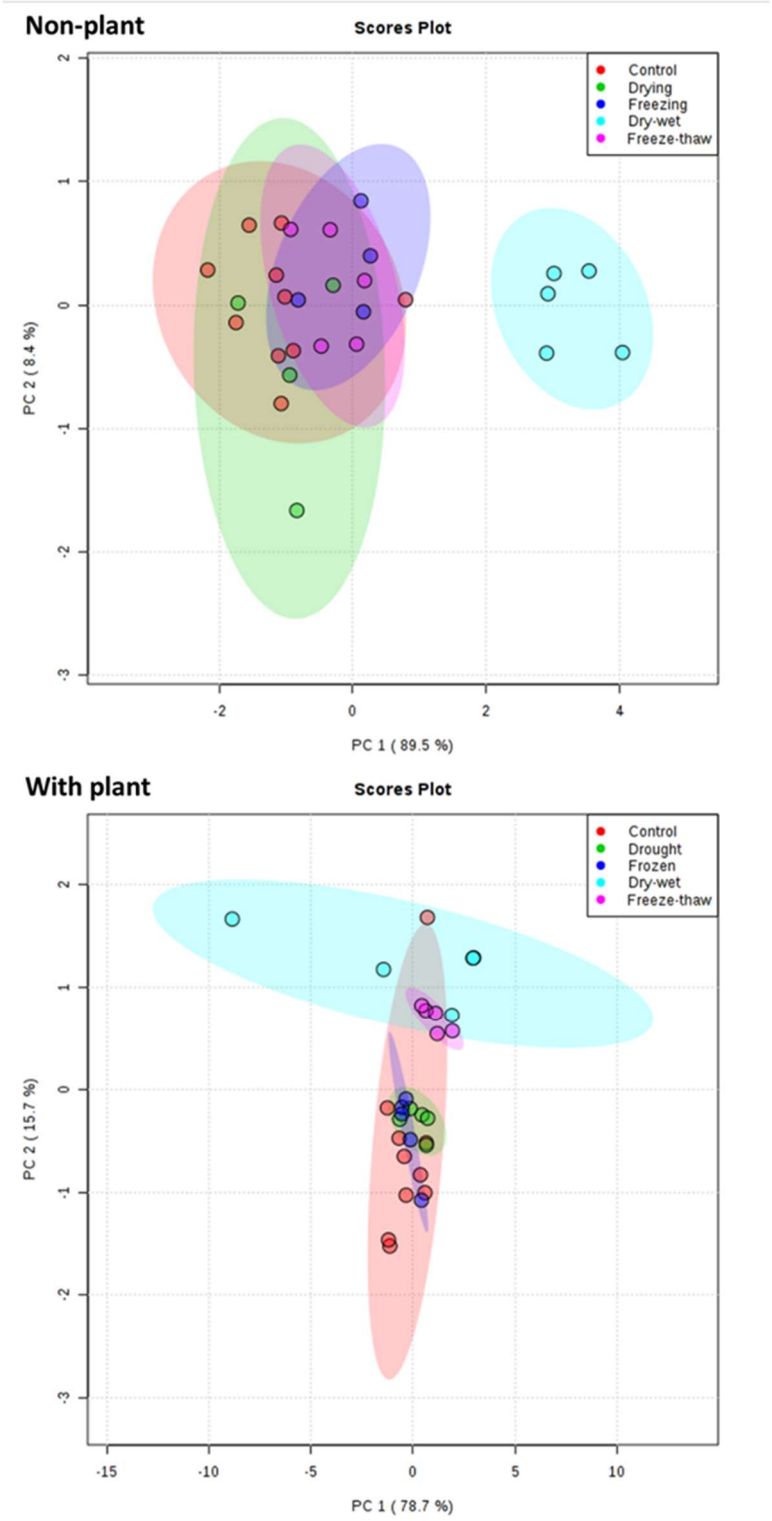

Figure S3. Principal component analysis (PCA) for nucleosides' metabolomics in response to a single freeze-thaw or wet-dry event. Values represent means ± SEM (n = 10 for control and n = 5 for treatments).

Appendix 5

Supplementary material for Chapter 7

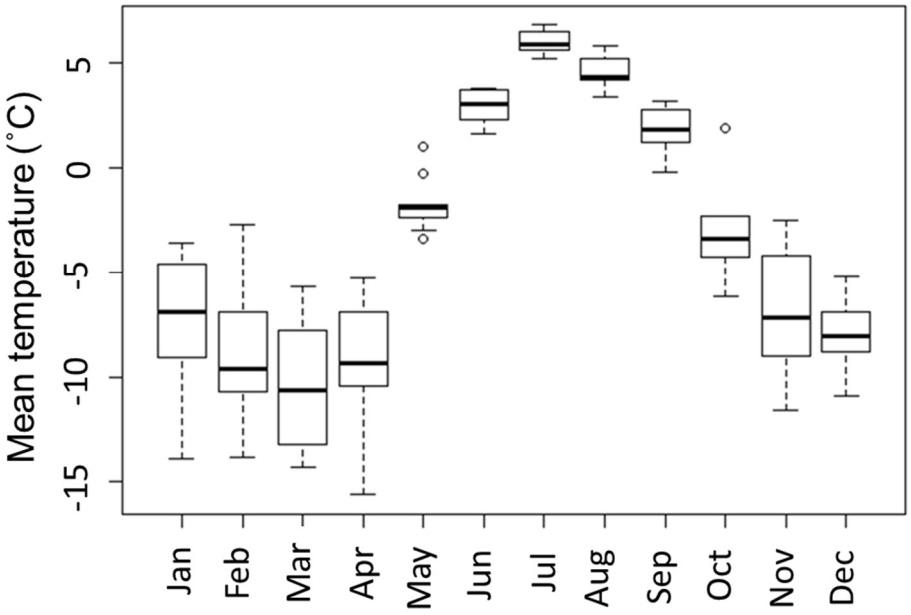

Figure S1. Mean monthly temperature in Ny-Ålesund for the period January 2007 to December 2016. Data were provided by Felleskonto Klimavakten (MET Norway).

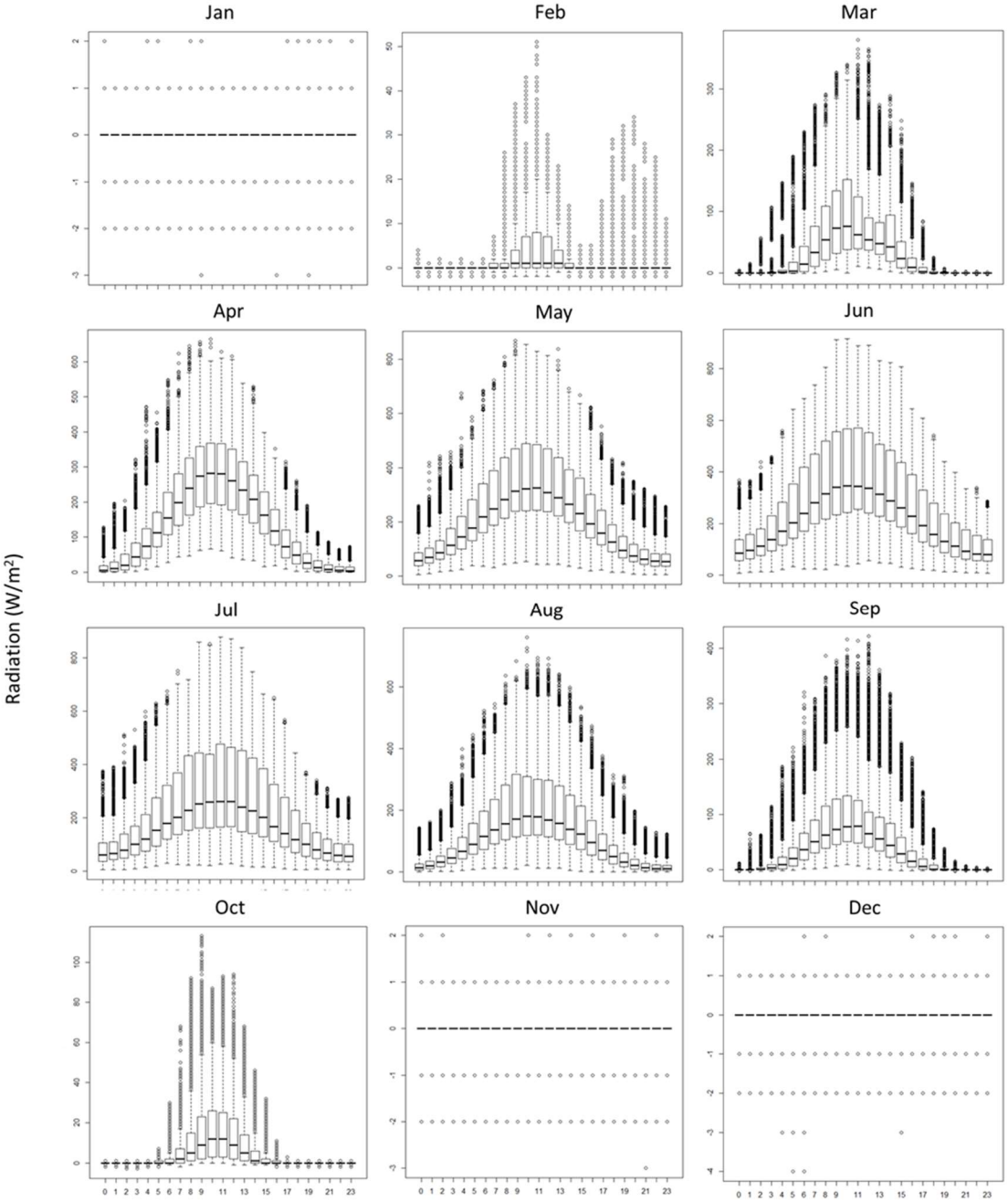

Figure S2. Radiation in Ny-Ålesund for 10 years (the period January 2007 to December 2016) of 1-minute resolution. Data were provided by Marion Maturilli (AWI).

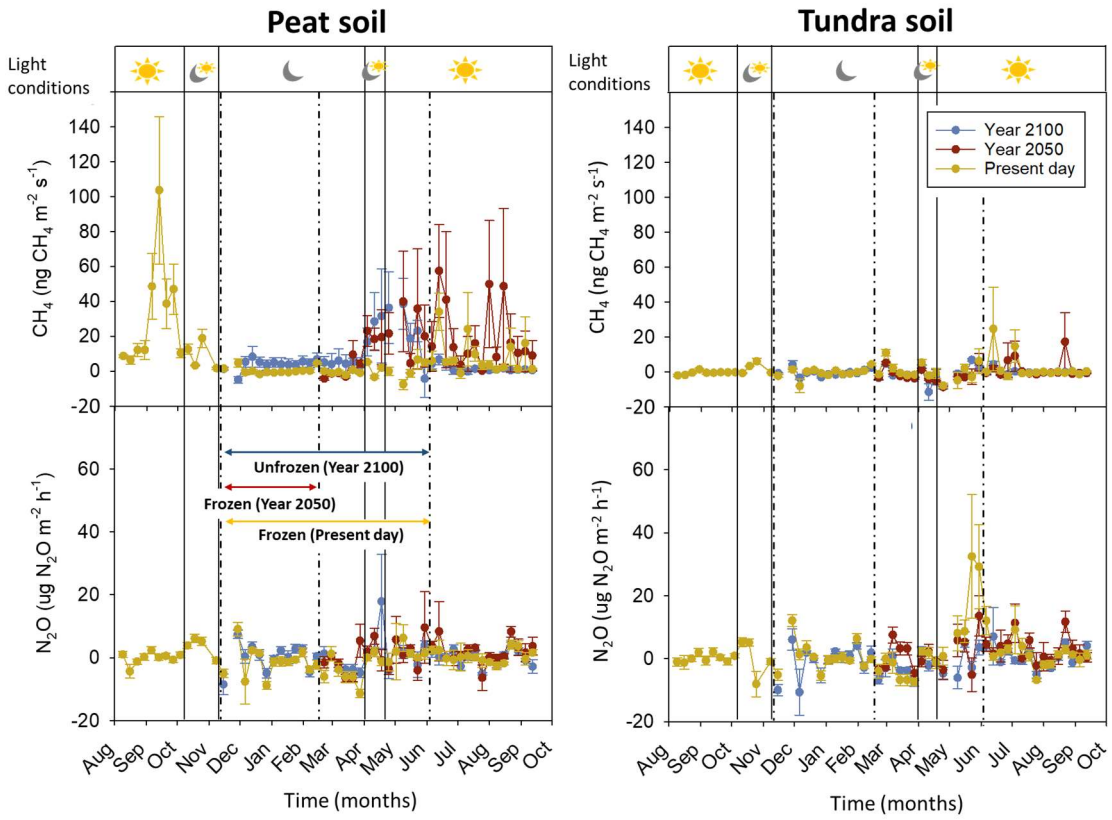

Figure S3. N_2O and CH_4 fluxes under three different simulated winter climate regimes (present day, year 2050 and year 2100) in a wetland peat soil and a mineral tundra soil. Values represent means ± SEM (n = 5). Present day = frozen during the dark and thawed under light condition (-10 °C / +7 °C). Year 2050 = Frozen and thawed under dark conditions (-10 °C / +7 °C). Year 2100 = Unfrozen during both the dark and light period (+7 °C). The dotted lines denote the times at which the temperature conditions were changed (Figure 7.1.).

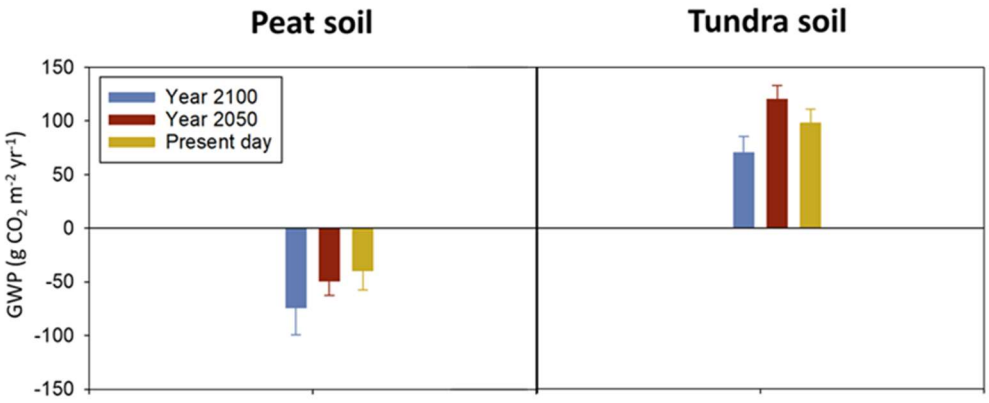

Figure S4. Global warming potential (GWP) under three different simulated winter climate regimes (present day, year 2050 and year 2100) in a wetland peat soil and a mineral tundra soil. Present day = frozen during the dark and thawed under light condition (-10 °C / +7 °C). Year 2050 = Frozen and thawed under dark conditions (-10 °C / +7 °C). Year 2100 = Unfrozen during both the dark and light period (+7 °C). Values represent means ± SEM (n = 5).

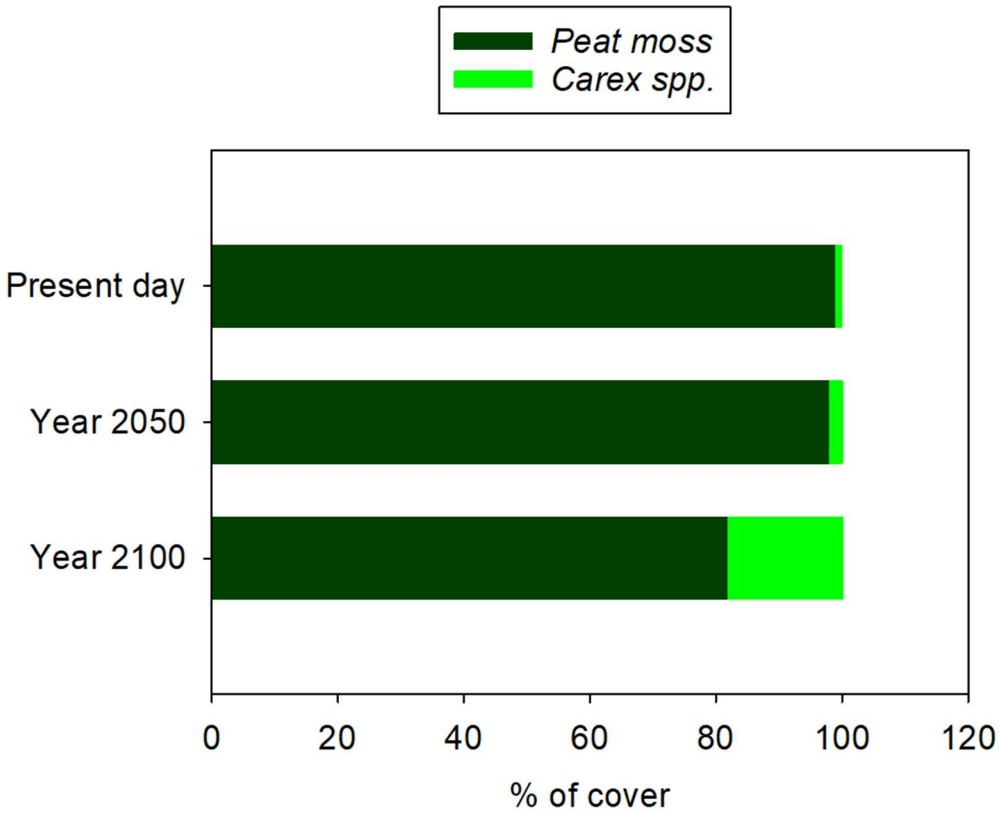

Figure S5. Plant biodiversity change in the different wetland organic core treatments. Present day = frozen during the dark and thawed under light condition (-10 °C / +7 °C). Year 2050 = Frozen and thawed under dark conditions (-10 °C / +7 °C). Year 2100 = Unfrozen during both the dark and light period (+7 °C). Values represent means (n = 5).

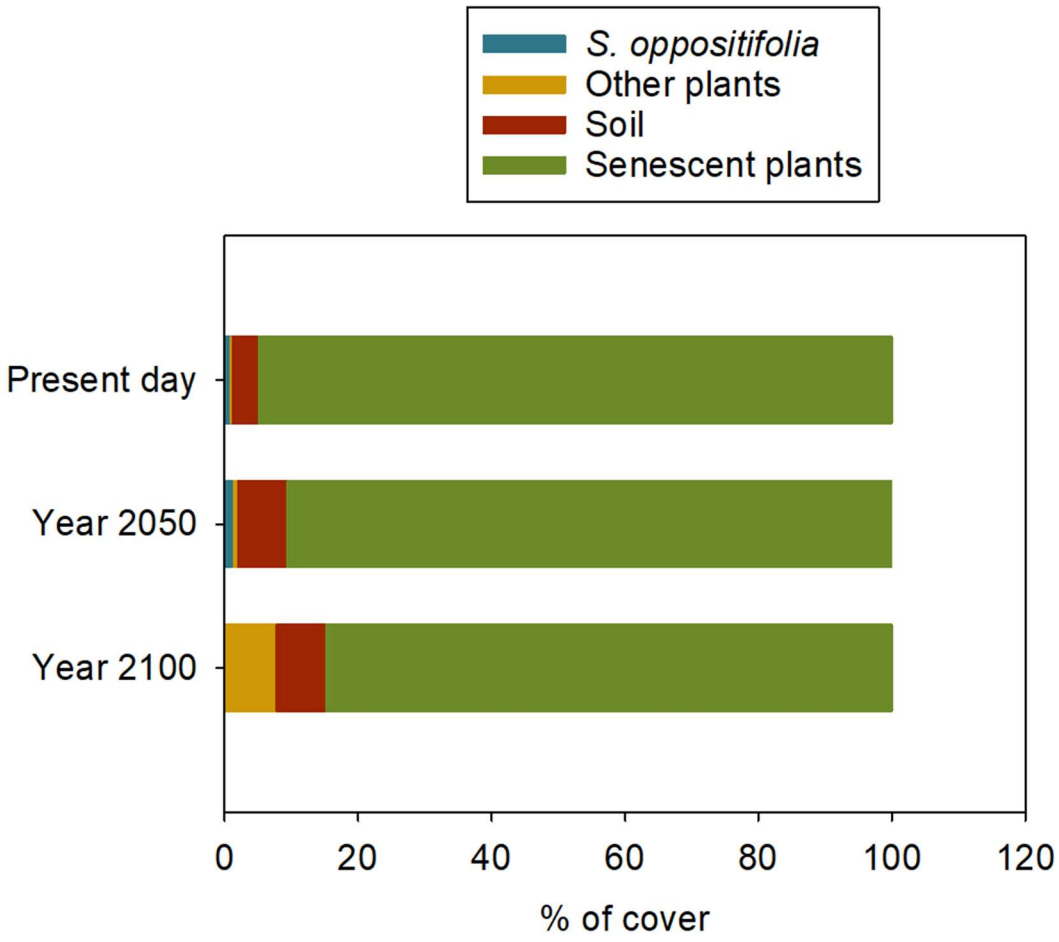

Fig. S6. Plant biodiversity change in the different tundra core treatments. Present day = frozen during the dark and thawed under light condition (-10 °C / +7 °C). Year 2050 = Frozen and thawed under dark conditions (-10 °C / +7 °C). Year 2100 = Unfrozen during both the dark and light period (+7 °C). Values represent means (n = 5).

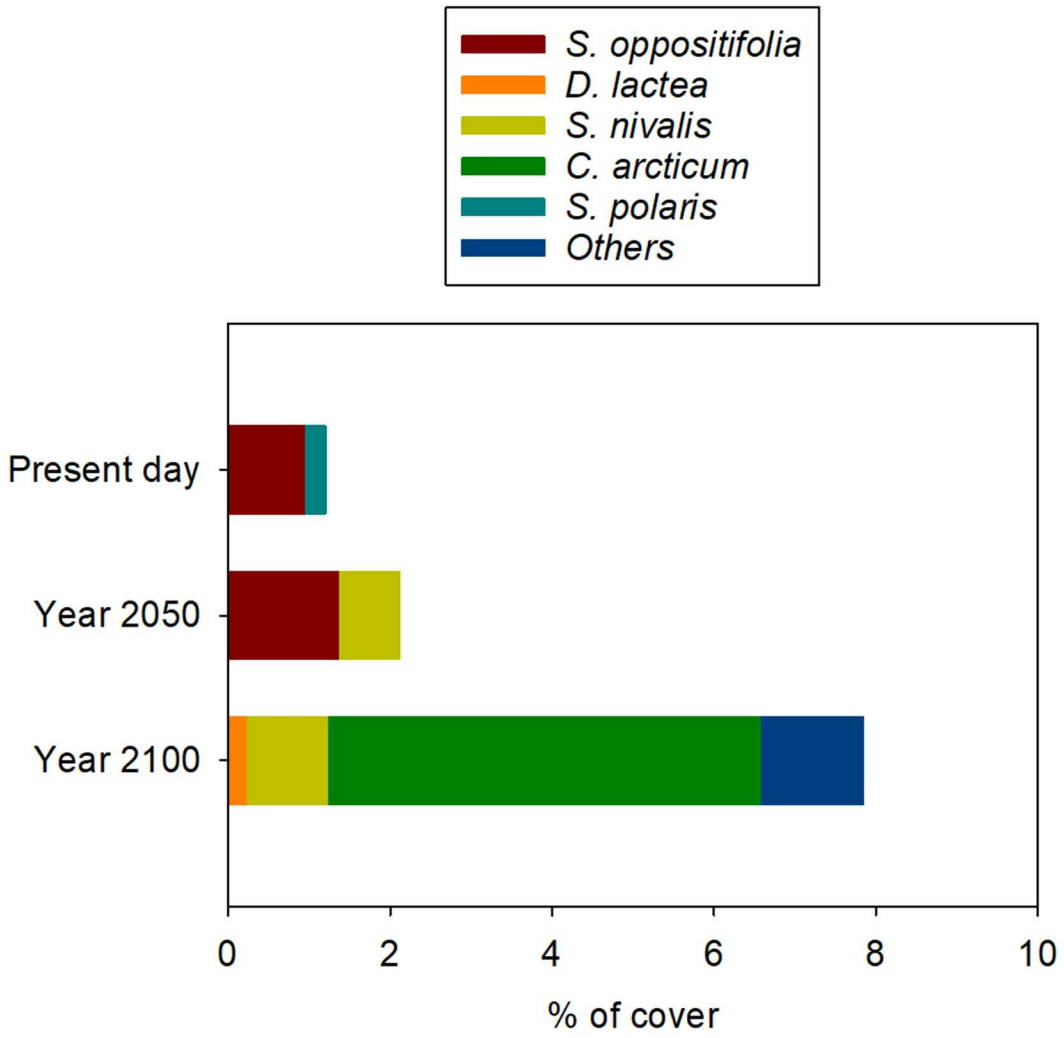

Fig. S7. Detailed plant species change in the core samples from tundra soil. The bare soil treatment and senescent plant is not shown for clarity. Present day = frozen during the dark and thawed under light condition (-10 °C / +7 °C). Year 2050 = Frozen and thawed under dark conditions (-10 °C / +7 °C). Year 2100 = Unfrozen during both the dark and light period (+7 °C). Values represent means (n = 5).