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DOCTOR OF PHILOSOPHY

The effects of freeze-thaw cycles on the composition and concentration of low molecular weight dissolved organic carbon in Arctic tundra soils

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The Effects of Freeze-Thaw Cycles on the Composition and
Concentration of Low Molecular Weight Dissolved Organic
Carbon in Arctic Tundra Soils

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PhD Thesis

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Summary

The arctic is currently an important store of terrestrial carbon, but may become a net source of carbon to the atmosphere in the future in response to climate change. However, the intricacies of how the carbon cycle is likely to respond to predicted changes are unclear, as soil organic carbon (SOC) is a mixture of many different pools and compounds. Low molecular weight dissolved organic carbon (LMW-DOC) is a particularly important and dynamic C pool as it is highly available to microorganisms. This thesis explored the effects of freezing temperature and freeze-thaw cycles (FTC) on the composition, concentration and fate of this SOC pool, and on the carbon and nitrogen cycles in general, in arctic tundra soils.

FTC were shown to have the potential to increase LMW-DOC in arctic tundra soils. This was only observed after 1 or 2 FTC and this also depended on tundra type. The composition of this LMW-DOC also changed with new LMW-DOC compounds, changes to aromaticity and the fluorescence spectrum being observed. Plants were a source of LMW-DOC during FTC. *Salix polaris* vegetation produced similar compounds and fluorescence spectra to FTC subjected soil. Initial experiments on sterilised *Zea mays* showed plants as well as microbes could be a source of glucose and amino acids released into soil after FTC. The fate of some rapidly utilised LMW-DOC compounds was relatively unaffected by FTC in tundra soils suggesting rapid recovery of C and N cycling and the microbial community within 12 hours of thaw.

Freezing temperature decreased uptake and mineralisation of LMW-DOC in a manner that was substrate specific. Progress was made in measuring and modelling unfrozen water content. The mineralisation rate of glucose was modelled using temperature and unfrozen water content. Warmer winters seemed to increase nitrification and amino acid turnover early in the growing season and also to decrease microbial biomass carbon. Earlier thaws could promote soil CO₂ emissions and increase susceptibility to FTC.

In conclusion, LMW DOC concentration, composition and dynamics can be affected by FTC with the vegetation being a notable source. Microbial use of LMW DOC is affected by freezing temperature and, whilst this recovers quickly on thaw, over winter freezing temperature can have longer term impacts on C and N cycling in the arctic tundra.

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Abbreviations

ANOSIM	Analysis of Similarity
ANOVA	Analysis of Variance
ARISA	Automated Ribosomal Intergenic Spacer Analysis
DAMM	Dual Arrhenius Michaelis Menten
DHB	2,5-dihydroxybenzoic acid
DNA	deoxyribonucleic acid
DOC	Dissolved Organic Carbon
DON	Dissolved Organic Nitrogen
DW	Dry Weight
EEM	Excitation Emission Matrix
Em	Emission
Ex	Excitation
FT	Freeze-Thaw
FTC	Freeze-Thaw Cycle
FW	Fresh Weight
HMP	sodium hexametaphosphate
IC	Ion Chromatography
ICP-OES	Inductively Coupled Plasma Optical Emission Spectrometry
LMW	Low Molecular Weight
MALDI-TOF	Matrix Assisted Laser Desorption/Ionization – Time of Flight
MBC/N	Microbial Biomass Carbon or Nitrogen
NMDS	Non-Metric Multidimensional Scaling
PCR	Polymerase Chain Reaction
QSU	Quinine Sulphate Units

RISA	Ribosomal Intergenic Spacer Analysis
RR	Relative Recovery
SEM	Standard Error of the Mean
SOC	Soil Organic Carbon
SOM	Soil Organic Matter
SUVA	Specific Ultraviolet Absorption
TFA	Trifluoroacetic acid
TFAA	Total Free Amino Acids
TC	Total Carbon
TN	Total Nitrogen
TOC	Total Organic Carbon
WHC	Water Holding Capacity

Chapter 1

General Introduction

1. General Introduction

1.1. Introduction

Arctic winters are an important time for soil nutrient dynamics. Temperature and snow cover can radically affect processes such as carbon and nitrogen mineralisation (Brookes et al. 1997). Daylight hours decrease from 24 hour daylight between mid-May and mid-August to being completely absent from mid-November to mid-February. Soil temperatures also decrease with the active layer freezing, mostly from the surface down, around September/October. Soils can remain frozen until June/July. Snow varies in thickness and duration which can moderate soil temperatures (Cooper 2014).

Winter conditions in the arctic are predicted to change rapidly over the coming century (Anisimov et al. 2007). How this is manifested will depend on location and how these predicted changes will affect soil temperatures and processes is also uncertain (Cooper 2014). For Svalbard, increases in both temperature and precipitation are predicted (Førland et al. 2011). This could increase both soil temperatures and snow depth which can further increase soil temperature by insulating the soil from decreases in air temperature. Raising air temperatures can melt snow and cause precipitation to fall in the form of rain rather than snow. This could decrease soil temperatures when air temperatures fall and also increase the potential for freeze-thaw cycles (FTC) (Henry 2008). Changes to arctic conditions could have global ramifications as it is calculated that 44% of soil organic carbon is stored in Arctic soils (Tarnocai et al. 2009). This thesis will explore the effects of FTC on low molecular weight dissolved organic carbon, which is an important set of substrates, used for biological activity.

1.2. Thesis Overview

The layout of this thesis is summarised in Fig. 1.1. This thesis contains 9 chapters overall including this introduction. Immediately following this chapter will be a literature review which investigates current knowledge on soil freezing and thawing, its physical effects and how it affects soil biological processes. It will also cover the carbon and nitrogen cycle in the arctic tundra, particularly dissolved organic carbon and dissolved organic nitrogen and identify where knowledge gaps exist in the effects of FT. The 6 experimental chapters that follow will explore some of the knowledge gaps identified in the literature review. Chapter 9 will contain a general discussion of the results of the preceding chapters and how they relate to previous studies.

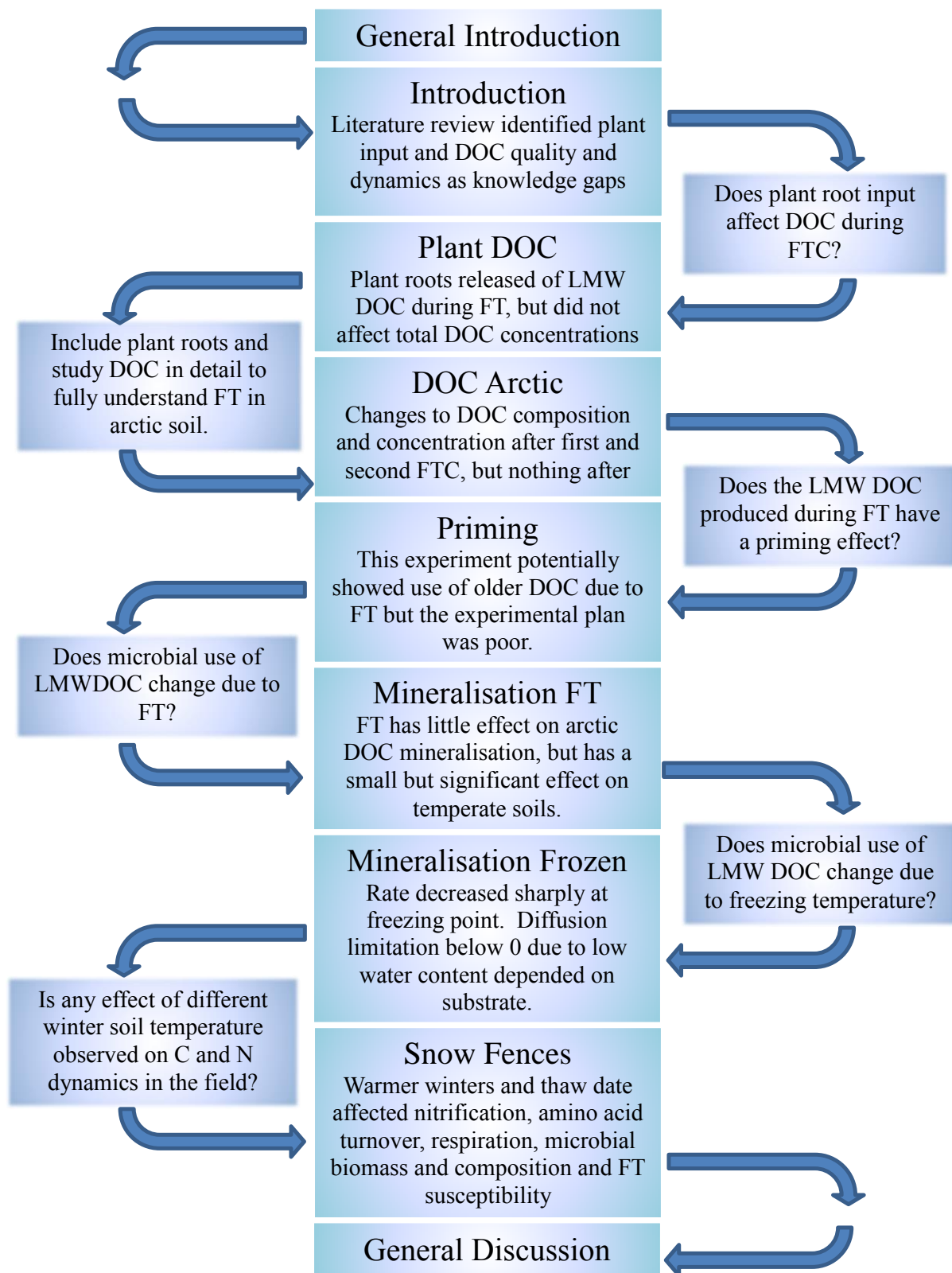


Fig. 1.1 A summary of the chapters in this thesis

The first experimental chapters explore the production and fate of low molecular weight dissolved organic carbon and nitrogen (LMW DOC/N). Chapter 3 investigates the potential

for plant roots to produce DOC and LMW DOC during FTC. Chapter 4 applies this information to arctic tundra soils with the exploration of repeated FTC cycles and how they affect the composition and concentration of DOC and DON. The fate of this DOC and DON is explored in chapters 5 and 6 with chapter 5 looking at whether FTC could have a priming effect and chapter 6 examining the microbial use of LMW DOC and DON after FTC. Chapter 7 focusses on the frozen part of FTC exploring the dynamics of LMW DOC and DON in arctic tundra soil frozen to different temperatures. This chapter also tries to model the mineralisation of LMW DOC with respect to temperature and soil water content. The final experimental chapter is a field study where winter soil temperatures in the arctic tundra were manipulated and monitored, then the differences in growing season C and N cycling were observed.

Chapter 2

Literature Review

2. Literature Review

2.1. Introduction

Climate change is predicted to cause an increase in the average temperature of the arctic which could lead to milder winters and reduced snow cover duration. This could result in reduced insulation from extreme air temperatures and solar radiation which could increase the occurrence of freeze-thaw cycles (FTC) in the arctic (Henry 2008). This could be countered by increased precipitation which could increase snow depth, but this precipitation could also fall as rain, reducing snow cover duration or causing the formation of less insulating ice rather than snow (Cooper 2014, Førland et al. 2011). Laboratory experiments and *in-situ* measurements have linked FTC with emissions of CO₂ and N₂O (Holst et al. 2008, Schimel and Clein 1996; Zhu et al. 2009). It is important to study frozen soil and FTC in order to determine future feedback to climate change and to improve current climate models. The FTC could also change nutrient concentrations in the soil which has implications for agriculture (DeLuca et al. 1992). This report reviews existing literature on frozen soil, FTC and their effect on the carbon and nitrogen cycles. It will focus on observed changes in dissolved C and N concentrations and the suggested causes of these changes. It also aims to identify where knowledge is lacking.

2.1.1. Freeze Thaw (FT)

Freeze-Thaw Cycles (FTC) can be defined as when soil temperature drops below and then rises above 0°C. Freezing in winter and then thawing in spring can be considered to be a FTC, although the term is also associated with more rapid fluctuations, such as diurnal and fortnightly cycles. These shorter FTC occur regularly at high latitudes during autumn and spring, and can occur during milder winters. Mid-latitudes also experience FTC, mostly in late winter and early spring. The temperature of the soil at the surface experiences the most severe temperature changes and more frequent FTC, whilst soils at 5cm depth are less affected (Baker and Ruschy, 1995; Henry, 2007). Most diurnal FTC cause only small changes in soil temperatures and do not penetrate to a great depth (Vestgarden and Austnes 2009). These are largely caused by changes in incoming solar radiation (Gradwell 1955). Longer cycles occur due to changes in the weather (Henry 2007). These longer cycles could cause colder soil temperatures and extend to greater depth in the soil (Vestgarden and Austnes 2009). Over arctic winter, the entire active layer, which can be over 1 m in depth can freeze.

2.1.2. Soil Freezing

Above FT was described as when soil temperatures dip below and rise above 0°C. However soil solutions do not necessarily freeze at 0°C. Homogeneous nucleation of water, where stable ice crystals can form spontaneously, does not occur until below -39°C. The presence of ice crystal nuclei is required for water to freeze between 0°C and -39°C. Ice crystal nuclei are ubiquitous in soils and include: certain minerals and compounds with the right molecular shape; rough edges and cracks in mineral and organic matter particles; hydrophobic materials and materials with low surface charge (Kawahara 2013). These ice crystal nuclei allow the bulk soil solution to commence freezing between 0°C and -1°C. Soil freezing and thawing also exhibits hysteresis, freezing is delayed by low temperature kinetics and the requirement for ice crystal nuclei which allows the water to supercool, whilst ice thaws very close to 0°C (Sparman et al. 2004). Soil solution freezing is also offset from decreases in air temperature due to the high thermal capacity of water which needs to be overcome for ice to form and warming caused by the latent heat of fusion (Romanovsky and Osterkamp 2000). For ice crystals to grow water molecules must move towards the freezing front. This is delayed by the hydraulic conductivity of the soil, slow kinetics and greater viscosity at low temperatures (Kurylyk and Watanbe 2013).

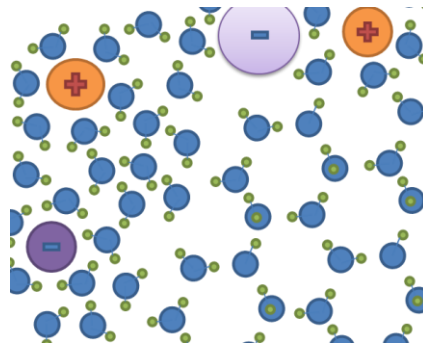


Fig. 2.1. Schematic diagram demonstrating the disruptive effect of solutes on the formation and growth of ice crystals (right of figure) as they attract water molecules.

Even when the bulk soil solution has frozen, some liquid water remains. The amount of unfrozen water remaining in soil after it has frozen depends on a number of factors including: the ionic strength and composition of the solution, mineral type, organic matter content, the age of the organic matter and grainsize (Hansen-Goos and Wettlaufer 2010; Harrysson-Drotz

et al. 2010). Ice formation concentrates solutes in the remaining solution, which lowers the freezing temperature due to interaction between water molecules and solutes and between solutes and ice crystal surfaces (Fig 2.1). This is particularly apparent for smaller ions (Chen 1987). As the salt content increases polar organic compounds such as proteins can precipitate from solution and inorganic minerals can form as the solution saturates (Marion 1997). This could decrease the effect of solutes with decreasing freezing temperature.

A film of unfrozen water a few nm wide is adsorbed to the surface of ice and similar films are adsorbed to mineral surfaces. These thin liquid layers exist between soil minerals and ice crystals due to repulsive interactions between their surfaces due to differences in their crystal structure (Osipov 2012). Soil minerals and ice crystals tend to have negative zeta potential at a pH greater than 4 suggesting that they have a negative surface charge which could contribute to surface repulsion (Drzymala et al. 1999; Wang et al 1997). These overcome any attractive Van der Waals forces between the grains, surface tension and that ice is thermodynamically more stable than water below 0°C. It has been shown by Thomson et al. (2013) that the number of ions in the solution increases the thickness of the liquid layer, except at low concentrations where the surface charge density plays an important role. Solutes would screen the interactions between the surfaces at high concentrations. This partly supports the findings of Harrysson-Drotz et al. (2009) who suggested that both osmotic and matric potential contribute to unfrozen water content in soil.

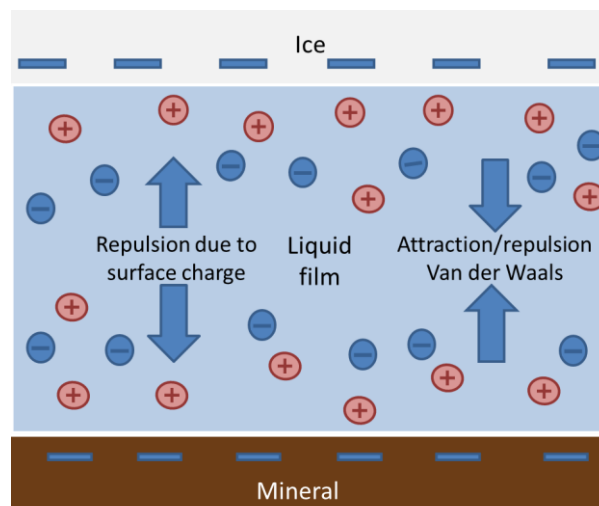


Fig. 2.2 Schematic diagram illustrating how thin films of water exist between ice and mineral grains in frozen soil

Pore shape does not appear to affect the film thickness but greater roughness of the surface increases film thickness (Dash et al. 2006; Morishige et al. 2010). This film does not form hexagonal or cubic ice, but vitrifies when temperatures decrease enough (Christenson, 2001). Theoretically, this thin film can collapse at low temperatures if the van der Waals forces between the mineral and ice crystals are attractive as in the case of quartz (Hansen-Goos and Wettlaufer, 2010). Wei et al. (2002) found little evidence for surface pre-melting between ice and quartz below 272K. Prior to this ultimate freezing temperature being reached, the film maintains fluidity (Klein et al. 2004). In unfrozen conditions the viscosity of this film is 3-5 times greater than that of the bulk pore water solution, but ions can get trapped by the charged surfaces and the dissolving capacity is less (Klein et al. 2004; Osipov 2012). Thus the amount of liquid water available is dependent on not only the temperature, but on mineral surface area and composition, and soil solution composition.

Studies have shown that in nm scale pores ice forms into cubic ice crystals by homogeneous nucleation which occurs at and below -39°C (Christenson, 2001). Potentially such small pores lack ice crystal nuclei. Also, small ice crystals are less stable than larger ones and their continued existence requires a lower freezing temperature. Similarly liquid water exists below zero in tight spaces between grains as the small, high curvature ice crystals required to fit the space are unstable at warm freezing temperatures (Hansen-Goos and Wettlaufer 2010). Ice in larger pores could propagate into the nanopores, but most studies have focussed on uniform pore sizes (Zeng et al. 2015).

2.1.3. The Carbon Cycle

For the purpose of this topic the carbon cycle can be described as follows: Atmospheric CO_2 is fixed by autotrophic organisms into organic carbon (Fig. 2.3). Senescence, death and waste removal input this organic carbon into the soil in dissolved and particulate forms. It is altered into different organic forms by microbial utilisation and degradation. Ultimately, it is either released back into the atmosphere, during respiration, or buried. The rate at which carbon is mineralised in the soil is partially dependent on the organic form (Coûteaux et al. 1995). Dissolved Organic Carbon (DOC) is considered to be particularly labile (Marschner and Kalbitz 2003; van Hees et al. 2005). Schmidt et al. (2011) suggest that, in the long term, turnover is more dependent on factors such as pH, temperature, moisture and microbial activity than on chemical structure. A compound's position in the microenvironment could

also protect it from degradation. Physical protection by soil aggregates or cell wall material and sorption to minerals can delay degradation (Bachmann et al. 2008; Papa et al. 2014).

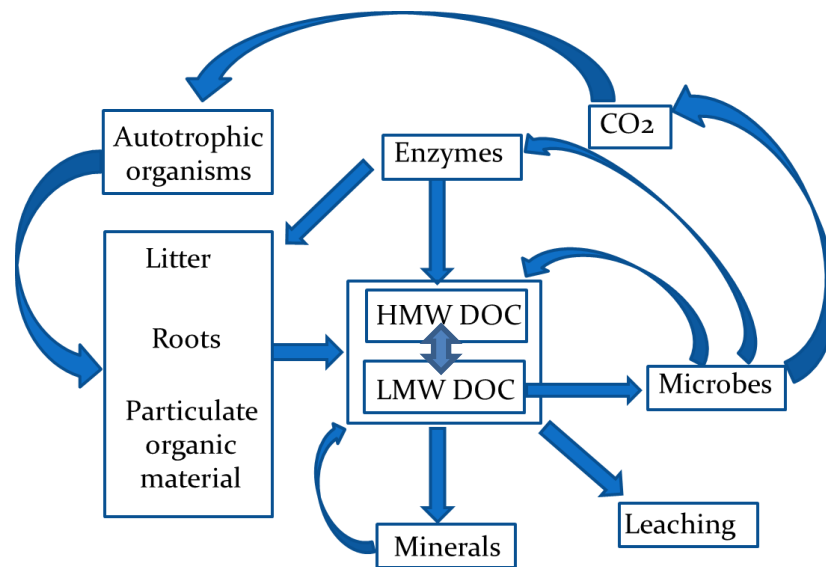


Fig. 2.3. A simplified version of the carbon cycle in soil

The DOC pool is a mixture of many different soluble carbon molecules of varying size. The low molecular weight (LMW) DOC is an important component of the C cycle as only compounds smaller than 650 MW can pass through the microbial membrane (Payne and Smith 1994). Therefore, it is generally considered to be the most accessible form of C for heterotrophic microorganisms. Certain LMW-DOC compounds, such as glucose and amino acids, have been found to be rapidly utilised by the microbial community, with half lives in the soil solution as low as between 1 and 2 hours (Boddy et al. 2008; van Hees et al. 2005). These compounds tend to be maintained at low concentrations in soil solution, glucose and amino acids represent about 5% of total DOC (Boddy et al. 2007). High molecular weight compounds have longer residence times in soil solution, with half lives greater than 100 days (Kalbitz et al. 2003) These and particulate organic matter need to be broken down, by extracellular enzymes, before they can be utilised by microbes (Schimel and Bennett 2004).

There is uncertainty as to the main sources and sinks of DOC. Plant litter is a large source of DOC at the soil surface (Uselman et al. 2008). This DOC is subject to alteration in the organic layer as it is used by microbes for catabolism and anabolism. This makes the DOC more aromatic (Hanson et al. 2010). Carbon isotope studies suggest that DOC at depth is locally produced rather than having leached from the surface (Sanderman et al. 2008). Tree girdling experiments have shown that root exudates can contribute up to 40% of DOC

(Giesler et al. 2007). Root exudates contain a relatively large proportion of LMW DOC, such as sugars, amino and organic acids (Farrar et al. 2003). Root biomass has also been shown to contribute to soil DOC (Uselman et al. 2008). The DOC has been shown to adsorb to mineral grains, particularly metal oxyhydroxides and smectite. However, mineral grains have only a limited capacity for sorption and the DOC can desorb (Guggenberger and Kaiser 2003; Sanderman et al. 2008). Microorganisms are also both sources and sinks of DOC (Sanderman et al. 2008). Thus DOC is a constantly changing pool of carbon with inputs from litter, roots, soil organic material and microbes. Losses are from mineralisation, leaching and adsorption by minerals and microbes.

2.1.4. The Nitrogen Cycle

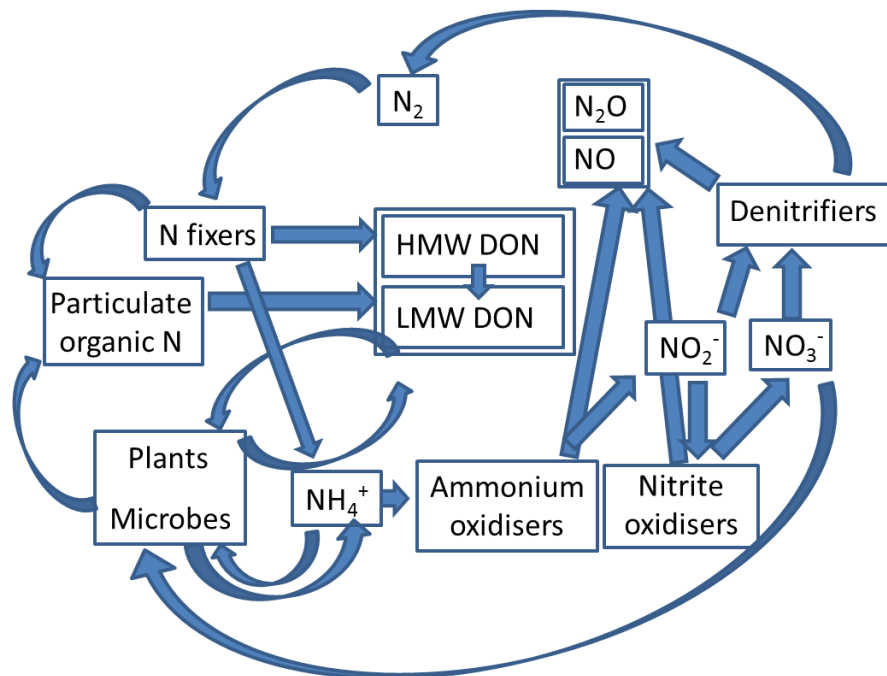


Fig. 2.4. A simplified version of the soil nitrogen cycle

Nitrogen is a vital nutrient for organisms. The biotic part of the nitrogen cycle is outlined in Fig. 2.4. Inputs into the soil are via nitrogen fixation and as organic nitrogen from biomass, in the form of chitin, peptiglycan and protein (Geisseler et al. 2010). Nitrification transforms NH_4^+ to NO_3^- by the combined activity of ammonium and nitrite oxidising bacteria. NO_3^- can also be transformed back to NH_4^+ by nitrate ammonifying bacteria which can also produce N_2O where the C/N ratio is low (Stremińska et al. 2012). N_2O can also be produced by ammonium oxidising microbes (Blackmer et al. 1980). Denitrifying bacteria transform NO_3^- into N_2 . This occurs in low oxygen conditions where NO_3^- is used as an electron

accepter by heterotrophic bacteria. Anaerobic ammonium oxidation, nitrification and denitrification release N to the atmosphere in the forms of N_2 , N_2O and NO .

Nitrogen is absorbed by organisms in the forms of NO_3^- , NH_4^+ and LMW dissolved organic nitrogen (DON). Traditionally plants were thought to rely on the inorganic forms of nitrogen, but recently LMW-DON has been shown to be a source of N for some plants, as well as for microbes, particularly where N concentrations are low (Hill et al. 2011; Schimel and Bennet 2004). Microbes will take up NH_4^+ in preference to NO_3^- as NO_3^- must be reduced to NH_4^+ , by nitrate and nitrite reductase enzymes, before it can be used (Marzluf 1997; Merrick and Edwards 1995). Bacteria are better at using NO_3^- than fungi (Myrold and Posavatz 2007). As with DOC, molecules of DON larger than 650MW must be broken down by extracellular enzymes before they can be utilised by microbes. It is more energy efficient for microbes to take up peptides whole than to take up the NH_4^+ and amino acids required to make them (Geisseler et al. 2010). However, taking up peptides and amino acids require many specific proteins in the cell membrane, whereas taking up NH_4^+ only requires one type (Geisseler et al. 2010). When C is limiting DON will be used as both a carbon and a nitrogen source. This would potentially reduce the uptake of NH_4^+ and also produce it.

2.1.5. The Arctic Tundra

The arctic tundra is found north of the boreal forests. This covers a large area and there is approximately $10^\circ C$ difference in average July temperature between the sub/low arctic in the South and the high arctic in the North (Walker 2000). The active layer above the permafrost can vary in thickness (c.a. 0.2-2m) depending on the temperatures, the number of days above freezing, the thermal conductivity of the soil and snow and vegetation cover (Hinkel and Nelson 2003; Zhang et al 2005). In September-October the active layer freezes from the surface down until all the ground is frozen. The surface then thaws around May-June. Vegetation is limited and stunted, with most of a plants biomass ($> 90\%$) occurring below ground as roots, predominantly as fine roots (Wallen 1986). The most common plant types are dwarf shrubs, graminoids, lichen, moss and forbs (Elmendorf et al. 2012; Walker 2000). Sphagnum is common in the bogs and low pH soils of more southern/ low/ sub arctic regions, but tends not to occur in high arctic regions where the mean average temperature is above $-6^\circ C$ (Gignac et al. 2000). Bacteria found in the active layer are typically proteobacteria, which dominate neutral and basic soil, acidobacteria, which are more prominent in acidic soil, and gammatimonadates (Campbell et al. 2010; Coolen et al. 2011). Proteobacteria

were found to dominate shrub soils whilst acidobacteria dominated tussock soils (Wallenstein et al. 2007). Eukaryotes in the active layer include cercozoa, alveolata, fungi, and nematodes. There are few archaea in the active layer (Coolen et al. 2011). The permafrost has a different microbial community to the active layer. In addition to the above, bacterioidetes are present in the upper permafrost, actinobacteria, firmicutes and, an uncultivated type of bacteria, OP5 exist deeper in the permafrost. Stramenophiles, protists, amoeba, algae and plants are also found, though this may be in the form of detritus in some cases (Coolen et al. 2011). Methanogens are also present in the permafrost (Mackelprang et al. 2011).

2.2. Fluxes in C and N due to FT

2.2.1. Fluxes in C due to FT

Studies have shown that dissolved organic carbon (DOC) in the soil increases after FTC (Feng et al. 2007; Gao et al. 2015; Urakawa et al. 2014; Wang et al. 2014; Yu et al. 2011). However, the amount of DOC released has varied between studies and some studies have shown no change or even decreases (Grogan et al. 2004; Sehy et al. 2004; Urakawa et al. 2014). When successive FTC have been investigated increased DOC fluxes have ceased after a varying number of repeated cycles (Wang et al 2014; Yu et al. 2011). Few studies have investigated the composition or quality of the DOC. Austnes and Vestgarden (2008) found that the DOC/DON ratio and the aromaticity decreased following 8 weeks frozen (-5°C) then increased, suggesting an increase in labile DOC during or immediately after freezing, but found no affects for more rapid FTC. Hentschel et al. (2008) found that the aromaticity of the DOC was not significantly affected or that it increased after 1, 2 and 3 FTC at -3°C, -8°C and -13°C. However, in both cases they did not take their measurements immediately after thaw, waiting at least 60 hours in the former and 7 days in the later, so the more labile C released during the FTC may have already degraded.

Flushes of CO₂ have been reported during the thaw in FTC (Schimel and Clein 1996; Wang et al. 2014b). These were also smaller when less severe FTC were applied and, as with DOC, emissions of CO₂ decrease with repeated FTC (Goldberg et al. 2008; Larsen et al. 2002, Skogland et al. 1988). Most studies which have used milder FTC, that are more representative of *in-situ* soil conditions, have shown that they do not significantly increase annual CO₂ emissions (Goldberg et al. 2008). Emissions are much greater during the summer

than are released by FTC (Matzner and Borken 2008). In some cases FTC cause a decrease in CO₂ release as less is released during the time when the soil is frozen than if it were unfrozen (Reinmann et al. 2012; Wang et al. 2014b). Methane emissions have been reported during FTC, but these have tended to be from wetlands (Gao et al. 2015; Song et al. 2008). Studies from grasslands have shown methane uptake during FTC (Holst et al. 2008).

2.2.2. Fluxes in N due to FT

Nitrogen dynamics have varied in studies of FT. DeLuca et al. (1992) found the rate of N mineralisation to increase after FTC. Many studies have shown increases in NH₄⁺ due to FTC (Grogan et al. 2004; Koponen et al. 2006; Soulides and Alison 1961; Yu et al. 2011; Zhou et al. 2011). Fewer studies have shown an increase in NO₃⁻ with more studies showing it to be unaffected (Koponen et al. 2006; Soulides and Alison 1961; Yu et al. 2011; Zhou et al. 2011). Some have shown a decrease in NO₃⁻ and NH₄⁺ (Hentschel et al. 2008). Most studies have focused on the inorganic soluble forms. Elliot and Henry (2009) found that 2 FTC to -10°C caused an increase in extractable N as did rapid freezing cycles (3°C/hr and 30°C/hr) whilst slower and milder FTC did not significantly increase extractable N. The increase during the more severe and rapid cycles was caused by DON rather than NO₃⁻ and NH₄⁺. The results have varied with soil type and moisture content. Koponen et al. (2006) found that NO₃⁻ and NH₄⁺ increased in peat after FTC, but that only NH₄⁺ increased in a sandy soil. Zhou et al. (2011) concluded that NH₄⁺ increased with FTC particularly in wetter soil and during more severe temperature change (to -25°C). The NO₃⁻ concentrations only increased in one soil type at low water content. Hentschel et al. (2008) found NO₃⁻ and NH₄⁺ to decrease due to FTC in the organic layer. In the organic + mineral layer FTC increased NH₄⁺ and NO₃⁻ also increased but not as much as the control which saw a large increase in NO₃⁻ and a decrease in NH₄⁺.

Few studies measure both soluble and gaseous N fluxes so may give an incomplete picture of FTC effects on N. Fluxes in N₂O have occurred during FTC but not consistently (Gao et al. 2015; Grogan et al. 2004; Holst et al. 2008; Kammann et al. 2008; Song et al. 2008). There have been few measurements of NO during FTC. Goldberg et al. (2008) found more NO than N₂O was emitted during FTC, but it did not occur as a pulse during thaw nor was it greater than the control. The N₂O may have been converted to N₂, the emissions of which have not been studied. The results of studies into N fluxes due to FTC are not consistent but

emissions of N₂O, where they do occur, could be annually significant (Matzner and Borken 2008).

2.2.3. Arctic C and N fluxes due to FT

A few studies have looked at the C and N fluxes due to FT in the subarctic tundra (Grogan et al. 2004; Larsen et al. 2002; Schimel and Clein, 1996). These showed that repeated FTC decreased microbial biomass carbon (Grogan et al. 2004; Larsen et al. 2002). CO₂ emissions were generally less from FT treatments than from unfrozen controls, save in the wet meadow tundra investigated by Schimel and Clein (1996). Inorganic nitrogen dynamics have varied. Larsen et al. (2002) found NH₄⁺ decreased after repeated FTC in Graminoid and dwarf shrub heath tundra. Schimel and Clein (1996) found immobilisation after the first cycle and mineralisation after the second cycle in wet meadow tundra and after the third in tussock tundra. This was in sieved soil samples and the control also showed a change from immobilisation to mineralisation. The process was slowed by FT in the tussock tundra but accelerated in the wet meadow. Grogan et al. (2004) found that a single deep freeze increased NH₄⁺, but repeated cycles did not until a few days after. Larsen et al. (2007) found little difference in NH₄⁺, but did find DON and DOC were higher in soil subjected to fluctuations in air temperature. Grogan et al. (2004) found DOC and DON to increase due to a single deep freeze, but that DOC and DON decreased due to multiple FTC.

2.2.4. Gas fluxes in frozen soil

C and N fluxes still occur at temperatures below 0°C. Emissions of CO₂ have been observed from frozen soils (Elberling and Brandt 2003; Fahnestock et al. 1998; Grogan 2012; Mikan et al. 2002; Schimel and Clein 1996; Sullivan et al. 2008). Changes in NO₃⁻ and NH₄⁺ have been measured over winter suggesting that N mineralisation occurs in frozen soils (Schimel et al. 2004). Evidence for C and N emissions at -39°C exist with Panikov et al. 2006 measuring ¹⁴CO₂ emissions from soil at -33°C and ¹²CO₂ at -39°C temperature and Price and Sowers (2004) interpreting relatively high concentrations of CO₂, N₂O and CH₄ from deep in ice cores to be generated *in-situ* at around -40°C. However, emissions of CO₂ could be inorganic in nature or have been produced at warmer temperatures, but trapped by the soil (Elberling and Brandt 2003; Shanahun et al. 2012).

2.3. Causes of C and N Fluxes

The release of DOC during FTC affects C and N dynamics. An increase in labile DOC can stimulate microbial activity and cause a sharp increase in gas emissions. The sources of this labile DOC are summarised in Fig. 2.5. Herrmann and Witter (2002) found that 65% of DOC released after a FTC was of microbial origin. The source of the other 35% was uncertain, but was suggested to be due to aggregate break up. However, this study did not include vegetation which is a potential source of DOC. Feng et al. (2007) showed that free lipids increased as a result of FTC which could come from the disruption of soil aggregates and also from fresh litter. Damage to fine plant roots could also be a source of DOC during FTC (Matzner and Borken 2008). Gases produced in the subsoil, but trapped by the frozen surface could also contribute to the observed emissions in the field (Morgner et al. 2010)

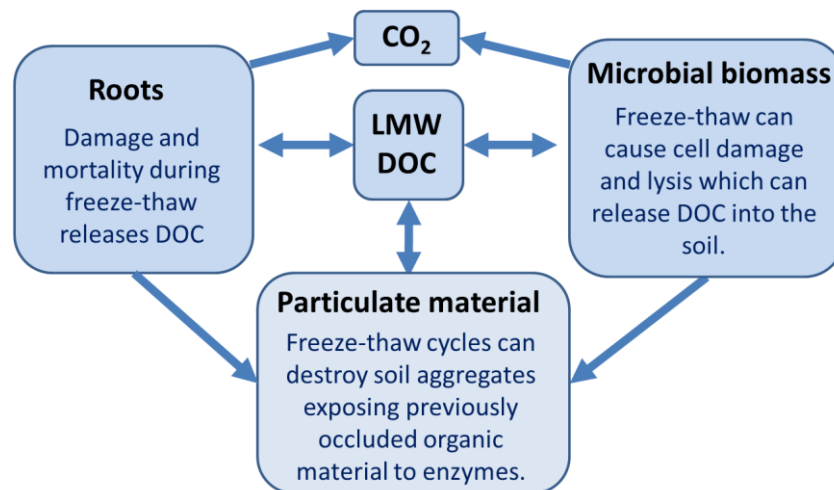


Fig. 2.5. The sources of carbon fluxes during and after freeze-thaw cycles

2.3.1. Microbial response to FT

Respiration and cell growth still occur below 0°C, but at a much slower rate (Drotz et al. 2010; Elberling and Brandt 2003; McMahon et al. 2009). Freezing of water increases the solute concentration in the remaining solution, lowering its freezing point. Microbes can survive in films of water which form on surfaces. McMahon et al. (2009) found that fungi incorporated more ¹³C-glucose into PLFA at -2°C than bacteria. Gram negative bacteria also grew at -2°C but gram positive bacteria did not. It is thought that fungi may grow better than prokaryotes at freezing temperatures as they only have to use their outer membrane for substrate uptake, whereas bacteria must also use theirs for energy generation (Hall et al.

2008; McMahon et al. 2009). This allows fungi's cell membrane to adapt more to lower temperatures. Fungi may also have greater access to substrate due to their hyphae. Gram positive bacteria maybe unable to grow below freezing as their thick cell wall could reduce diffusion (McMahon et al. 2009). It has been suggested that microbes utilise different substrates as temperatures decrease below 2°C, using more N rich substances (Schimel and Mikan 2005).

In order to survive FTC microbes need to tolerate changes in temperature, ice formation and also changes in salinity. Microbes are thought to survive freezing by producing ice-nucleation and anti-freeze proteins, by increasing their internal salinity and by altering membrane compounds (Wilson and Walker 2010). Some antifreeze proteins can cause freezing point depression although in most bacteria this is only by around 0.01-0.3°C (He and Liu 2013). Large freezing point depression can lead to the rapid growth of large, damaging ice crystals if this temperature decrease is exceeded. Antifreeze proteins can also prevent ice recrystallisation which keeps ice crystals small. It is suggested that antifreeze proteins work by binding to ice crystals and ice crystal nuclei, but this is still uncertain (He and Liu 2013). Ice-nucleation proteins can have a pathogenic function, causing ice growth and cell damage in plant tissues or can be used in combination with antifreeze proteins to keep ice crystals small (Lindow et al. 1982; Lorv et al. 2014). Both ice-nucleation proteins and antifreeze proteins appear to work by having the binding sites of the right structure for ice crystals, with molecular size being the determining factor as to their function (Lorv et al. 2014). Drotz et al. (2010) found increased carbon saturation in membrane compounds and higher levels of glycerol at -4°C compared to +4°C. They also found that microbes take time to adjust to a change in temperature. It took 2 days after a drop to 9°C and 4°C for maximum respiration rates to be reached. It took 60 days at -4°C. The rate at which freezing occurs influences microbial survival, overly rapid freezing causes the inside of the cell to freeze and overly slow freezing leads to ice forming around the cell causing the cell to dehydrate (Rodrigues and Tiedje 2008). Desiccation and starvation could occur during freezing. Increases in the concentration of certain molecules, such as trehalose, glycerol, mannitol and antifreeze proteins may help alleviate these effects (Robinson 2001). Increase in water during thaw could cause cell lysis, due to cells being unable to adjust to new osmosis gradients, (Jefferies et al. 2010). Which of these processes is more important in causing cell death is uncertain.

A number of studies have shown an adverse effect of FT on the microbial population. Skogland et al. (1988), using viable plate counts, found a 70% decrease in bacteria due to FT

(-7°C for 24 hrs, thawed at 25°C). Morley et al. (1983) found that FT to -9°C and -27°C (for 12hrs, 23°C for 12hrs) decreased bacterial numbers by 50% in a mixed community but that it took 2 repeated cycles to -9°C to cause a decrease in *Pseudomonas paucimobilis*. A second FTC caused further decrease in the mixed community, whilst after a third microbial numbers increased. The results suggest that bacterial species differ in their tolerance to FTC and that only a certain proportion is vulnerable. However, viable plate counts are poorly representative of the true microbial population of the soil. Larson et al. (2002) found a significant decrease in microbial biomass carbon of about 20%, after 18 mild, diurnal FTC (2°C for 9 hours, -4°C for 15 hours). Herman and Witter (2002) suggested that a 5% microbial mortality was responsible for the increase in DOC they observed. Stres et al. (2010) found that 50 diurnal FTC, to -4°C, caused no change to the microbial abundance in a Himalayan soil, but in a temperate soil the same treatment caused a 7 fold decrease. It has been shown that the microbial population recovers rapidly from FT (Feng et al. 2007). This could be due to the increase in labile DOC supporting new growth or helping surviving microbes adapt to further FTC (Schimel et al. 2007). However, Grogan et al. (2004) saw no recovery of microbial biomass carbon in ten days after 5 FTC. How much of the released substrate is utilised for growth and how much for respiration is unclear. An increase in LMW-DOC can cause priming of more recalcitrant carbon (Farrar et al. 2012). Whether this occurs during FT is unknown.

Few studies have investigated the effects of FT on the microbial community. Wilson and Walker (2010) found that the diversity of the bacterial community significantly decreased after 48 severe and rapid FTC (-18°C to 5°C in 2 hours). The surviving bacteria were found to be of low abundance prior to the FTC. However, this severity of FTC does not occur in the natural environment. Using more realistic FTC temperatures, two studies using PLFA analysis have shown a decrease in fungal biomarkers after freezing, whilst bacterial biomarkers were relatively unaffected (Feng et al. 2007; Haei et al. 2011). Microbial methods have also revealed only small changes in the bacterial community due to FT (Mannistö et al. 2009). These studies suggest that microbial communities are tolerant to FTC. However, these studies only investigated soils from high latitudes. Mid latitude soils may contain less FT tolerant microbial communities, as shown by Stres et al. (2010). The fungal community has been shown to recover quickly from FTC and to have a faster growth rate than bacteria during thaw (Feng et al. 2007; Haei et al. 2011). McMahon et al. (2011) found that only a small proportion of the microbial community, of arctic tundra, was active in

frozen soil. The dominant microbes found in normal PCR analysis were dormant. As dormant microbes are less susceptible to changing conditions it is likely that it is the active proportion of the microbial community that suffers from FTC and a change in this group may not show up in normal microbial methods (Schimel et al. 2007). No studies have investigated the effects of FT on archaea.

2.3.2. Plant response to FT

Plants survive FT by being in a dormant state and due to the process of frost hardening. This process changes the chemistry of plant cells increasing concentrations of phenols, lignin, flavonoids, sucrose, stachyose, raffinose, pinitol, proline, arginine and histidine (Bertrand et al. 2007; Klimov et al. 2008). After frost hardening plants can survive at temperatures of -30°C. Frost hardening occurs when the air temperature is between 5°C and -10°C. Severe frost prior to frost hardening and dormancy can cause plant death (Klimov et al. 2008). Damage to roots can occur during FTC. This can make plants more sensitive to drought and increase mortality, particularly in mature trees (Auclair et al. 1996). Giesler et al. (2007) found that the increase in DOC after FTC was 50% greater in soils where trees were not girdled, allowing plant exudates to the roots. This shows that plant roots could be a large contributor to the carbon flux after FTC. Root turnover has been shown to increase due to FT which could increase the soil organic carbon content (Gaul et al. 2008; Tierney et al. 2001). Senesced roots degrade during FTC. Wu et al. (2010) found up to 20% of fine root mass was lost over the FT season.

Different plant species may have individual responses to FTC. For example, Fitzhugh et al. (2001) found different fates of NO_3^- and NH_4^+ to FTC in the soil under sugar maple and yellow birch trees. They suggested that part of the reason for this could be the different root types of the tree species. Sugar maple are endomycorrhizal, whilst yellow birch are ectomycorrhizal. As ectomycorrhizal hyphae spread out further in the soil they may be more prone to damage from FTC. Also different vegetation cover could affect the composition of DOC in the soil (Feng et al. 2007) and the microbial community. Vestgarden and Austnes (2009) found different effects of FTC beneath different types of vegetation. However, they did not take into account other differences in the soil which may affect both FTC response and the dominant type of vegetation. Plants can also provide shading from radiation and insulate the soil from changing temperature – reducing FTC (Gradwell 1955).

2.3.3. Physical response to FT

The physical disruption to the soil during FTC could break up soil aggregates. This could expose previously protected organic material to microbes and external enzymes. As the position of organic matter is considered to be one of the main factors in its degradation, possibly more so than its chemical form as inaccessibility can protect even sugars from degradation, the above could be a significant factor in increased CO₂ emissions after FT (Schmidt et al. 2011). Studies have shown FT to decrease aggregate stability (Soulides and Alison 1961), but only in moist soil not in dry soil (Bullock et al. 1988; Hinman and Bisal 1968). However, repeated FTC have been shown to increase aggregate stability (Lehrsch et al. 1991; Oztas and Fayertobay 2003). Thawing could increase aggregate stability, but as Bullock et al. (1988) and Lehrsch et al. (1991) thawed their soil for the same length of time, yet achieved contrasting results, this cannot be the sole cause of the contradictory results. Soils with increased clay content appear to be less adversely affected by FT than other soils. Clay has greater particle-particle interface area and charged surfaces increasing cohesion (Lehrsch et al. 1991). Bullock et al. (1988) found that FT in constrained soil caused greater disruption than in unconstrained soil. The literature suggests that freezing causes physical disruption to soils but only in moist soils. These studies have been done using severe FTC. There is little evidence on to what extent more moderate FTC affect the soil structure.

Different types of soils could have different responses to FTC. Soil with higher total organic carbon (TOC) could produce greater CO₂ after FTC but unfrozen controls would also produce more CO₂. FTC might be more significant from low TOC soils (Schimel and Clein 1996). The soil water content could also have a large effect. Öquist et al. (2009) found that the unfrozen water content of frozen soils controlled CO₂ production and that the unfrozen water content increased as the soil organic matter quality decreased. Haei et al. (2011) found that respiration after frost increased with water contents, being greater at 90% water holding capacity (WHC) than at 30%WHC and 60%WHC. A greater volume of water freezing causes greater disruption of soil aggregates (Hinman and Bisal 1968). It would also limit the diffusion of gases (Tucker 2014). The latter could lead to anaerobic conditions changing the processes occurring in the soil and leading to emissions of CH₄ and N₂O. This could also trap gases in the soil causing emission when the ice melts. Phillips et al. (2012) showed that emissions in a core during thaw correlated positively with porosity. Increased water content could also reduce the response of the soil to temperature due to its high heat capacity, decreasing the effects of FTC.

2.3.4. Causes of N fluxes

As with DOC, damage to microorganisms, roots and soil aggregates during FTC could input DON to the soil. This could explain why Elliot and Henry (2009) found an increase in DON at extreme FTC. Degradation of DON could produce NH_4^+ which would explain observed increases in NH_4^+ after FTC (Geisseler et al. 2010; Koponen et al. 2006; Souliides and Alison 1961). However, input of DOC could require increased N uptake to support a growing microbial community, reducing NH_4^+ and NO_3^- . Therefore the DOC/DON ratio could control the effect of FT on NH_4^+ . Damage to roots could cause a decrease in the uptake of NH_4^+ and NO_3^- after FTC (Fitzhugh et al. 2001). Nitrification could decrease NH_4^+ .

Nitrification could increase as a result of increased NH_4^+ . Agricultural soils seem to increase in NO_3^- and NH_4^+ after FTC (Matzner and Borken 2008). High levels of N input in to these systems would support a large community of nitrifiers which, if undamaged by FT, could benefit from an increase in NH_4^+ . Nitrifiers compete poorly for NH_4^+ so an increase in NO_3^- after FT may only be likely in high N systems, although it is possible that FT damage to other organisms could decrease their NH_4^+ uptake leaving more available for nitrifiers (Schimel and Bennet 2004). Nitrification would be reduced in anaerobic conditions which could explain why Zhou et al. (2011) found NO_3^- only increased in dry soil. Also increased denitrification could reduce nitrate concentrations. This would be more likely to occur in water logged soils. Zhou et al. (2011) found that NO_3^- only increased in a soil of low water content. Therefore, the existing microbial community and the water content could control N fluxes.

Emissions of N_2O can occur during nitrification or denitrification. Zhu et al. (2009) found increased emissions of N_2O during FTC under anaerobic conditions which suggests denitrification is important. A model simulation by de Bruijn et al. (2009) also found denitrification to be important in N_2O release during FT. They suggested that a combination of limited O_2 diffusion into the soil due to ice and thaw water and increased microbial activity caused by cell death was responsible for N_2O fluxes. Observed N_2O emissions during FTC from wetlands and grasslands with a higher soil moisture content support this conclusion (Holst et al. 2008; Song et al. 2008). Sehy et al. (2004) found that adding DOC to saturated soil caused a significant increase in N_2O emissions, although not as much as after FT. This was attributed to more labile carbon being released and a better distribution of C throughout the soil after FT. However, Reinmann et al. (2012) did not observe any pulses of N_2O in an

experiment where FTC and snowmelt were considered. Buckeridge et al. (2010) found that fertilisation increased N₂O emissions but did not cause high fluxes after FTC. These measurements were made after FTC had already occurred so it is possible that the early cycles caused pulses that were not measured. It has also been suggested that N₂O emissions are from the subsoil.

2.3.5. Experimental Artefacts

The experimental design has a large effect on the results obtained. The temperature minima and rate of temperature change are important parameters. Colder freezing temperatures appear to result in greater DOC concentrations as do more rapid freezing rates (Henry 2007). The temperature that the control is kept at can affect the significance of the results as a higher temperature control will produce more CO₂ than one at a lower temperature (Matzner and Borken 2008). In many areas only the surface soil experiences FTC so experiments where the subsoil freezes may not be realistic. Temperature changes occur from the soil surface; cores not insulated on the sides would freeze overly rapidly. The season that the samples are collected also affect the results (Henry 2007). As fluxes can be brief, when after thaw the measurement takes place will influence results. What constitutes thaw is unclear in many papers. Is it when the soil temperature reaches above freezing or when the air temperature reaches that of the control?

Many experiments sieve the soil to achieve a homogenous mix. This breaks up soil aggregates, removes roots, reduces soil density and increases porosity. This makes laboratory experiments unrepresentative of field conditions. They could potentially reduce the effects of FTC as C input from plant roots is removed (Giesler et al. 2007). Furthermore, the decrease in soil density puts less constraint on the soil which would decrease the physical disruption of the soil by FT (Bullock et al. 1988). The increased porosity reduces the possibility of anaerobic conditions forming or of gases being trapped in the soil (Phillips et al. 2012).

2.4. Conclusion

Freeze thaw cycles can cause an increase in DOC concentration, but this result is not always observed. Colder freezing temperatures have been shown to produce more DOC and possibly DON. How DOC concentrations and quality vary after thaw is less clear as studies are

lacking in temporal resolution. Little is known about the long term effects of increased FTC. Peaks in CO₂ and N₂O emissions have been observed on thaw, but as with DOC this is not always the case. Fluxes of CO₂ due to FTC are probably not significant annually. However, FTC could contribute substantially to N₂O emissions although different experiments have given conflicting results. FTC can also affect NH₄⁺ and NO₃⁻ concentrations, but both increases and decreases have been reported. What controls N dynamics during and after FT is open to debate as few studies have examined all the individual components. DON concentrations are rarely measured after FTC despite it being an important N source for both plants and microbes.

The mechanisms that cause the fluxes of C and N during FTC are not fully understood. It is believed that cell lysis releases DOC which stimulates microbial activity during the thaw. Labile DOC could be released by damage to both microbes or roots after FT and physical disruption of aggregates could also provide fresh degradable carbon. The relative importance of these sources could depend on numerous factors such as temperature change, soil type, vegetation, water content and microbial community. Further work is required to find the most important DOC sources in different environments and ecosystems.

Microbial dynamics during and after FT are poorly understood. The active microbial community is different in frozen soil compared to unfrozen soil and it is possible that their substrate use changes with an increased reliance on DOC and microbially produced compounds. They survive in thin films of water on the surface of the ice. How this affects microbes is unclear. Studies on the microbial community suggest that fungi are most affected by FTC. The microbial community appears to not be overly affected by FTC, but past studies may not have represented the active community which could be more affected. Microbes seem to recover quite rapidly from FTC, but this still requires further investigation.

In order to address the knowledge gaps identified in this literature review the chapters of this thesis investigate the following main hypotheses:

- Vegetation is an important source of the DOC and DON produced during FTC.
- The composition of DOC in arctic tundra soils changes due to FTC, with an increase in LMW DOC concentrations and DON concentrations.
- FTC affect the microbial use of LMW DOC.
- FTC can increase degradation of old soil organic carbon.
- Freezing temperature affects microbial use of LMW DOC in arctic tundra soil.

- Winter freezing temperatures affect DOC and DON concentrations and have further ramifications for arctic C and N cycles.

Chapter 3

Are Plants an Important Source of DOC to Soil During Freeze-Thaw Events?

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3 Are Plants an Important Source of DOC to Soil During Freeze-Thaw Events?

Abstract

Damage to plant roots has been suggested as one of the contributors to the observed increases in soil dissolved organic carbon (DOC) and resulting emissions of greenhouse gasses after Freeze-Thaw cycles (FTC). Here the effect of different freezing temperatures on the concentration and composition of DOC produced by freezing plant roots is assessed. Results show that plant roots can be a significant source of glucose and amino acids during freezing to -3°C and -5°C . Changes to the fluorescent components of the extracted DOC were also observed after freezing to -5°C . However, no significant change to total DOC concentration could be attributed to roots. Amino acid concentration was particularly affected by FTC with concentrations increasing more than 15-fold at both -3°C and -5°C whilst glucose concentrations increased by 4.9 and 5.9-fold, respectively. No increase in concentration was observed when the plants were frozen at -0.1°C suggesting that mild frosts are unlikely to cause soil DOC to increase. This study shows both the importance of plant roots to soil LMW-DOC after freeze thaw and the importance of studying the composition of DOC in detail to gain a full understanding of its dynamics after a freeze-thaw event.

3.1. Introduction

Freeze-thaw cycles (FTC) can produce changes to the concentration and composition of soil dissolved organic carbon (DOC) (Austnes and Vestgarden 2008; Haei et al. 2012). Plant roots have been suggested as a potential source of this DOC (Giesler et al. 2007; Tierney et al 2001), but many studies on DOC concentrations after FTC remove roots and surface vegetation prior to their experiment (Hentschel et al. 2008; Hermann and Witter 2002; Vestgarden and Austnes, 2009). As plants are largely present in the topsoil they can experience much colder freezing temperatures and more temperature fluctuation than the underlying soil which can be insulated from the air temperature (Oliva et al. 2014). Giesler et al. (2007) showed that soil containing tree roots that were still alive produced much more DOC after a FTC than soil that contained dead roots. However, this does not distinguish between DOC produced by the roots and DOC produced by microbes in the rhizosphere that the plant roots support.

Plants can be damaged and killed by freezing temperatures and non-cold tolerant varieties can even be damaged by chilling (0-5°C; Kocsy et al. 2011). There is still uncertainty about how plants adapt to and survive freezing temperatures. Plant buds can supercool rather than freeze due to structural barriers that prevent the propagation of ice. Little is known about these barriers, but it may involve reduced xylem connectivity, thicker cell walls and tightly packed cells with no air spaces (Kuprian et al. 2014). Other strategies that plants use to avoid internal ice formation include growing mostly below the ground surface or growing in cushion form; these methods insulate plant cells from air temperatures. As with microbes, plants have been shown to increase the concentration of certain compounds in their tissues that could prevent ice re-crystallisation and the concentration of reactive oxygen species (Bertrand et al. 2007; Klimov et al. 2008; Kocsy et al. 2011).

It has been shown that damage to soil microbes by freezing produces DOC (Hermann and Witter, 2002). This has been shown to be more apparent at colder freezing temperatures, -5°C to -8°C, whilst milder freezing temperatures such as -3°C have often been reported to cause no increase in DOC (Hentschel et al. 2008; Vestgarden and Austnes, 2009). Here we used plants grown in sterile conditions to show whether plant roots without surrounding micro-organisms can affect the concentration and composition of DOC in soil solution after FTC. Different freezing temperatures were used to investigate whether more intense freezing causes more damage as is observed in soil without roots.

3.2. Methods

3.2.1. Growing plants in sterile conditions

Procedures were conducted in a laminar flow cabinet under sterile conditions. Maize was chosen as it was easy to sterilise. Maize seeds were sterilised by shaking for 5 minutes in 50% sodium hypochlorite with 20 µl Tween 20. Seeds were then washed in autoclaved deionised water to remove the bleach and left at 5°C overnight. Seeds were transferred to sterile Petri-dishes and left over night to germinate. Individual seeds were placed in a sterile phytatray (Sigma-Aldrich) and covered with, autoclaved sand (150 g, size <2 mm). Sand-only samples were also prepared as no-root controls. These were watered with 27 ml autoclaved, 20% Long Ashton solution so that the sand was at 75% water holding capacity. Plants were grown in a growth cabinet at 20°C, with 16 hours a day (430 µmol m⁻² s⁻¹) light

for 2 weeks. After 10 days, a further 2 ml of autoclaved, 20% Long Ashton solution was added.

3.2.2. Freeze-thaw treatment

Intact plants and sand ($n = 6$) were frozen at a rate of 1°C h^{-1} to either -0.1°C , -3°C , -5°C or kept as a control at 5°C . Minimum temperature was maintained for 40 h. The temperature was then raised at the same rate as for freezing to 5°C . Plants were removed 4 h after the air temperature reached 5°C , the sand was homogenised and 110 g was centrifuged at 2057 g for 15 minutes through a Whatman 541 filter to extract soil solution. Soil solutions were then centrifuged at 8228 g for 8 min to remove particulate material. Solutions were stored at -20°C prior to analysis

3.2.3. Check for sterility

Approximately 10 g sand was taken for each plant and background sand 4 hours after FT treatment. To this 200 nmol of glucose labelled with $0.03 \mu\text{Ci ml}^{-1} \text{}^{14}\text{C}$ was added. Vials containing 1 ml, 1 M NaOH were added to trap any $^{14}\text{CO}_2$ emissions. Traps were removed after 40 min. To measure the ^{14}C content of the vials, scintillation fluid (Hi-Safe OptiPhase scintillation cocktail; PerkinElmer Corp.) was added and decays per minute were detected by a Wallac 1409 scintillation counter (PerkinElmer Corp., Waltham, MA). Decays per minute greater than 40 which is equal to the lower limit of detection (the average blank concentration + 3 standard deviations) were considered to indicate a non-sterile sample and that sample was discarded and repeated.

3.2.4. Analysis of soil solution

The DOC concentration was measured by multi N/C 2100S (Analytic Jena AG, Jena, Germany). Free amino acid concentration was measured according to the fluorescence method described in Jones et al. (2002) and glucose according to the method provided with Amplex® Red Glucose/Glucose Oxidase Assay Kit (Invitrogen™). The composition of the extracted DOC was assessed using its Excitation Emission Matrix (EEM), for the FTC treatment to -5°C and the control solutions. EEM were measured at wavelengths between 200 nm and 400 nm excitation and between 270 nm and 550 nm emission using a Cary Eclipse Fluorescence Spectrophotometer. Inner-filter effects were removed according to Westerhoff et al. (2001) by the following equation:

$$\frac{I}{\left(10^{-\frac{A_{ex}}{2}} + 10^{-\frac{A_{em}}{2}}\right)}$$

where I is the emission intensity, A_{ex} and A_{em} are the absorbance at the excitation and emission wavelengths respectively.

Intensities were corrected for Rayleigh scattering by removing all values where emission < (excitation + 10 nm) or emission > (2*excitation – 20 nm). Raman effects were corrected by subtracting inner-filter corrected blank intensities as described by Bro and Vidal (2011). Potential changes in lamp intensity were correct by converting emission intensities into quinine sulphate units (QSU). Quinine sulphate (4 µg l⁻¹ in 0.05 M H₂SO₄) samples were run alongside soil solution samples and corrected as described above. Soil solution emission intensities were divided by ¼ the average maximum emission intensity at an excitation wavelength of 350 nm of the quinine sulphate samples to make the resulting intensities relative to 1 µg l⁻¹ quinine sulphate (Yamashita and Tanoue 2003).

The EEM were assembled as a multidimensional array in MatLab (version R2014a) and the components were found using PARAFAC (Andersson and Bro 2000). The integrity of the model was analysed using CORCOND and by comparison with models comparing only half the samples (Bro and Kiers 2003). The relative intensities of the components were calculated as follows:

$$Relative\ intensity = \frac{component\ intensity}{\sum\ component\ intensity} \times 100$$

Solutions were also analysed by Matrix Assisted Laser Desorption/Ionization – Time of Flight (MALDI-TOF) to assess whether new compounds are produced and released by the plant roots during FT. MALDI-TOF was chosen as a method for assessing whether FT produced any particular compounds as this method causes little fragmentation of the organic compounds and requires no special extraction methods that could modify the solutions. The matrix chosen was 10 g l⁻¹ 2,5-dihydroxybenzoic acid (DHB) in water matrix. A 10 g l⁻¹ DHB in Trifluoroacetic acid (TFA) matrix were also tested, but the DHB in TFA had more matrix interference than the chosen matrix. A 10 g l⁻¹ TiO (10 µl) in TFA matrix had even less matrix interference than the chosen matrix, but the inulin standard was not resolved which lead to a poor calibration and suggests that higher LMW compounds were not resolved in this matrix. Soil solutions (10 µl) were mixed with an equal amount of matrix solution and

analysed by MALDI-TOF set to positive ion, reflector mode in the Flexcontrol program. The mass to charge ratio was calibrated using sucrose, glucose and inulin as standards. The resulting m/z list were analysed between samples using Euclidean distance and then hierarchical clustering using Wards algorithm in the statistics software R.

3.2.8. Statistical analysis

Factorial ANOVA was performed using SPSS (version 22) for each substrate and for each EEM component. Amino acid and glucose concentrations were analysed together to assess different effects of FTC on their concentrations. Where homogeneity of variances could not be achieved values were transformed using a square root or log transformation. This was unsuccessful for glucose analysis so weighted least squares general linear model was performed using the inverse of the squared standard deviation as the weight as suggested by (Gurevitch and Hedges 1999; Rosopa et al. 2013). Significant differences were located using TUKEY's post hoc test. Significant difference was assumed where $p < 0.05$.

3.3. Results

3.3.1. Changes to concentration of DOC and LMW DOC compounds due to FT of plant roots

The DOC in soil solution was significantly greater in samples with roots (by a factor of 2.6 when all results are included $p < 0.001$) and was significantly affected by freezing temperature ($p < 0.001$) (Fig. 3.1). No significant interaction between roots and temperature was observed ($p = 0.489$). Freezing samples with roots to -5°C produced a DOC concentration which was almost double all the other roots treatments, a difference which was either significant or close to significant ($p = 0.054$, $p < 0.001$, $p = 0.064$ for 5°C , -0.1°C and -3°C , respectively). However, its root-free control was also relatively high, between 4.1 and 1.8 times greater than the other controls, so this increase cannot be attributed to the presence of roots. As the sand was sterile, microorganisms could not be the source of this increase in DOC suggesting that it could be from mineral bound compounds. The other freezing temperatures had DOC concentrations which were not significantly different to each other or the unfrozen control.

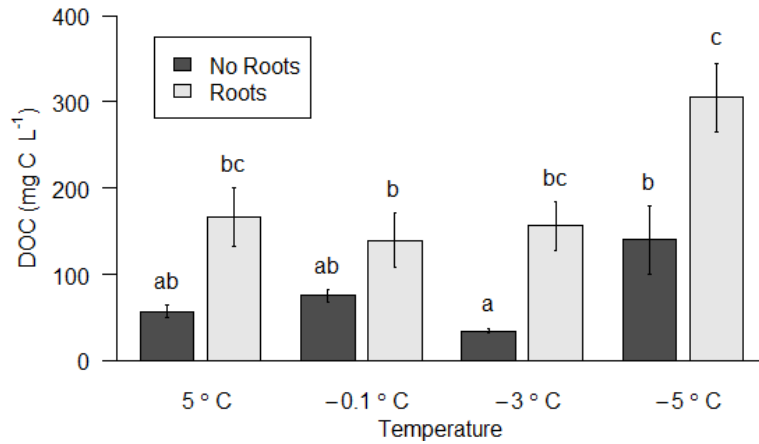


Fig. 3.1. The concentration of DOC in solutions for the controls and the Freeze-thaw treatments with and without roots. Values represent means \pm SEM ($n = 6$). Different letters indicate significant differences between treatments at the $p < 0.05$ level.

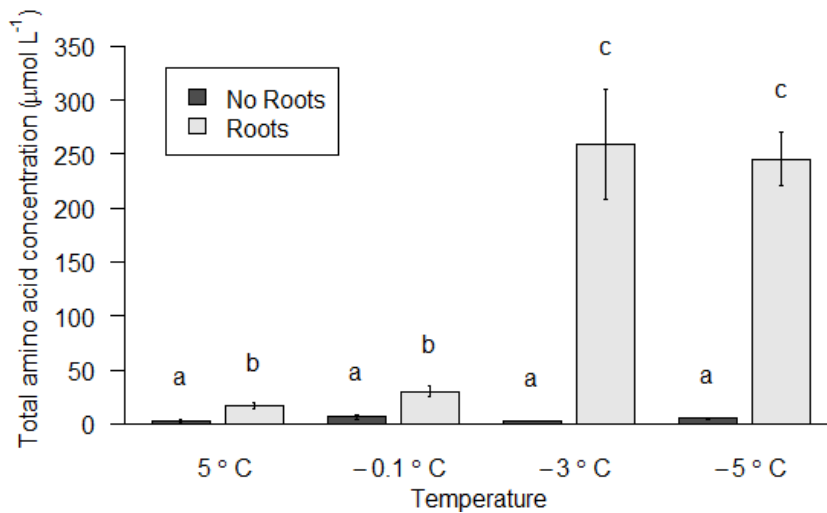


Fig. 3.2. The concentration of total amino acids in solutions for the controls and the Freeze-thaw treatments with and without roots. Values represent means \pm SEM ($n = 6$). Different letters indicate significant differences between treatments at the $p < 0.05$ level.

All treatments in which plants were present had significantly higher amino acid concentrations in extracted solutions than those that did not ($p < 0.001$) (Fig. 3.2). A significant effect of temperature ($p < 0.001$) and a significant interaction between roots and temperature ($p < 0.001$) was observed. Freezing temperatures of -3°C and -5°C significantly increased ($p < 0.001$) the concentration of amino acids in the sterilised sand solution of samples containing plants by a factor of 16.0 and 15.1 respectively. There was no significant difference between the amino acid concentration in the sterile sand solution from the

unfrozen plant samples and the samples frozen to -0.1°C ($p = 0.286$). Freezing samples with no plants did not significantly change soil solution amino acids concentrations.

As with the amino acid concentrations, both the presence of roots and the incubation temperature had a significant effect on glucose concentration (Fig. 3.3) and there was a significant interaction between them ($p < 0.001$ for all). Freezing plants to -5°C caused a significant increase ($p < 0.001$) in sterile sand solution glucose concentrations by a factor of 5.9. The increase caused by freezing to -3°C (a factor of 4.9) was also significant ($p < 0.001$). No significant difference in glucose concentrations was observed between the control plant samples and those frozen to -0.1°C ($p = 0.748$). Glucose concentrations were significantly greater than amino acids ($p < 0.001$), but amino acid concentrations increased more at the lower temperatures of -3°C and -5°C for the samples in which roots were present ($p = 0.008$).

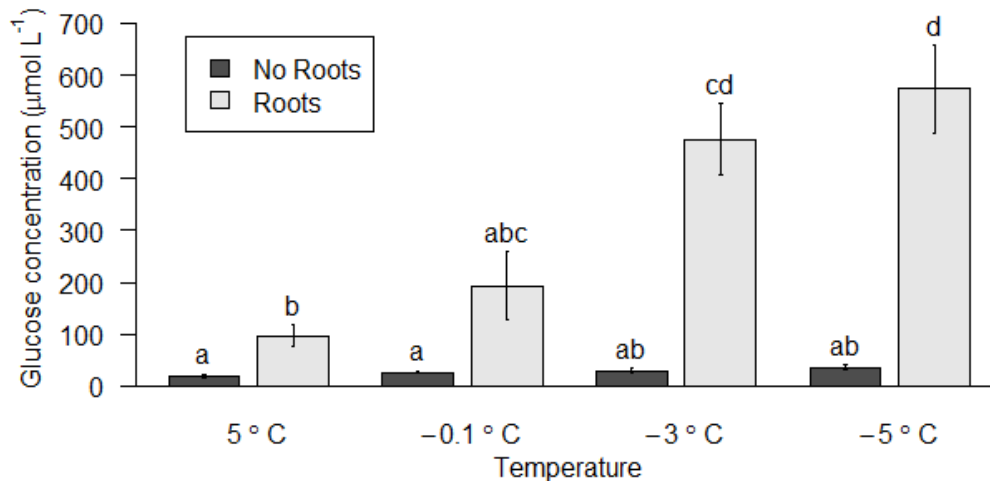


Fig. 3.3. The concentration of glucose in solutions for the controls and the Freeze- thaw treatments with and without roots. Values represent means \pm SEM ($n = 6$). Different letters indicate significant differences between treatments at the $p < 0.05$ level.

3.3.2. Changes to DOC compounds due to FT of plants

Analysis of EEM, which measures DOC fluorescence and can give information about its composition and source material, identified four distinct components (CORCOND = 73.86) (Fig. 3.4). Component 1 is a broad peak with excitation (Ex) 280-360 nm and emission (Em) 430-510 nm. Component 2 is peak with Ex/Em 250-260/460-480 nm. Component 3 is a narrow peak at Ex/Em 230/424-430 nm and component 4 is at Ex/Em 240/442-452 nm. A significant effect of roots was observed on the relative intensity of all components with roots showing an increase in components 1 ($p < 0.001$), 2 ($p < 0.001$) and 4 ($p = 0.041$) with a

decrease in component 3 ($p < 0.001$) compared to samples without roots (Fig. 3.5). FT treatment had a significant effect on the relative intensity of components 1, 2 and 3 with 1 and 2 increasing significantly ($p < 0.002$) and 3 decreasing significantly ($p < 0.001$). A significant interaction was observed between FT treatment and the presence of roots for the relative intensity of components 1, 2 and 3 ($p < 0.01$) with the freeze-thaw of plant samples showing a greater effect.

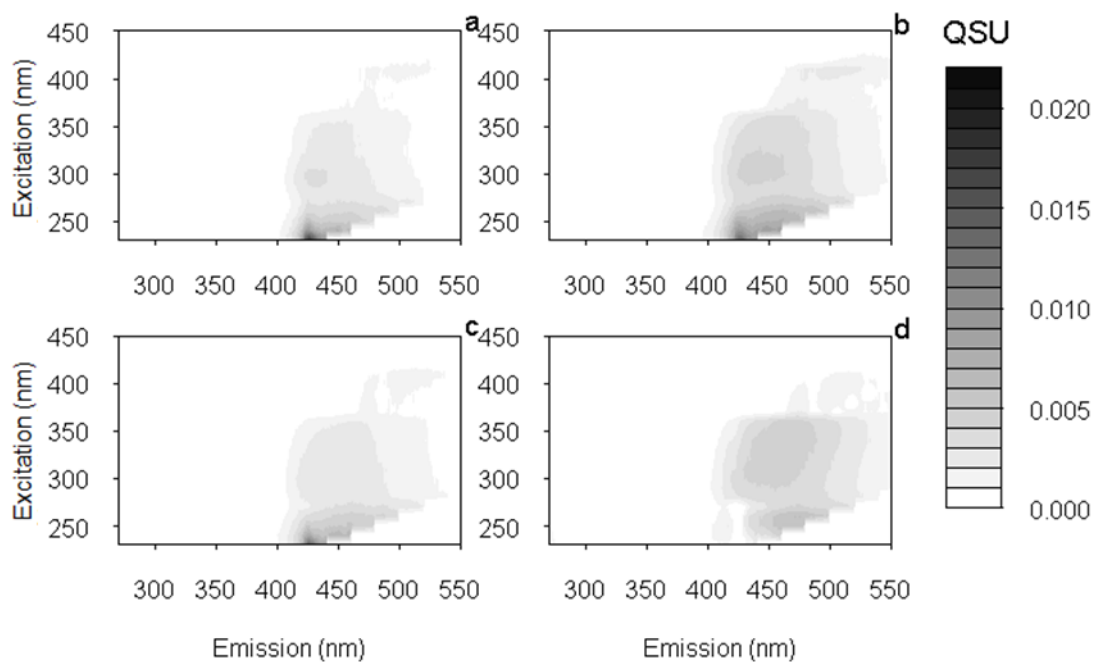


Fig. 3.4. Average Excitation Emission Matrices for; a) no Roots, no FT, b) Roots, no FT, c) no Roots, FT to -5°C and d) Roots, FT to -5°C (QSU = quinine sulphate units, which is the emission intensity relative to the maximum emission intensity of $1 \mu\text{g l}^{-1}$ quinine sulphate).

The MALDI-TOF results showed no clear clustering between roots and no roots or FT treatment (data not presented).

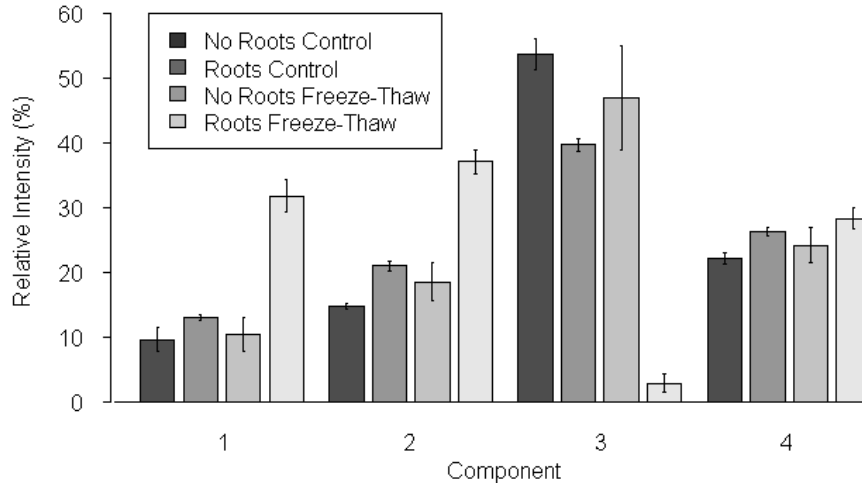


Fig. 3.5 Relative intensity of the four components from the EEM of extracted soil solutions for the controls and the Freeze- thaw to -5°C treatment in the presence and absence of roots. Values represent means \pm SEM ($n = 6$).

3.4. Discussion

The results presented above suggest that freeze-thaw of roots to -3°C and -5°C caused an increase in the concentration of the LMW DOC compounds, glucose and total amino acids. Such an increase was not clearly observed in the total DOC, although an increase at -5°C was observed for treatments with and without roots. Glucose and amino acids only make up a very small proportion of the total DOC (glucose C is $< 0.08\%$ of total DOC). Whilst they are only a small proportion of the DOC, they are considered to be very important as microbial substrates and, potentially, for CO_2 emissions. These compounds are turned over quickly in the soil so may only support a brief increase in CO_2 emissions (Boddy et al. 2007). The increase in glucose and amino acid concentrations indicate that increases of other root DOC compounds, such as other sugars (e.g. sucrose, fructose) and organic acids, is likely after FT events. It is possible that the DOC concentrations measured here after FT may be an underestimate as some may have remained within the dead root cells and potentially high concentrations of DOC near the root may have been removed with the root.

The difference in concentrations between the FTC treatment with roots and the unfrozen control treatment with roots may not have been solely caused by damage to roots. Root uptake of glucose and amino acids could have occurred in the unfrozen rooted controls (Jones and Darrah 1996; 1994; 1992). At the uptake rates reported by Jones and Darrah (1994; 1996)

of 59 nmol glucose mg⁻¹ root DW day⁻¹ and around 240 nmol amino acid mg⁻¹ root DW day⁻¹ maize roots could take up 15.7 μmol glucose and 64 μmol amino acids in the 64 hours that the plants were incubated separately. This is more than the size of the difference between the control with roots and the -3°C and -5°C with roots treatments which were about 12 and 8 μmol of glucose and amino acids, respectively (the average dry weight of the maize roots was approximately 100 mg). However, the uptake rate was shown to be quite low at 5°C so the actual uptake in this period was likely much less (Jones and Darrah 1996). Decreased growth rate in the dark is also likely to decrease uptake rates as has been shown for nitrate and ammonium (Gessler et al. 2002; Ourry et al. 1996). Uptake of organic compounds might increase in the dark due to C limitation (Jones and Darrah 2002). However, it is unlikely that increased uptake is the sole cause of the difference between the rooted controls and rooted FTC samples (at -3°C and -5°C). Plants would rapidly recapture glucose and amino acids so high concentrations probably would not accumulate without increased input.

Freezing at -0.1°C showed no increase in any of the measured compounds. This temperature may not have caused the sample to freeze. Therefore, no soil disruption could occur and no ice crystals could form within the roots. This result suggests that mild frosts would not affect soil solution DOC. Soil, and overlying snow and vegetation can insulate roots from freezing air temperatures so it would probably take a relatively prolonged frost period to cause the soil to reach -3°C even just below the surface (Oliva et al. 2014; Semenchuk et al. 2013). However, freezing air temperatures could cause ice to form in plant foliage and kill the plant which could then be a source of both soluble and insoluble carbon for the soil (Kocsy et al. 2011). Loss of the shoots may also ultimately lead to death of the roots. This study does not take into account potential increases in DOC or carbon output due to increased root senescence and turnover caused by FTC. This could be substantial and have longer lasting effect on C emissions than the LMW DOC generated immediately by FTC (Gaul et al. 2008; Tierney et al. 2001; Wu et al. 2010).

Root exudates can add a number of carbon compounds to the soil, particularly sugars, organic acids and amino acids (Jones et al. 2009). I feel this is one of the most likely causes for the increase in both glucose and amino acid solution concentrations in the sterile sand controls with roots compared to the controls without roots. Plants also reduced the moisture content of the sterile sand due to water uptake which was observable as up to a third less solution was extracted by centrifugal drainage. This would also have a small solute concentrating effect and could account for the high total DOC concentration in the control with roots compared to

the no root control, but not for the greater glucose and amino acid concentrations. Removal of plants from the soil could have caused some root damage and released some compounds. Less amino acids than glucose were produced by the roots, but the relative increase caused by FT was much larger for amino acids. Root exudation is thought to occur by passive diffusion and is thought to depend on the concentration gradient across the root-soil interface. Roots contain a greater concentration of glucose than amino acids which explains the higher concentration, but not the greater increase in amino acids due to FT (Walter et al. 2003). It has been shown for amino acids that relative concentrations differ between root extracts and root exudates (Lesuffleur et al. 2007; Paynel et al. 2001). A potential reason for this could be the position within the root where concentration is highest. Exudation occurs mostly at the root tip and from the outermost part of the cell (i.e. across the cytoplasm/apoplast boundary). If the ratio of amino acid concentration occurring in the vacuole rather than the cytoplasm or at the base of the root rather than the tip is greater than glucose this could explain the observed difference as FT damage is unlikely to have the same spatial distribution as exudation. FT could also damage the maize seed which could contain more amino acids than glucose (He and Burris 1992; Pettibone and Kennedy 1916). Plants are more efficient at amino acid uptake than at glucose uptake. Whilst recapture of glucose exudates is 80-90% efficient, previous studies suggest that amino acid recapture is greater than 99.9% efficient in sterile conditions (Jones and Darrah 1993; 1994; Krafczyk et al. 1984). This would mean the increase in amino acids after FT could be greater than glucose as amino acid concentrations in the soil solution would be kept lower in the control.

Nutrient fluxes relating to vegetation have generally been shown to correlate well with their concentration within the plant (Elliott 2013). The organic N content in plant roots can increase when in the dark. Chilling maize has been shown to increase the amino acid concentration in its leaves (Kocsy et al. 2011; Szalai et al. 1997). The leaves were present during FTC treatment and so could potentially be a source of amino acids for the soil solution if they leached into the soil. If the amino acid concentration also increased in the roots this could perhaps contribute to the greater increase in amino acids than glucose observed on FT. However, plants can accumulate sugars, particularly sucrose, when chilled (Pastorczyk et al. 2014). Glucose has been observed to increase in concentration in some plants due to frost hardening (Angelcheva et al. 2014; Astakhova et al. 2014).

Plants acclimatising to freezing change the concentration of certain metabolites, but do not create new ones (Angelcheva et al. 2014). This may explain why the MALDI-TOF analysis

did not find any difference in the compounds between the rooted control and the rooted FT samples. However, MALDI-TOF only identifies compounds which can be ionised so might not have found new compounds if they do not ionise readily in DHB, like very acidic oligomers (Ohara et al. 2009). The EEM results show that the roots have relatively more of components 1 and 2 which are likely to be composed of plant derived humic/fulvic material (Coble 1996; Cory and McKnight 2005). The high absorbance at UV wavelengths from FT released root DOC compounds could have quenched the fluorescence of component 3. Components 3 and 4 are in the fulvic acid region. They must either be from the Long Ashton solution, be whatever compounds were bound to the sand or could be noise caused by low absorbance.

Significant increases in DOC and LMW DOC components were observed at less extreme temperatures than have been observed in bulk soil experiments (Elliot and Henry 2009; Hentschel et al. 2008). Roots are likely to be more susceptible to FTC damage than bacteria as they could be damaged by the physical disruption to soil. This has been observed in filamentous fungi which are more affected by FTC than bacteria, potentially due to damage to hyphae (Feng et al. 2007). Such physical disruption could occur at warmer temperatures as ice formation is more likely in relatively large soil pores between soil aggregates than within cells. Ice crystals are less likely to grow in concentrated cytoplasmic solutions which lack ice crystal nuclei and organisms have some ability to prevent the growth of large, damaging ice crystals. However, Cleavitt et al. (2008) found that the tensile strength of fine roots of hardened sugar maple was not affected by displacement, representing ice formation, and that frost heave was not an important factor in root damage. Ice can form in extracellular spaces and in xylem tissue, potentially propagating rapidly through the plant. This can dehydrate cells which can damage the membrane and release DOC on thaw (Pearce 2001). As root cells are larger than microbial cells, they maybe more prone to forming intracellular ice crystals due the presence of more ice nuclei or less efficient dehydration (Prickett et al. 2015). Intracellular ice could damage the cell so root cells could be a source of DOC at warmer freezing temperatures than bacteria.

Maize has thick roots and is not frost hardy (Kocsy et al. 2011). Therefore, these results could be an overestimate of how much DOC can be produced by FTC damage to plant roots in Arctic tundra. Hardier plants could produce different results as some plants can survive freezing to -30°C and have adapted to survive dehydration and can control where ice formation occurs (Pearce 2001). This experiment was performed on 2 week-old seedlings,

more established plants with tougher (suberized) roots might produce less DOC. It has also been shown that actively growing plants produce more DOC than dead or dormant ones (Elliot 2013; Melick and Seppelt 1992). Plants need time to adjust to cold temperatures (Klimov et al. 2008). This experiment could be improved by growing cold tolerant plants at a chilled temperature prior to freezing to give them a chance to acclimatise.

3.5. Conclusions

Plants roots were shown to be a source of glucose and a larger source of amino acids after 1 FTC. However, the results for total DOC concentration did not show a significant increase when roots were subjected to FTC. This emphasises the importance of studying the DOC in more detail than just measuring the total concentration. A greater increase in amino acids than glucose was observed due to the FT of roots. This is partly attributed to more efficient recapture of amino acids than glucose in the control and potentially to the accumulation of amino acids during freezing and to the spatial distribution of the compound within the root. Roots also could be a source of DOC at milder freezing temperatures than soil microbes, but further work needs to be done on frost hardy plants. These results show that plants should be included in FT experiments of soil so as not to underestimate carbon fluxes.

Chapter 4

Composition and Concentration of Dissolved Organic Carbon Produced by Repeated Freeze-Thaw Cycles in Arctic Tundra Soils

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4. Composition and Concentration of Dissolved Organic Carbon Produced by Repeated Freeze-Thaw Cycles in Arctic Tundra Soils

Abstract

Freeze-thaw cycles (FTC) can affect the soil's dissolved organic carbon (DOC) concentration to differing extents depending upon a range of soil physicochemical and biological factors and the nature of the freeze-thaw event. This soluble carbon pool is composed of many compounds some of which are rapidly used by microbes and are potentially important for CO₂ emissions. In this study we examined the FTC-induced changes to the DOC pool in detail, paying particular attention to low molecular weight (MW) DOC, its composition and the concentration of some compounds which have been shown to be rapidly used by soil microbes. The results showed that Meadow tundra soil solutions showed pronounced changes to both DOC composition and concentration due to FTC. These changes consisted of increased DOC <1000 MW, glucose and amino acid concentrations and the appearance of new compounds in soil solution. Both microbes and vegetation could be sources of these compounds. Heath tundra soil solutions showed some changes to excitation emission matrix (EEM) component composition, but no other significant effect of FTC on the variables measured here. No elevated DOC concentrations were observed after more than two repeated FTC, although changes in EEM component composition were not clearly affected by cycle number. This suggests that an increase in the number of FTC that the arctic will potentially experience will not overly affect the LMW DOC pool.

4.1. Introduction

Dissolved organic carbon (DOC) in soil solution is a dynamic mixture of many compound types and it is arguably the most microbially-available pool of soil carbon (C) (Marschner and Kalbitz 2003). Certain compounds such as glucose, amino acids and small peptides can be taken up almost immediately from solution by soil microorganisms (Hill et al. 2008; 2012). It has been shown that compounds such as these are major contributors to soil respiration (Fujii et al. 2010). However, larger components of DOC (>650 MW) need to be broken down prior to utilisation by prokaryotes, giving them a much longer turnover time (Payne and Smith 1984; van Hees et al. 2005). Kiikkilä et al. (2014) have shown that the

composition of DOC can affect bacterial growth. For some compounds, such as glucosamine, uptake is affected by the presence of other organic C compounds in solution, such as glucose (Roberts and Jones 2012). Therefore, both changes to the concentration and composition of the DOC pool are important when considering its dynamics and events with the potential to influence DOC composition, such as freeze-thaw cycles, require further study.

A number of studies have examined the effects of freeze-thaw cycles (FTC) on the size of the DOC pool. These studies have produced contrasting results; some show FTC to increase DOC (Feng et al. 2007; Grogan et al. 2004; Hentschel et al. 2008; Vestgarden and Austnes 2009; Yu et al. 2011), some show no effect (Hentschel et al. 2008; Vestgarden and Austnes 2009) and in some a decrease is observed (Grogan et al. 2004). Some of this variability has been attributed to different freezing temperatures, the number of freeze-thaw cycles, different ecosystems and experimental differences such as the extraction time or method (Henry 2007; Grogan et al. 2004; Hentschel et al. 2008; Vestgarden and Austnes 2009). Increases in DOC have been attributed to damage and mortality of microbes and roots, disturbance to soil structure and microbial release of compounds due to changes in osmotic conditions (Hermann and Witter 2002; Tierney et al. 2001; Schimel et al. 2007). Decreases could be caused by increased microbial activity, or from sorption to freshly exposed mineral surfaces (Özgül et al. 2012; Sehy et al. 2004). The intensity of the FTC have also been shown to be an important factor in the effect of FTC on DOC with colder freezing temperatures producing more DOC (Hentschel et al. 2008). Fewer studies have examined the composition of DOC after FTC. These studies have been mostly limited to aromaticity and also show contrasting results (Austnes and Vestgarden 2008; Haei et al. 2012; Hentschel et al. 2008).

Here, the effect of repeated FTC on the DOC composition of arctic tundra soil was examined, both qualitatively and quantitatively. Arctic soils were selected as they are particularly vulnerable to climate change which could change the number of FTC they experience (Henry 2008). The following hypotheses were tested: (1) Freeze thaw increase the LMW-DOC concentration in arctic soils, and (2) this effect decreases with successive FTC. Excitation-Emission Spectroscopy Matrix (EEM), $SUVA_{255}$ and MALDI-TOF were used to qualitatively describe the DOC composition whilst the concentration of LMW-DOC, glucose and amino acids were measured after 1, 2, 3, 4 and 8 FTC. Changes in the composition of plant-extracted DOC and fumigation-extracted soil DOC were also tested to discover the potential source of DOC released by FTC.

4.2. Materials and methods

4.2.1. Field sites

Two sites near Ny-Ålesund, Svalbard were selected for soil sampling. These represent different arctic tundra environments. Westbyelva (78°55.4 'N; 11°54.4'E) is a meadow tundra, vegetated predominately with lichen, *Salix polaris* (Polar Willow), moss and *Ptilidium ciliare* (Liverwort). Kolhaugen (78°55.224'N; 11°52.439'E) is an example of heath tundra and is sparsely vegetated mostly with *Dryas octopetala* (Mountain Avens) and *Saxifraga oppositifolia* (Purple Saxifrage). Vegetation (Table 4.1) was analysed using 10 randomly placed 1 m² quadrats and identifying the vegetation every 10 cm. Both are Cryosols consisting of a shallow organic layer overlying a mineral soil. The organic layer varied in thickness being about 2-10cm deep at the Meadow site, but being often absent at the Heath site

Soil characteristics (summarised in table 4.2) were analysed from randomly sampled 5x5 cm core samples as follows: pH was measured in 1:2.5 (v/v soil:water) mixture. Soil microbial biomass C (MBC) was measured by fumigating 10g (FW) of soil with chloroform for 1 day. Along with non-fumigated soil samples the DOC was extracted by shaking in deionised water (25 ml). The total DOC in the non-fumigated soil was subtracted from that in the fumigated soil and the result was used to represent the MBC as described by Vance et al. (1997). DOC and total dissolved N (TDN) were measured on a TOC-V-TN analyser (Shimadzu Corp., Kyoto, Japan). DON was calculated by removing of NO₃⁻ and NH₄⁺ concentrations, measured according to Miranda et al. (2001) and Mulvaney (1996) respectively, from the TDN value. The soil C and N proportions were measured from soil dried at 105°C and sieved to < 2mm using a Carlo Erba NA 1500 Elemental Analyzer (Thermo Fisher Scientific, Milan, Italy).

Site	Bare earth	<i>Bistorta vivipara</i>	<i>Dryas octopetala</i>	Lichen	Moss	<i>Saxifraga oppositifolia</i>	<i>Salix polaris</i>	Other
Meadow	2.6	0.0	0.0	44.1	7.9	4.4	38.1	2.3
Heath	54.0	3.7	18.9	2.8	2.2	8.0	6.6	3.9

Table 4.1. Vegetation Cover at the experimental site where the soil was collected. Values equal average percentage cover ($n = 9$). Liverwort was included within the moss category.

Soil type	Depth (approx. cm)	pH	Total C (%)	Total N (%)	DOC mg/kg	DON mg/kg	MBC mg C/kg
Meadow Organic soil	0-10	6.40 ± 0.16	24.0 ± 1.4	1.77 ± 0.03	36.6 ± 5.1	6.94 ± 1.34	815 ± 25
Meadow Mineral soil	>2	6.28 ± 0.12	7.9 ± 1.2	0.67 ± 0.09	23.9 ± 2.3	4.18 ± 0.67	293 ± 58
Heath Organic soil	0-5	7.21 ± 0.02	19.7 ± 1.9	1.41 ± 0.12	43.5 ± 3.5	9.38 ± 2.71	856 ± 56
Heath Mineral soil	>0	7.39 ± 0.10	5.5 ± 1.0	0.38 ± 0.09	23.2 ± 1.2	4.39 ± 0.59	293 ± 50

Table 4.2. Properties of the soil used in the experiments including microbial biomass carbon (MBC) and the depths in the field at which they can be found, note that the organic layer varies in thickness. Values correspond to mean ($n = 4$) ± SEM

4.2.2. Soil and vegetation sampling

Soil samples were collected in mid July 2012 and early July 2013. Intact core samples of the top 5 cm were collected by driving steel rings (5cm diameter) in to the soil. Sampling location was selected randomly by throwing the ring and sampling where it landed, relocating if that area was too rocky or vegetation too woody to sample. Samples were transported to Bangor University and stored at 5°C in oxygen permeable polypropylene bags. Treatment began within 5 days of collection. *Salix polaris* leaves and *Ptilidium ciliare* were sampled from Westbyelva in late July 2014 and were stored for 1 day at 5°C prior to treatment. No vegetation samples were taken from Kolhaugen as cores were taken from areas of mostly bare earth.

4.2.3. Sample treatment

Intact soil cores were placed in individual, sealed O₂ permeable polypropylene bags and kept at either 5°C as a control or subjected to (1-8) freeze-thaw cycles. Samples ($n = 3 - 6$) were arranged in a randomised block design to counteract any potential temperature gradients in the incubator. A freeze-thaw cycle consisted of freezing to -7.5°C at a rate of 1°C hour⁻¹ and remaining at that temperature for 46 hours. Soils were then thawed at the same rate and kept at 5°C for 46 hours. These temperatures are representative of winter soil temperatures at the arctic sites (see chapter 6 for further discussion). Samples of each plant were frozen for 39 hours at -20°C or kept as a control at 5°C in an attempt to characterise DOC composition from arctic foliage and see if any changes were produced by FT. A -20°C is representative of winter air temperatures in Ny-Ålesund which plants with little snow cover might be subjected to.

4.2.4. Soil solution extraction

Soil solutions were extracted by centrifugal-drainage (Giesler and Lundström 1993). The soil solution was extracted 6 hours after thawing in the 1st, 2nd, 3rd, 4th and 8th cycle. At this time soils were completely thawed and had reached a temperature of 5°C. This was tested prior to the experiment by placing temperature probes in 120 g of soil (30% moisture content DW). MilliQ water was added to the plant samples (20:1 w/w) and vortexed for 1 minute, 2 ml were removed. The liquid was retained. Solutions were centrifuged at 8228 g to remove particulate organic material and stored frozen at -20°C to await further analysis.

4.2.5. Soil solution size fractionation

A sub-sample of the soil solutions and fumigated soil solutions (section 4.2.1) was ultrafiltered through a 1000 MW filter. This fraction was considered to consist of low MW organic material. High MW organic material was considered to be equal to the difference between total concentration and low MW concentration.

4.2.6. Determination of organic and inorganic C and N concentrations in solution

Total DOC and dissolved N of each fraction were measured by multi N/C 2100S (Analytic Jena AG, Jena, Germany). Inorganic N was measured using the VCl₃ method for NO₃ and the sodium salicylate method for NH₄ according to Miranda et al. (2001) and Mulvaney (1996), respectively. DON was determined by subtracting the sum of the inorganic N concentrations from the total dissolved N concentration. Amino acids were measured by fluorescence according to Jones et al. (2002). Glucose was measured using an Amplex® Red Glucose/Glucose Oxidase Assay Kit (Invitrogen™) according to the manufacturer's instructions.

4.2.7. SUVA₂₅₅ and EEM

Specific ultraviolet absorption (SUVA₂₅₅) allows quantification of the aromaticity of the soil solution and the excitation emission matrix (EEM) helps characterise fluorescent components providing insight in to the source of some DOC compounds. Aliquots of each ultrafiltered soil solution, fumigation extract and vegetation extract (300 µl) were pipetted in to 96 well plates (UV transparent and white). For SUVA₂₅₅, unfiltered samples from the first FTC were also analysed. The UV absorption was measured in 5 nm intervals between 200 nm and 550 nm on a Biotek PowerWave XS.

SUVA₂₅₅ was calculated for total and ultrafiltered samples using the following equation:

$$\text{SUVA}_{255} = \frac{A_{255}}{([\text{DOC}] \times \text{path length})}$$

where A_{255} is the UV absorbance at 255 nm, [DOC] is the DOC concentration and path length is in metres.

Excitation-Emission matrices were measured between 200 nm and 400 nm excitation wavelengths and between 270 nm and 550 nm emission wavelengths using a Cary Eclipse Fluorescence Spectrophotometer. Corrections for inner-filter, Raleigh and Raman effects and changes in lamp intensity were carried out as described in chapter 3. The intensity of component 3 in the control *Salix polaris* extracts was extremely high. To enable a visual comparison between the vegetation extracts the intensities were converted into the natural logarithms of their QSU so the intensity of the vegetation EEM is on a logarithmic scale. As in chapter 3 the EEM components were found using PARAFAC and verified using CORCOND (Andersson and Bro 2000; Bro and Kiers 2003). Then relative intensity of each component was found compared to the total intensity of the components

4.2.8. MALDI-TOF

Ultrafiltered soil solutions (10 µl) were mixed with 10 g l⁻¹ 2,5-dihydroxybenzoic acid (DHB) in a water matrix and analysed by MALDI-TOF in positive ion, reflector mode using the Flexcontrol program. The resulting mass-to-charge ratios were calibrated against a solution containing sucrose, glucose and inulin. The significance and the degree of similarity between the soil solutions from the soils subjected to FT, fumigated soil solutions and plant extracts were analysed by ANOSIM and NMDS using Bray Curtis distance in PRIMER 6 and R.

4.2.9. Statistical analysis

Outliers within each soil type were identified using Grubbs test in R. Differences between sites, treatment and cycle number on soil solution concentrations, SUVA₂₅₅, component and relative component intensities were analysed using weighted least squares ANOVA in SPSS (version 22) and differences were located using TUKEY's post hoc test for sample sets that include outliers and for those where they were removed. Where removal of outliers changed the result both are reported. Where no change was observed the result from the total set was reported. Significant difference was assumed where $p < 0.05$. The controls for 2012 and 2013 tended to have different compound concentrations in extracted solution. It was considered

that this variability obscured treatment trends so the 2012 results were not included in the analysis. This led to some unequal sample sizes ($n = 3-6$) as cycles 1 and 3 were not analysed in 2012. Unequal sample sizes can lead to misleading results in factorial ANOVA, but as only one factor was affected (cycle) no correlation between factors due to sample size existed. Therefore, the results of type 3 ANOVA should be reliable.

4.3. Results

4.3.1. Freeze-thaw changes LMW-DOC composition

Results showed that $SUVA_{255}$ was significantly affected by site, size and FT treatment ($p < 0.001$, $p = 0.001$ and $p = 0.010$, respectively) (Fig. 4.1). There was significant interaction between site and size ($p = 0.001$), site and FT treatment ($p < 0.001$), but not between size and FT treatment. The $SUVA_{255}$ of the DOC significantly ($p < 0.001$) decreased by 1.33 due to 1 FT at the Meadow site and the LMW-DOC tended to have a lower $SUVA_{255}$ than HMW-DOC at the Meadow site ($p < 0.001$). No significant effects were observed at the Heath site. The LMW-DOC (Fig. 4.2) was significantly affected by site with the Heath site having a slightly lower $SUVA_{255}$ than the Meadow site ($p = 0.013$). There was a significant interaction between site, cycle and FT treatment ($p = 0.048$). In the meadow soil cycle there was a significant interaction between cycle and treatment ($p = 0.033$). This could be due to the large $SUVA_{255}$ reported after 3 FTC (2.93). This value is not significantly greater than any of the other values. After 1 FTC in the meadow soil the $SUVA_{255}$ is significantly lower than after a number of the controls ($p < 0.003$ for controls 2, 3 and 4), but not its individual control.

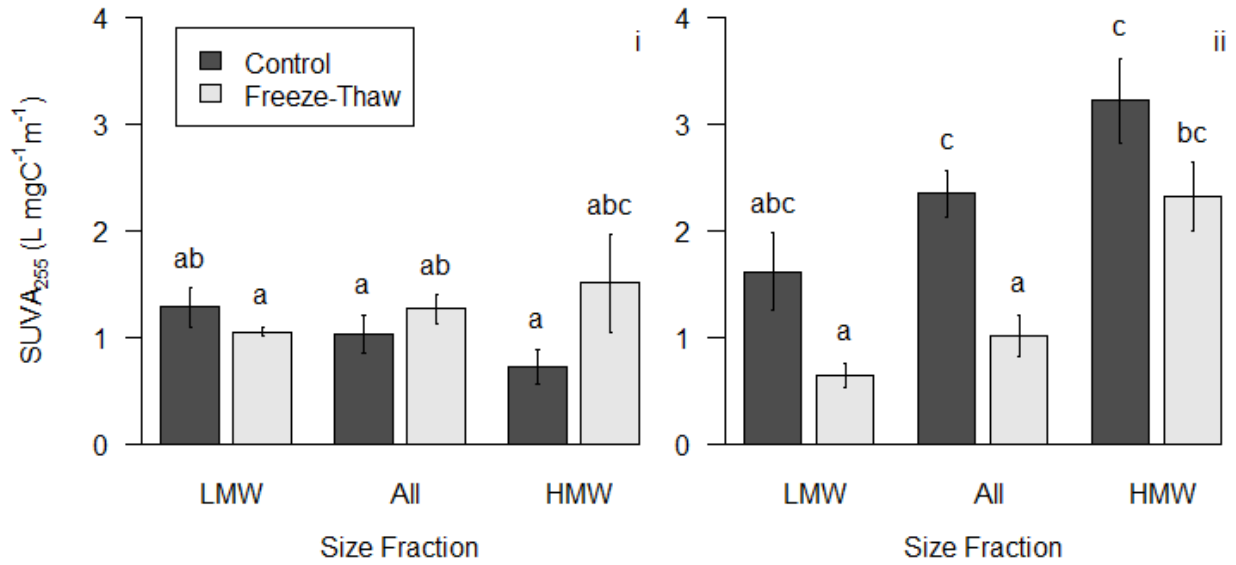


Fig. 4.1. SUVA₂₅₅ of fractionated soil solutions in (i) Heath or (ii) Meadow soils that were either kept as a control at 5°C or subjected to 1 freeze-thaw cycle to -7.5°C. Lower case a, b and c letters show significant difference between treatments size fractions and soil type.

Values equal mean, error bars show ± SEM (*n* = 4-5)

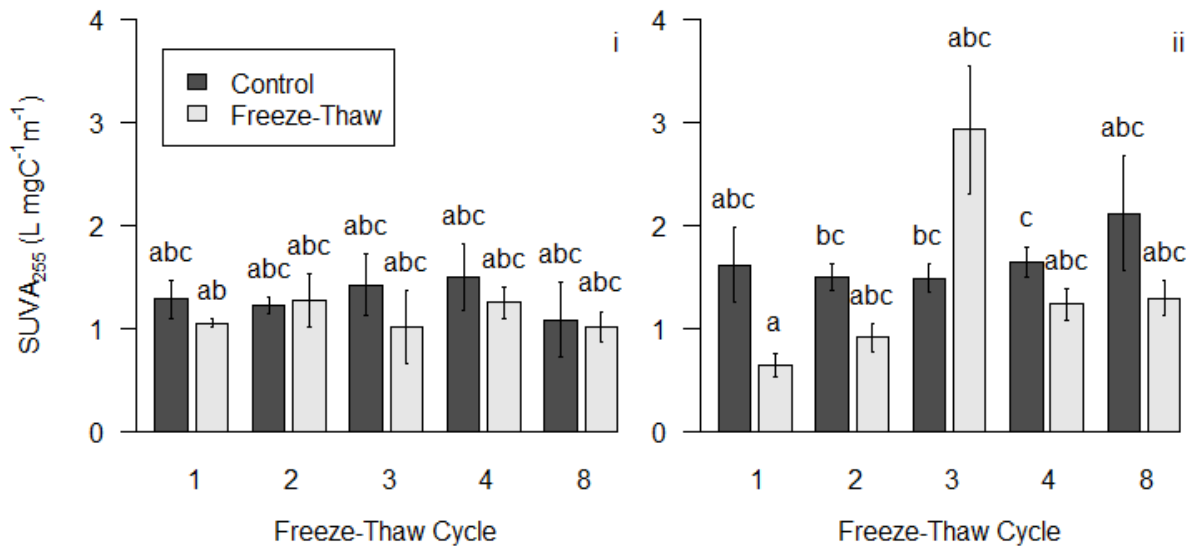


Fig. 4.2. SUVA₂₅₅ of LMW-DOC of soil solutions in (i) Heath or (ii) Meadow soils that were either kept at 5°C (Control) or subjected to 1-8 repeated freeze-thaw cycles to -7.5°C (FT). Lower case letters a, b and c show significant difference between treatments and soil type.

Values equal mean, error bars show ± SEM (*n* = 3-6)

The PARAFAC analysis of soil solutions identified 2 components (CORCOND = 100) in the EEM with the following excitation-emission peaks: component 1- excitation 300 nm, emission 431-437 nm; component 2 - excitation 320 nm, emission 457-462 nm (Fig 4.3 and 4). PARAFAC analysis of excitation emission matrix of DOC extracted from arctic plants identified 4 potential components (CORCOND = 77.4). The position of component 1 (excitation 300-310 nm, emission 435-445 nm) is similar to that of the soil solution analysis. Component 2 (260-360 nm/460-480 nm) is also similar to that found in the soil solution. The other component peaks identified here are component 3 (230-240 nm/440-460 nm) and component 4 (230-240 nm/425-430 nm) (Fig. 4.3 and 4.6). Whilst these peaks do appear in the EEM of soil solution they were not identified by PARAFAC analysis. Similar components were observed for fumigated soil solutions (Fig. 4.5).

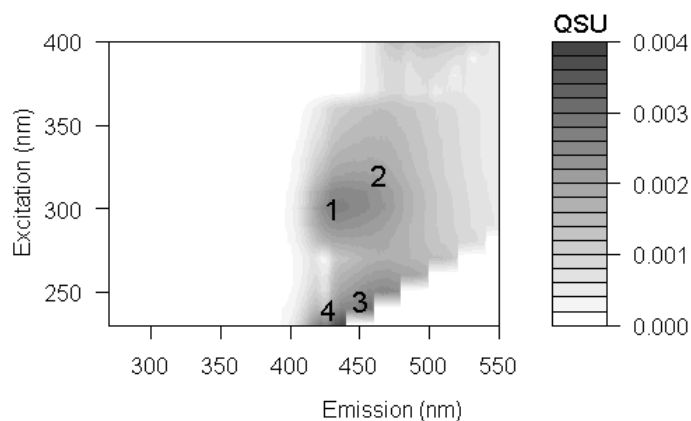


Fig. 4.3. Location of peak fluorescent components (indicated by numbers 1-4) identified by PARAFAC in plant extracts (all components) and soil solution (only components 1 and 2 were identified in soil solution). Illustration uses the average Meadow control soil solution with the position of components 3 and 4 from the plant extracts included for reference. EEM with intensities relative to the maximum quinine sulphate emission intensity (QSU).

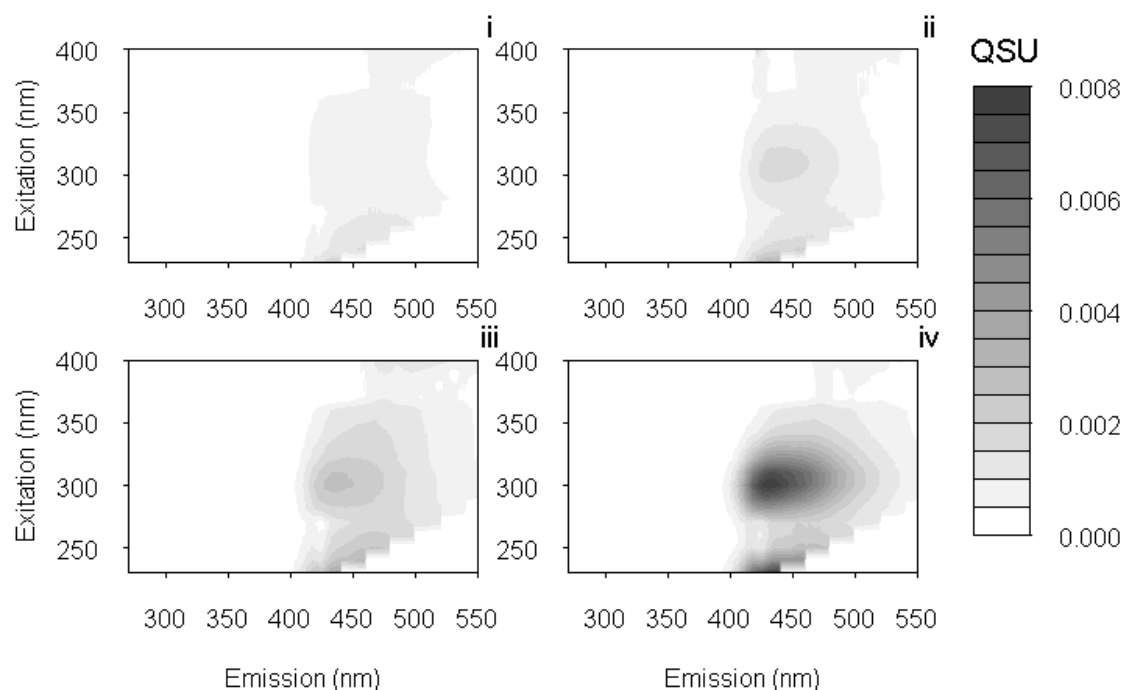


Fig. 4.4. Average Excitation-Emission Matrices for the soil solutions from; i) Heath Control, ii) Heath Freeze-Thaw, iii) Meadow Control and iv) Meadow Freeze-Thaw. Intensities are shown relative to maximum quinine sulphate intensity at 350 nm excitation.

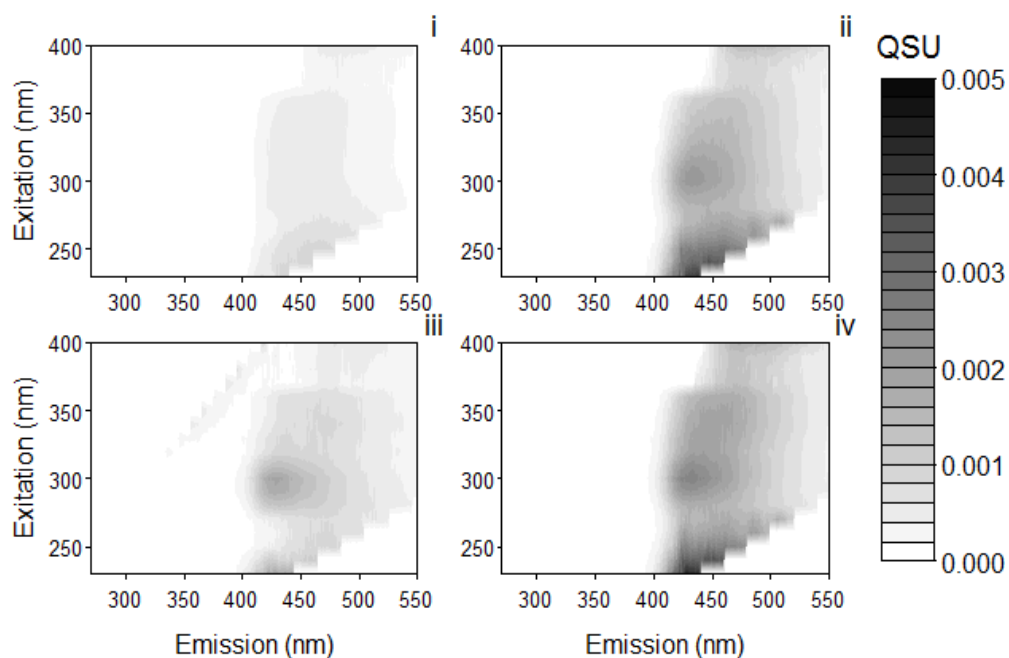


Fig. 4.5. Average Excitation Emission Matrices for; i) non fumigated Heath soil, ii) fumigated Heath soil, iii) non fumigated Meadow soil and iv) fumigated Meadow soil. Intensities are shown relative to maximum quinine sulphate intensity at 350 nm excitation.

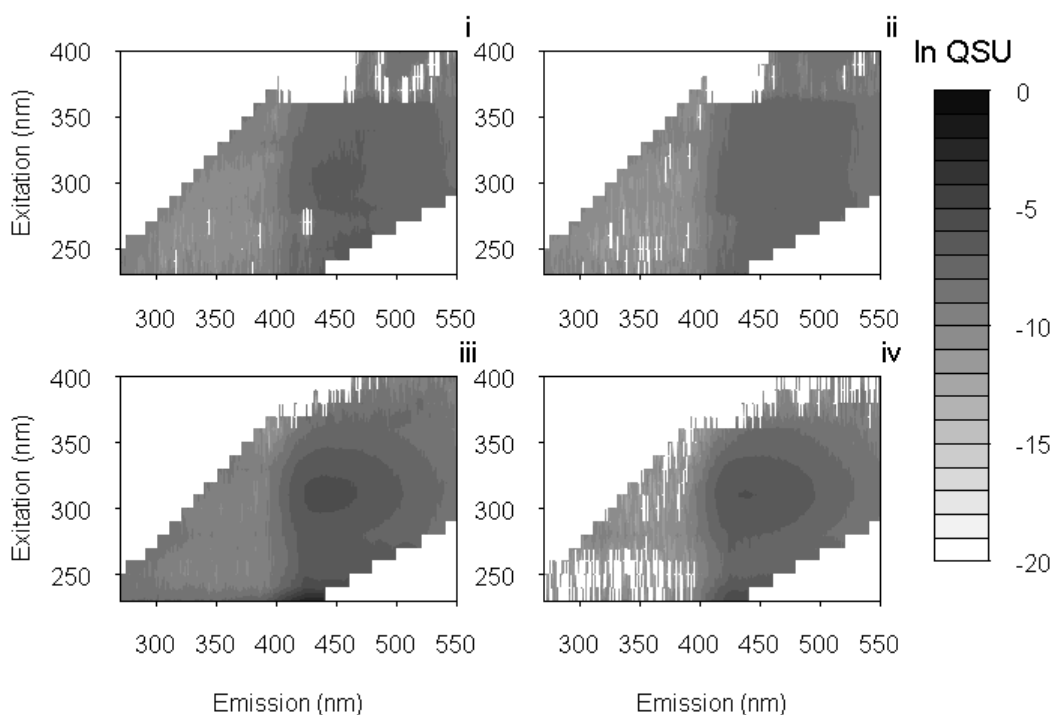


Fig. 4.6. Average Excitation Emission Matrices for plant extractable DOC from; i) *Ptilidium* Control, ii) *Ptilidium* Freeze-Thaw, iii) *Salix* Control and iv) *Salix* Freeze-Thaw. The natural logarithm of the intensities relative to maximum quinine sulphate intensity at 350 nm excitation are presented.

All 4 components were analysed for intensity and relative intensity. The intensity of all components increased significantly due to FT and were significantly greater at the meadow site ($p < 0.05$; Fig. 1). The EEM of the soil solution from the soil of both sites showed significant differences in component composition due to freeze-thaw. The relative intensity (Fig. 4.7) of component 1 increased significantly due to freeze-thaw ($p = 0.002$) whilst component 4 decreased ($p < 0.001$). No interaction was observed between treatment and soil type or between treatment and cycle. The proximity of component 1 to component 2 could have resulted in interference, with an increase in component 1 causing an apparent increase in intensity of component 2. No significant difference in intensity or relative intensity was observed between successive FTC at the heath site.

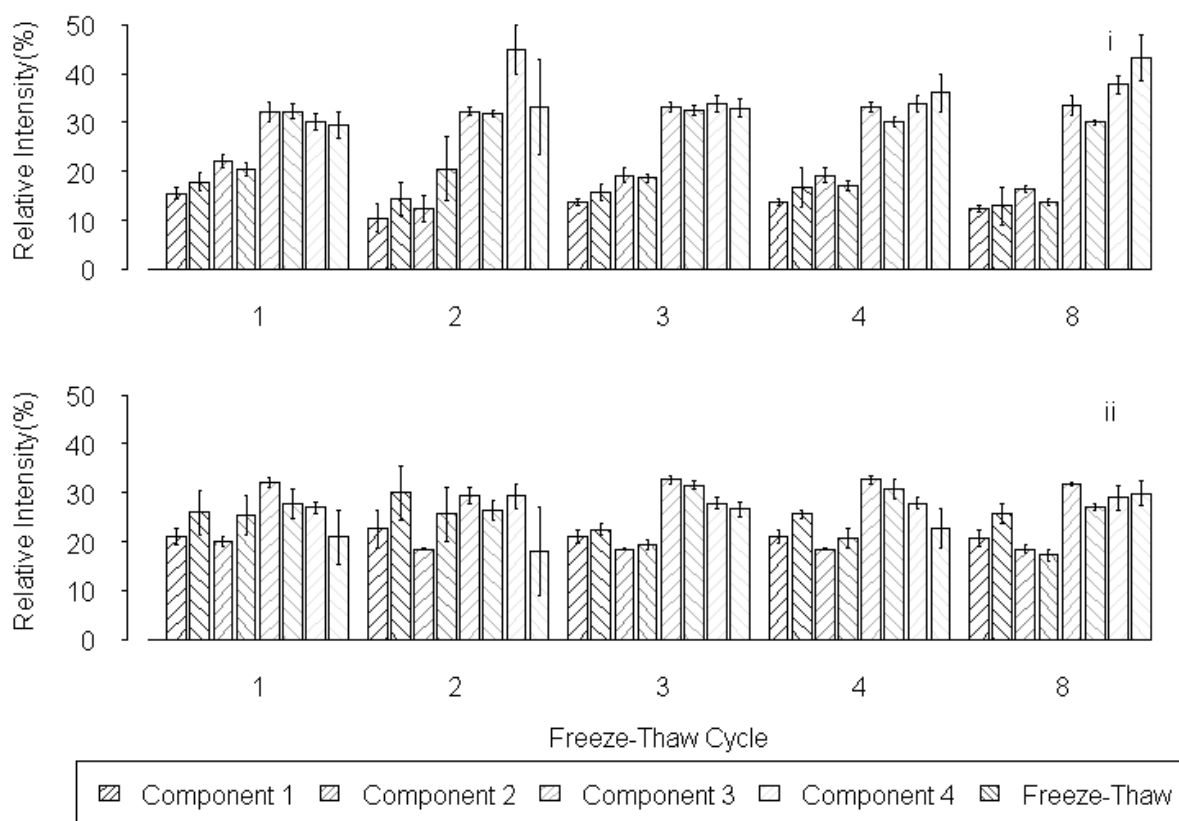


Fig. 4.7. Relative Intensity of components of the EEM from the soil solution from i) Heath soil and ii) Meadow soil that were either kept at 5°C (Control) or subjected to 1, 2, 3, 4 or 8 repeated freeze-thaw cycles to -7.5°C (FT). Treatment is identified by fill line angle with controls having lines that increase to the right and FT decreases to the right, whilst PARAFAC components are differentiated by fill line colour.

The MALDI-TOF results show certain LMW compounds in the FT solutions that are not present in the controls. This occurs for the meadow soil (Fig. 4.8), but not for the heath soil (data not presented).

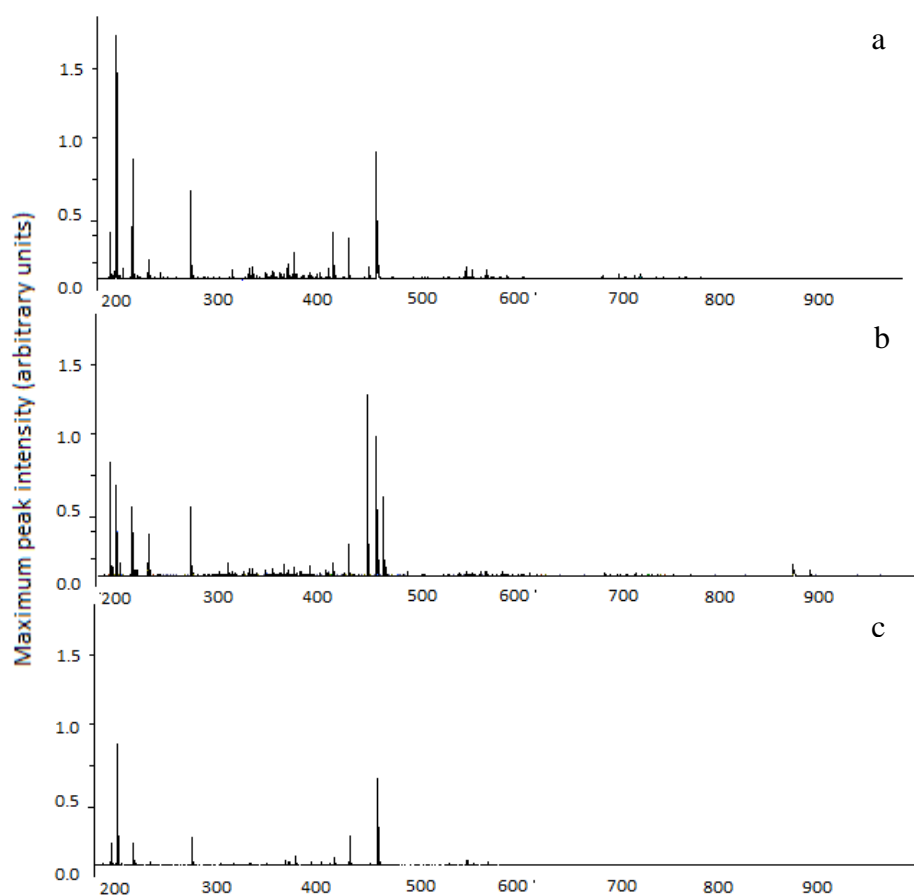


Fig. 4.8. m/z charts for the maximum MALDI-TOF results for a) the Meadow soil control samples (5°C), b) the meadow soil samples after one freeze-thaw cycle to -7.5°C and c) blank DHB samples ($n = 5$).

The m/z measurements likely represent the compound's mass plus that of either sodium or potassium. Compounds found in meadow soil solutions but not in the controls give the following m/z 203.2, 309.4, 447.5 and 463.4. 203.2 could be glucose or fructose plus a sodium ion. The m/z 203.1 was also identified in all the fumigated soil samples, most of the *Ptilidium* samples and 1 *Salix* sample. The m/z values of 463.4 and 447.5 could be 424.3 plus a potassium and a sodium ion, respectively. Or they could be a type of glucoside, a plant storage product for glucose combining a flavonoid with various sugar molecules (quercetin-3-O-glucoside and quercetin-3-O-rhaminoside, with molecular weights of 463.4 and 448.4 respectively) which have previously been found in the other *Salix* species (Mizuno et al. 1991), but this cannot be confirmed without more detailed analysis. A value of 463.8 was found in the *Salix* samples, which is close to the 463.4 value obtained from the FT arctic soil solutions and could be the same compound. This suggests that the vegetation could be the

source of this compound. Nothing was found near 309.4 in the fumigated or vegetation samples. So there is no clear source for this compound.

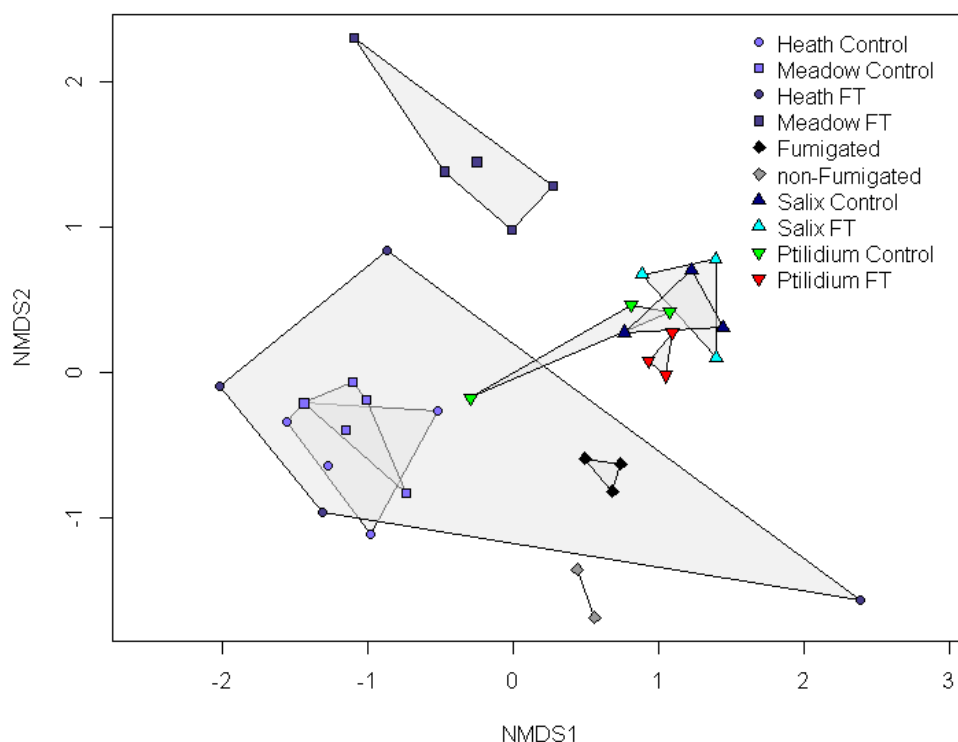


Fig. 4.9. NMDS showing ranked distances (Bray Curtis) in 2 dimensional space between MALDI-TOF results for the extracted soil solutions after 1 Freeze-thaw (FT) cycle or 1 control period (C); the extracted soil solutions from the arctic plants (*Salix* and *Ptilidium*); the extracted soil solutions from fumigated and non-fumigated Meadow soil (F and nF).

The NMDS of the MALDI-TOF (Fig. 4.9) results show that the Meadow FT samples have a different spectrum to the other soil samples. This is confirmed by ANOSIM results (Appendix A) which shows high and significant R values between the Meadow FT and the Heath and Meadow controls (0.89 and 0.896, $p = 0.008$ for both). R values close to 1 indicate separation whilst those close to 0 indicate that there is no clear separation between samples. Heath FT samples produced quite variable results and the R values were not significant. The vegetation samples cluster together and appear different to the soil samples (R values between 0.55-1 for soils not including Heath FT, $p < 0.05$) the same is true for the fumigated and non-fumigated samples (R values between 0.70-1, $p < 0.05$). These analyses were done at different times and the laser intensity may have been different. This could possibly ionise

more compounds or break up more compounds on one analysis than the other so it is difficult to compare between them.

4.3.2 Freeze-thaw changes to DOC concentration

A significant effect of site ($p = 0.021$) was observed on DOC concentration with the Meadow site having a $1.8 \times$ greater DOC concentration than the Heath site. Significant interaction was observed between FT treatment and number of FT cycles ($p < 0.001$). No significant interaction was observed between site and FT treatment, but one was observed between site, cycle number and FT treatment ($p = 0.021$). The Meadow soil showed a significant effect of FT treatment ($p = 0.011$) and significant interaction between treatment and cycle number ($p < 0.001$) whilst the Heath site did not ($p = 0.399$ and $p = 0.534$). The concentration of DOC was significantly higher after 1 and 2 FTC than in the control for the Meadow soil (3.7 and 1.6 times greater, respectively; $p < 0.006$), but later were not different to their controls ($p > 0.969$). The heath site did show slightly elevated DOC concentrations after 1 FTC, but this was not significantly greater than the control. When outliers were removed, an almost significant effect of FT treatment was observed at the heath site ($p = 0.067$) on DOC with FT tending to produce higher concentrations, but no difference was observed between individual FTC and controls. A similar effect of FT was observed on the LMW DOC concentration (Fig. 4.10). The HMW DOC concentration showed no FT treatment effect at either site ($p > 0.197$) nor was any interaction observed between treatment and cycle number (Fig. 4.10). Solution concentrations of glucose (Fig. 4.11) and amino acids (Fig. 4.12) showed significant interaction between soil type and FT treatment ($p = 0.045$ and $p = 0.027$ for glucose and amino acids, respectively) and between soil type, FT treatment and cycle number ($p = 0.009$ and $p = 0.001$ for glucose and amino acids, respectively). A significant increase in amino acids and glucose is observed after the first FTC in solution from the meadow soil (5.3 and 8.6 times, respectively; $p < 0.001$), but not the heath soil. The concentration of glucose after the second FTC in the meadow soil was significantly greater than in most of the controls for the Meadow soil and subsequent FTC ($p < 0.001$) except 8 FT by approximately a factor of 5. There was no significant difference between the control and freeze-thaw treatment in amino acid or glucose concentrations after 3, 4 or 8 FTCs.

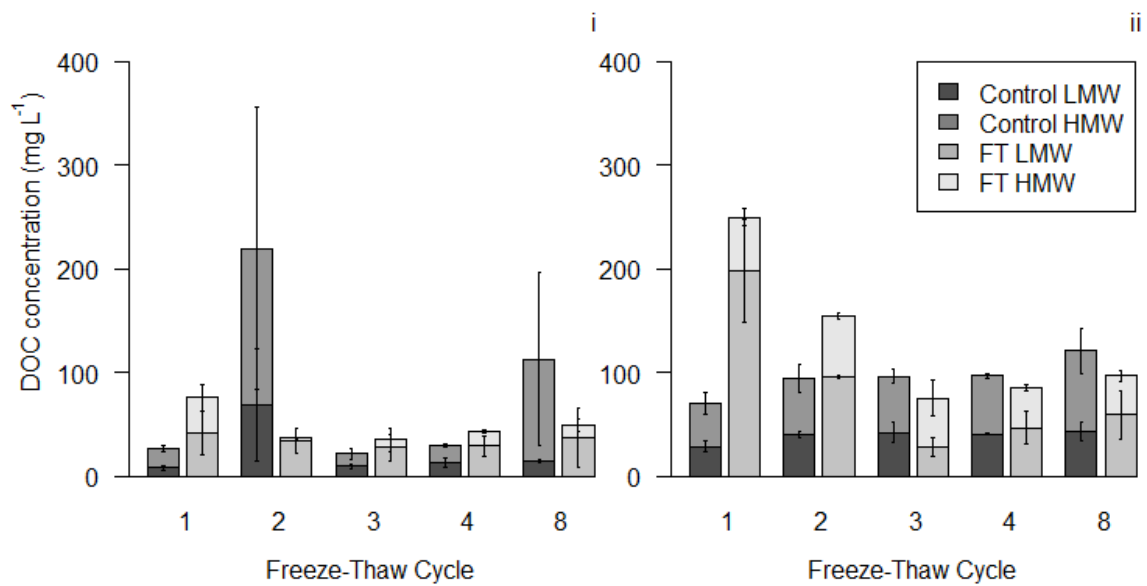


Fig. 4.10. Concentrations of LMW and HMW DOC in soil solution in (i) Heath or (ii) Meadow soils that were either kept at 5°C (Control) or subjected to freeze-thaw cycles to -7.5°C (FT). Values equal mean, error bars show ± SEM ($n = 3-6$).

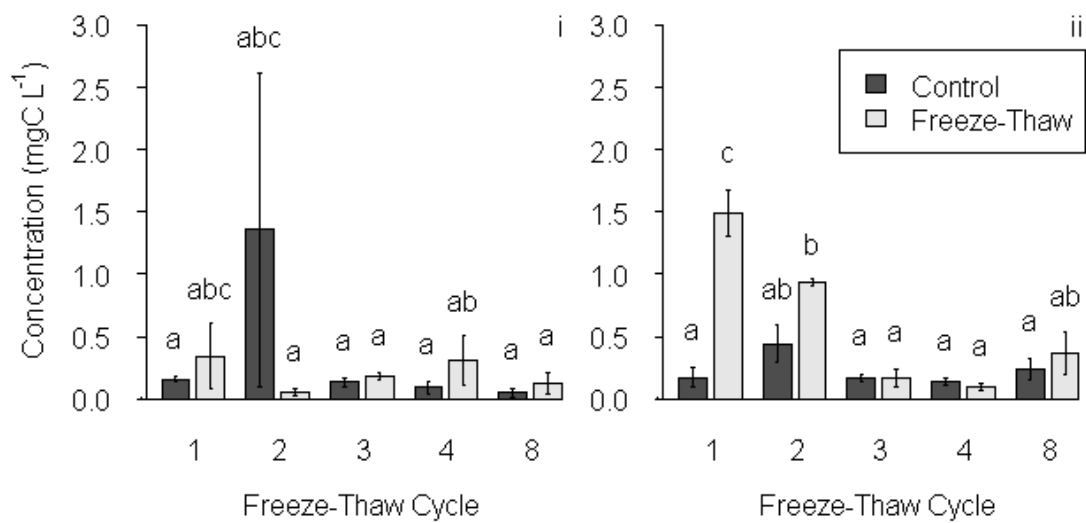


Fig. 4.11. Glucose concentrations in soil solution in (i) Heath or (ii) Meadow soils that were either kept at 5°C (Control) or subjected to freeze-thaw cycles to -7.5°C (FT). Lower case letters a, b and c show significant difference between treatments size fractions and soil type. Values equal mean, error bars show ± SEM ($n = 3-6$).

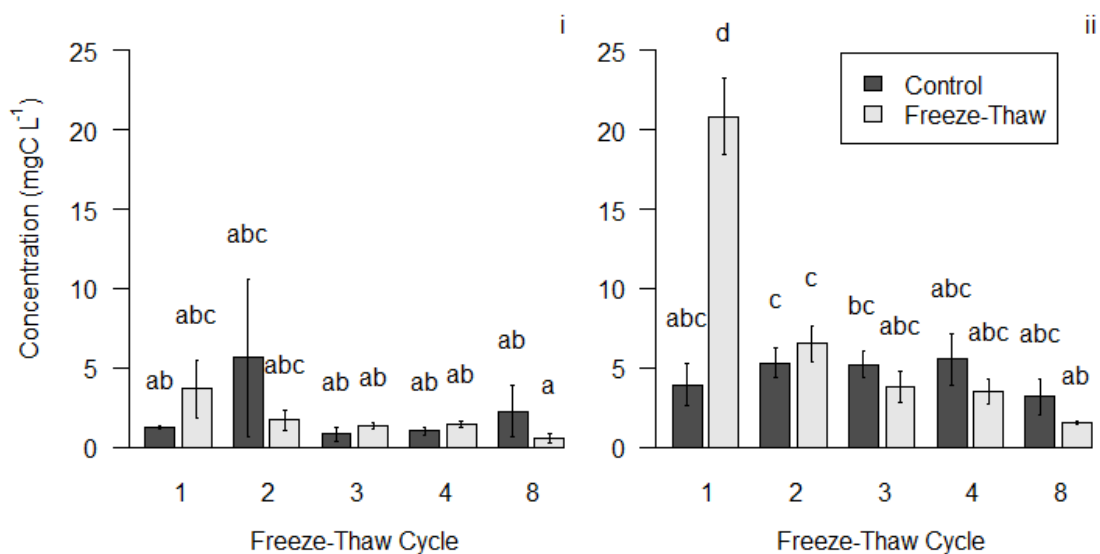


Fig. 4.12. Total amino acid concentrations in soil solution in (i) Heath or (ii) Meadow soils that were either kept at 5°C (Control) or subjected to repeated freeze-thaw cycles to -7.5°C (FT). Lower case letters a, b, c and d show significant difference between treatments size fractions and soil type. Values equal mean, error bars show \pm SEM ($n = 3-6$).

4.3.3. Freeze-thaw changes to N concentration

Due to a laboratory flood the second FTC extraction had to be redone. As the total dissolved N concentrations changed over time this result was excluded from the analysis. Freeze-thaw caused a significant increase in total DON after the first FTC at the Meadow site (+ 6.6 mg N l^{-1} ; $p = 0.031$), but no difference was observed with subsequent cycles (Fig. 4.13). A similar effect was observed at the Heath site but this was not significant. NH_4^+ concentrations were significantly ($p < 0.001$) greater at the Meadow site than the Heath site by a factor of 2.1 (Fig. 4.13). There was a significant interaction ($p = 0.037$) between FT treatment and cycle number with NH_4^+ tending to increase with 1 FTC but decrease later. However, no significant differences were observed between FTC and their respective control at either site. A significant increase ($p < 0.001$) in NO_3^- was observed over the course of the experiment in both the controls and FT treatments (Fig. 4.13). The Heath soil had a significantly greater NO_3^- content than the Meadow site (greater by a factor of 2.1; $p = 0.003$). FT treatment produced an effect that was almost significant ($p = 0.083$) with NO_3^- tending to be slightly greater in FTC samples. No significant interaction of cycle with FT treatment was observed for NO_3^- . Whilst NO_3^- values were generally low, occasional very high values were measured. Measurements of total dissolved nitrogen particularly by high temperature

catalytic oxidation is not 100% efficient and high inorganic N values can lead to high variability and negative results in calculated DON values (Vandenbruwane et al. 2007). This was observed particularly in the 1000 MW filtered samples where the ratio of inorganic to organic N increases.

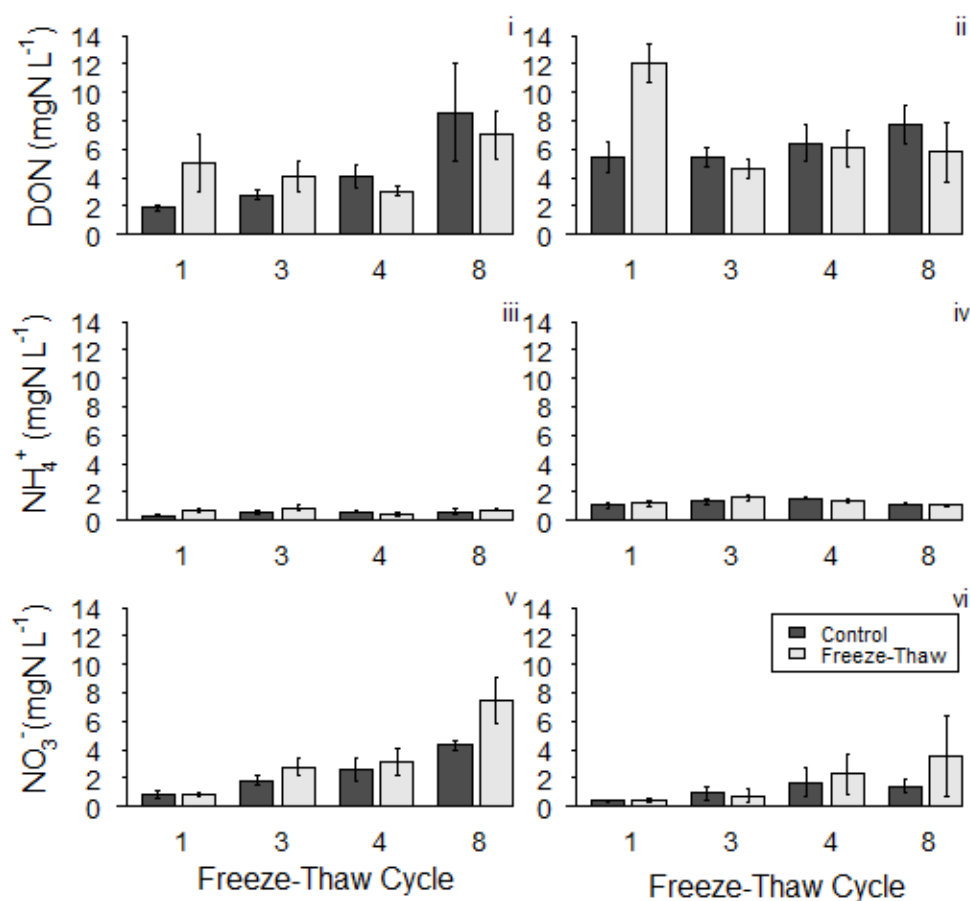


Fig. 4.13. DON (i & ii), Ammonium (iii & iv) and Nitrate (v & vi) concentrations in soil solution in Heath (i, iii, v) or Meadow (ii, iv, vi) soils that were either kept at 5°C (Control) or subjected to 1, 3, 4 or 8 freeze-thaw cycles to -7.5°C. Values equal mean, error bars show ± SEM ($n = 3-6$)

4.4. Discussion

4.4.1. Changes to DOC composition

The results presented here clearly show that freeze-thaw can affect DOC composition with changes to aromaticity, EEM components and the appearance of new compounds observed. However, the effect was not clear for all environments as the Heath soil solutions showed

changes only to EEM component composition, but no change in ionisable compounds or $SUVA_{255}$. The decrease in $SUVA_{255}$ in the meadow soil solution suggests that the initial FT cycle at the meadow site released aliphatic material making the DOC less aromatic. This aliphatic material was utilised rapidly as $SUVA_{255}$ values quickly returned to control levels. The results of LMW-DOC $SUVA_{255}$ for the Meadow soil were similar to those observed by Austnes and Vestgarden (2008) for $SUVA_{254}$ for relatively long FTC (125 hours at -5°C) in that a decrease in $SUVA_{254}$ was initially observed, but was short lived. Haei et al. (2012) also found freezing decreased $SUVA_{254}$ and that this was more apparent at greater frost intensities, but they found no effect of repeated FTC. No significant increase in $SUVA_{255}$ due to FT was observed in this experiment which is counter to Hentschel et al. (2008) and to the results of Austnes and Vestgarden (2008) after 5 long FTC and 14 days after thawing of soil frozen at -5°C for 56 days. These latter experiments simulated soil leaching which could produce different results than those presented here as DOC changes with depth in the soil column. Whilst the $SUVA_{255}$ used here may give slightly lower values than $SUVA_{254}$, the trends should be the same.

The LMW-DOC had a lower $SUVA_{255}$ than the HMW-DOC in the Meadow site suggesting that the HMW-DOC was more aromatic. This is different to that reported by Iliina et al. (2014), but they measured the $SUVA_{254}$ of DOC from river water which possibly has less LMW-DOC from microbial products and would therefore be more aromatic. As no significant interaction between MW and FT treatment was observed in $SUVA_{255}$ at the Meadow site this suggests that even the HMW-DOC got less aromatic after 1 FTC. Thus despite no significant change in HMW-DOC concentration due to freeze-thaw, dynamic change to this pool may have occurred. Potentially, enhanced breakdown of aromatic material (although the breakdown products would have to still remain in the $> 1\text{kDa}$ DOC fraction) or an input of aliphatic material and a removal of aromatic material through degradation or sorption to surfaces made available by physical disruption to soil may have occurred. The Heath site had a lower $SUVA_{255}$ than the Meadow site which would be consistent with the degradation of microbial material which is observed in mineral soils where there is an absence of fresh plant material (Sanderman et al. 2008). It is possible that if the main source of DOC is microbial, fresh input due to FT would not change the aromaticity and would require a more detailed study of DOC composition to identify.

The EEM results indicated changes to LMW-DOC composition occurred in soils from both sites after FTC. Components 1 and 2 identified by EEM are in the humic acid-like area of the

spectrum (Chen et al. 2003). Components 3 and 4 could be from the same compounds being excited by the UV spectrum or they could be representing Fulvic-acid like compounds (Chen et al. 2003; Coble 1996). Surprisingly, only in a few isolated samples were peaks observed in the protein-like area even after FT despite a clear increase in amino acid concentration. Fumigated soil samples did not show any peaks in the protein-like areas either. There is the potential that proteins precipitated out of solution when the extracted solutions were frozen for storage (Spencer et al. 2007). Peaks representing protein like components may have existed at excitation wavelengths below 230 nm, but there was a lot of analytical or experimental noise in this area so the results were not included in the analysis. The observed components are similar to those observed in soil extracts by Guigue et al. (2014). They have been suggested to be lignin degradation products although, as they were also found in the spectra of the fresh *Salix* vegetation, a portion of the signal is likely fresh, rather than degraded, plant-derived DOC. Component 1 has also been linked with marine/microbial humic material and plant leaves suggesting that the observed increase in this component is due to cellular damage (Coble 1996; Cuss and Guéguen 2013). The *Salix* leaves produced a broader emission peak (Fig. 4.6 iii and iv) for component 1 than the fumigated soil (Fig. 4.5) which is similar to the results of Cory and McKnight, (2005) who found broader peaks for plant material as plants contain a wider range of quinones. Component 1 is also quite broad in FTC samples from the meadow soil (Fig. 4.4 iv) which is consistent with plants being an important source of the DOC. This is not the case for the Heath soil where component 1 is narrower and more suggestive of a microbial source (Fig. 4.4 ii; Fig. 4.5). However, as both peaks from plants and microbes occur in the same place they are difficult to separate quantitatively.

The changes to the composition of DOC reveal FTC effects that may not have been apparent if only total DOC concentration was measured. For example, it has given clues as to the sources of the DOC flux. The potential for vegetation to provide a large proportion of DOC after FT is important as, being either from the foliage at the soil surface or in the shallow rhizosphere, the vegetation is potentially less protected from FTC than microbes and could experience colder freezing temperatures. DOC release from the roots could also be localised in areas of high microbial population that can use it immediately for CO₂ production. This could increase the soils oxygen demand, potentially leading to anoxic conditions forming in microsites and near the roots, especially if soil moisture content is high as might occur in the future as increased precipitation is predicted or after snowmelt in spring (Førland et al. 2011;

Uteau et al. 2015). This could potentially lead to N₂O emissions (Sehy et al. 2004). Release of DOC from foliage could lead to increased LMW DOC in the moss/lichen/litter layer than in the soil and could potentially stimulate degradation of fresh litter incorporated in this layer (Jackson et al. 2012; Lindo and Gonzalez 2010; Wilson and Coxson 1999). This may make LMW-DOC less likely to be released deeper in the soil where it might stimulate the turnover of old organic carbon, particularly where it has been stored by cryoturbation (Hugelius et al. 2010).

EEM does not provide precise information as to the structure of the compounds which make up the components making speculation on their fate difficult. Barančíková et al. (1997) suggested that higher emission wavelengths could be linked to more aromatic and high molecular weight material. They found that of the humic acid extracts from 6 soils, the 2 with the highest emission wavelengths were more aromatic and carboxylic in nature whilst the other 4 had lower emission wavelengths and were more aliphatic, containing more polysaccharide and amide groups. Therefore, the relative increase in component 1 due to FTC could suggest an increase in the bioavailability of the LMW DOC. Composition as well as the concentration of DOC can influence soil CO₂ emissions so this increase in the proportion of bioavailable LMW DOC at the meadow site after FT could support higher CO₂ emissions than an increase in total DOC (Glatzel et al. 2003; Kalbitz et al. 2003). However, Barančíková et al. (1997) were measuring at higher wavelengths than where the peaks are located here so this may not apply.

Whilst Chen and Jaffe (2014) observed clear changes in components due to degradation no significant change was observed here with increasing FTC. This could partly be due to the soil being frozen for half the time and thus slowing degradation. Compared with the rapid decrease in amino acids and glucose this suggests that the compounds contributing to component 1 are less microbially available. This is consistent with Michaelson et al. (1998) who found increases in humic acids after FT which degraded less than low molecular weight neutral material after 14 days. Thus, whilst FTC produces rapidly turned over carbon and a general increase in the availability of DOC, more recalcitrant compounds are produced as well even in the LMW fraction. The MALDI-TOF results suggest that sugar storage compounds could be released from plants due to FTC. These can have both an aromatic nature and also contain sugar molecules that would be a good C source for microbes. These compounds could be partially degraded leaving the more aromatic part of the molecule behind. This less microbially desirable DOC could potentially be leached from the soil and

lost from the terrestrial ecosystem, even if it is not mineralised, as increased DOC in rivers has been observed during spring thaw (Townsend-Small et al. 2011). DOC produced from foliage due to FTC could be more susceptible to being transported to water bodies than DOC produced in the soil as the soil can remain frozen during snow melt when the greatest water input into rivers occurs and not contribute to the nutrient output.

4.4.2. FT changes to DOC concentration

No significant change in concentration of DOC was observed in soil from the Heath site whilst FTC caused increased DOC concentrations in the Meadow soil. This is consistent with previous results which have shown both increased DOC and no change (Austnes and Vestgarden 2008; Feng et al. 2007; Grogan et al. 2004; Haei et al. 2012; Hentschel et al. 2008; Michaelson et al. 1998; Yu et al. 2011). The Heath site is more exposed which may mean it is more resistant to FTC. The thicker organic layer at the Meadow site means that the C content in the surface 5cm is greater than at the Heath site so the Meadow site probably has more potential DOC sources. Herrmann and Witter (2002) showed that 65% of released DOC can come from microbes in soil without roots. The Meadow site is also more vegetated than the Heath site. Plants are another potential source of DOC, as suggested by the EEM and MALDI-TOF results. Roots and moss have been shown to produce DOC after FT (Giesler et al. 2007; Melick and Seppelt, 1992). As freeze-thaw cycles are most severe at the soil surface this could be a very important source of DOC. The MALDI-TOF results suggest that plants can supply new compounds to the soil due to FT. It should be mentioned that collecting the soil cores in July could make this study less representative of real FTC as they are more likely to occur in the cold season when plants are dormant or dead. Dead moss produced less DOC than living moss (Melick and Seppelt, 1992). However, an earlier thaw date, which is predicted with climate change, could mean plants develop earlier in the spring which is still a time when FTC could occur. Therefore these results maybe representative of a future climate

Unlike in many previous studies, which typically collect soil solution days after thaw or after the soil solution has leached through the soil column, here soil solutions were extracted 6 hours after thaw in order to capture potential increases in rapidly utilised DOC. However, previous studies have shown that glucose and amino acids are removed from soil solution within minutes even when the substrates are added at quite high concentrations (Hill et al. 2008; 2012). That an elevated concentration was observed for these compounds after freeze-thaw is perhaps surprising. This suggests that either uptake was delayed by slower microbial

uptake, greater production was stimulated or that there was a physical barrier delaying microbial uptake. It has been shown that microbial use of amino acids and glucose in these soils was not significantly affected by freeze-thaw (Chapter 6). However, these were conducted 12 hours after air temperature returned to 5°C which may have given the microbes more time to recover. Studies on enzyme activity after FTC might help show if glucose or amino acid production increased. It has also been suggested that DOC may leak slowly out of damaged cells, keeping soil solution concentrations elevated for longer time periods than if it was released all at once (Buckeridge and Grogan 2010). Extraction of DOC does not completely describe its dynamics as rapid utilisation of a compound can keep its concentration low. Continuous extraction of DOC over a time period by techniques such as microdialysis could provide a better idea as to which compounds are being rapidly turned over (Inselsbacher and Näsholm 2012).

Changes to DOC concentration were most pronounced after a single rather than repeated FTC. This is in line with past studies on microbial mortality after FT, which show greater damage after one or two FTC with the surviving microbial population being resistant to further FT damage (Morley et al. 1983; Skogland et al. 1988). Previous studies of total DOC concentration have also shown the first two cycles to produce increased concentrations and that single cycles show increased DOC concentrations whilst multiple did not (Feng et al. 2007; Grogan et al. 2004; Hentschel et al. 2008; Yu et al. 2011). This suggests that an increase in FTC in the arctic which could occur due to warmer winters and reduced snow cover would not affect the DOC or related CO₂ emissions as suggested by Matzner and Borken (2008). However, this study has only studied FTC that have a short duration and short thaw time. Over the arctic winter in a warmer climate it is possible that long winter warming periods could be followed by long freezing periods. How these would affect DOC is not examined here.

4.4.3. FT Changes to dissolved N

Previous studies have shown changes to nitrogen concentrations due to FT. Studies have mostly focussed on inorganic N, with NH₄⁺ often being shown to increase due to FT whilst the response of NO₃⁻ has shown increases, but more often no change (Grogan et al. 2004; Koponen et al. 2006; Souliades and Alison 1961; Yu et al. 2011; Zhou et al. 2011). Hentschel et al. (2008) observed a decrease in inorganic N after FT. The results presented here show an increase in LMW-DON after 1 FTC. Elliot and Henry (2009) also found increases in DON,

but only after rapid or intense freezing. This increase in DON mirrors the increase in DOC and is most likely from the same source. The soils studied here are very low in N (Batjes 1996). The biota of such soils have been shown to compete for and prefer organic N to inorganic N (Hill et al. 2011). Glanville et al. (2012) found ammonia oxidising archaea in soils similar to those analysed here and the increase in NO_3^- concentrations over the experiment shows that ammonium oxidisers are active in these soils and resilient to FTC's. The NO_3^- results presented here tentatively suggest that FTC could increase NO_3^- concentrations, whilst NH_4^+ showed no change suggesting a rapid turnover of ammonium. It is possible that the NH_4^+ results are underestimates as the Berthelot method used has been shown to be affected by high amino acid concentrations (Husted et al. 2000). The increase in NO_3^- makes it possible that N_2O emissions during FTC could be a concern. It should be mentioned that this experiment does not simulate mass flow or include plant uptake so maybe overestimating the NO_3^- which would remain in the soil in the field. The potential for N_2O fluxes requires further research as FTC emissions could be a significant proportion of total emissions and N_2O is a powerful greenhouse gas (Matzner and Borcken 2008). These results might change if N concentrations increase in arctic soils and effect microbial communities (Horz et al. 2004).

4.4.4. Further notes on methodology

Whilst using intact cores helped maintain soil structure, the results presented here may not be entirely representative of *in situ* FT effects. Soil cores were not physically confined which may have reduced FT damage (Bullock et al. 1988). The cores were not insulated on the sides or base so they were frozen from all sides rather than for the top down. This insured all the core reached temperature, but is not what the soil would experience in the field (Henry 2007). Unfrozen water with its high heat capacity and enthalpy of fusion is attracted toward the freezing front which slows freezing in the field. This does not occur to the same extent in the lab so soils freeze more rapidly than they do in the field. This may have caused a bigger flux of dissolved organic matter than if the soils froze more slowly, although Elliot and Henry (2009) found that only really rapid freezing rates caused a significant increase in DON. They found that the 1°C per hour freezing rate we used in this experiment did not have a significant effect. Therefore, the results produced here should not be an overestimate of FT to the temperature of -7.5°C .

4.5. Conclusions

The effect of freeze-thaw cycles on DOC depended on the number of freeze thaw cycles and on the particular soil-vegetation system investigated. Any damage appeared to be limited to early freeze-thaw cycles. This has the potential to increase LMW DOC concentration and change DOC composition. The HMW DOC was not as clearly affected by FTC as the LMW DOC, but still may have experienced a change in composition. However, not all systems were strongly affected by freeze-thaw cycles as the more exposed, less vegetated Heath site soil showed little response to FT whilst the Meadow site was effected as described above. Quantitative evidence suggests both microbial biomass and vegetation could be source for DOC.

Chapter 5

Do Freeze-Thaw Cycles Induce Soil Organic Matter Priming?

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5. Do Freeze-Thaw Cycles Induce Soil Organic Matter Priming?

Abstract

An input of low molecular weight dissolved organic carbon (LMW-DOC) can stimulate the degradation of old, stable, soil organic carbon (SOC) in a positive priming effect. Freeze-thaw cycles (FTC) have been shown, in some circumstances, to cause an increase in LMW-DOC. Here, the extent to which FTC could induce priming of 6 and 18 month old SOC in arctic tundra soils was investigated. Soils were labelled with ^{14}C -glucose and incubated at 5°C to allow the ^{14}C to become incorporated into the soil microbial biomass and SOC. Soils were then frozen to -7.5°C for 46 hours. During this FTC, $^{14}\text{CO}_2$ emissions were continuously monitored. FTC appeared to increase $^{14}\text{CO}_2$ emissions from both the mineral soil labelled for 6 months and that labelled for 18 months. These results suggest that FTC could produce a positive priming effect in arctic soil. However, whether this is a real or apparent priming effect is open to interpretation. Suggestions for improvements to the method which would help clarify this issue are included.

5.1. Introduction

Soil organic carbon (SOC) represents a large storage pool of global carbon (Post et al. 1982). This is especially true at high latitudes which could contain up to 44% of global SOC and are an important sink of atmospheric C (Anisimov et al. 2007; Tarnocai et al. 2009). High water contents, low temperatures, low N and P levels as well as poor C input quality leads to decreased degradation rates and makes these areas particularly good stores of C (Billings et al. 1982; Ping et al. 1997). Processes that could destabilise this SOC store might be important for atmospheric CO_2 levels and need to be better understood.

Positive priming has been suggested as a mechanism which might enhance the degradation of stable SOC (Fontaine et al. 2004). This effect is caused by the input of labile C substrate which stimulates microbial growth, causing the production of more extracellular enzymes leading to the enhanced degradation of more stable SOC (Fontaine et al. 2003). However, negative priming effects have also been observed where the input of labile organic C has decreased the degradation of stable SOC, potentially due to the rapid response of fast growing microbes that use the fresh organic C, but cannot use the more complex SOC

(Guenet et al. 2010). If positive priming were to increase with predicted climate change this could have consequences for soil C storage and greenhouse gas emissions.

Some studies have shown that freeze-thaw cycles (FTC) can release dissolved organic C (DOC), although this result has not been achieved consistently (Feng et al. 2007; Grogan et al. 2004; Yu et al. 2011). They can also make available C that was previously physically protected, as FTC have been shown to break up soil aggregates (Soulides and Alison 1961). Therefore, it is possible that FTC could induce a priming effect. Arctic soils experience at least one FTC per year and the number could potential increase due to climate change (Henry 2008). This could have implications for C storage in arctic regions where a large proportion of global C is stored.

In this chapter I explored the hypothesis that a single FTC had positive priming effect on arctic soils. It also investigated whether different winter freezing temperatures affected whether FTC had a priming effect.

5.2. Methods

5.2.1. Soil sampling and preparation

In July 2012 and 2013 soil was collected from the mineral (Heath (m)) layer from the Heath tundra at Kolhaugen (78°55.224'N; 11°52.439'E), whilst soils from both the mineral (Meadow (m)) and organic (Meadow (o)) layers were sampled from the Meadow tundra at Westbyelva (78°55.4'N; 11°54.4'E) near Ny-Ålesund in Svalbard. For soil and site characteristics see chapter 4. The soils collected in 2013 were from either side of snow fences and so experienced different winter temperatures as described in chapter 8. Soils were sieved to 2 mm to remove stones and coarse roots. They were then stored in O₂ permeable bags at 5°C until ¹⁴C labelling commenced.

5.2.2. ¹⁴C labelling of soil

Soil (2 g FW) was weighed into 50 ml polypropylene tubes. To this, 0.2 ml ¹⁴C labelled glucose was added (100 µM of 1.2 kBq ml⁻¹ and 12 kBq ml⁻¹ for 2012 and 2013 respectively). Emitted CO₂ was trapped in vials of 1 M NaOH which were exchanged at increasing intervals (1, 2, 4, 8, 24 and 32 hours 2, 3, 4, 7, 45 and 190 days or 2, 5, 9, 26, 90, 386 and 531 days for the 6 months and 18 months treatments, respectively) over the measurement period of either 18 months for the soils collected in 2012 or 6 months for the

soils collected in 2013. Soils were kept at 5°C throughout the monitoring period. Hi-Safe OptiPhase scintillation cocktail (PerkinElmer Corp.) was mixed with the NaOH and the ¹⁴C content of the NaOH traps was measured by a Wallac 1409 scintillation counter (PerkinElmer Corp., Waltham, MA). The monitored ¹⁴C emissions over either the 18 or 6 month incubation period were fitted with a triple first order decay equation.

$$y = (a_1 \times e^{-k_1 t}) + (a_2 \times e^{-k_2 t}) + (a_3 \times e^{-k_3 t})$$

Where y is the percentage of the added ¹⁴C remaining in the soil, a_1 , a_2 and a_3 are the relative proportions of the fast, medium and slow soil carbon turnover pools respectively and k_1 , k_2 and k_3 are the rate constants describing their rate of turnover. The fast turnover pool (a_1) represents the added ¹⁴C that is immediately respired by microorganisms, whilst the medium turnover pool (a_2) characterises the substrate that is initially incorporated into the microbial biomass prior to being broken down and respired (i.e. short term intracellular C storage pool). Pool a_3 represents C that is converted to new biomass and turned over very slowly (i.e. structural cell wall C). Separating the microbially-incorporated ¹⁴C into 2 pools (a_2 and a_3) attempts to represent the breakdown of different biomolecules and the potential for recycling within the biomass. This modelling approach was used as it has been shown that a triple first order decay model is a better fit over long measurement periods than a double first order decay model (Farrar et al. 2012). As emissions were measured over either a 6 or 18 month period the triple first order decay model was chosen. This model was then used as a control to compare with the ¹⁴C emissions from the FT treatment in order to discern whether a priming effect had occurred. The average calculated equation parameters can be found in Appendix B.

5.2.3. Freeze-thaw treatment

6 months or 18 months after ¹⁴C-labelling, soils were frozen at a rate of 1°C h⁻¹ to a temperature of -7.5°C. They were maintained at this temperature for 46 hours, after which they were thawed at 1°C h⁻¹ to a temperature of 5°C. A NaOH vial was frozen with the soil and exchanged 1 hour after the soils had reached 5°C. Vials were exchanged at 1, 2, 4, 8, 26, 70, 118, 166, 242 and 406 hours and their ¹⁴C content was determined as mentioned in section 5.2.2. No control samples were physically measured so the FTC samples were compared with modelled controls - projected emissions for each sample using the triple first order decay equation described above. For the 18 month labelled soil, cumulative ¹⁴CO₂ emissions between 118-406 hours were compared as these were above detection limits. For

the 6 month old soil, emissions between individual time points were compared for the measured FTC samples and their projected controls.

5.2.4. Statistical analysis

The triple exponential decay model was fitted to the data in SigmaPlot (version 12.5). Differences in emissions between 118-406 hours were compared between the modelled control and measured emissions after FT by repeated measures ANOVA for the 18 month emissions with soil type as a within group factor. Differences in modelled emissions and measured emissions from the 6 month soil due to FTC were analysed by repeated measures ANOVA with soil type and snow cover as within group factors. To keep the assumption of sphericity, the Greenhouse Geisser correction was applied. Significance was assumed where $p < 0.05$. Emissions from the 6 month soil at 4, 26, 166 and 406 hours after FTC were analysed by paired sample t-test to identify significant differences caused by FTC in the individual soil/snow cover types. No correction for multiple comparisons was applied to the p values for these t-tests so some of the results could be false positives. Analysis was performed in SPSS (version 22).

5.3. Results

5.3.1. $^{14}\text{CO}_2$ emissions after FT from the 18 month old SOC

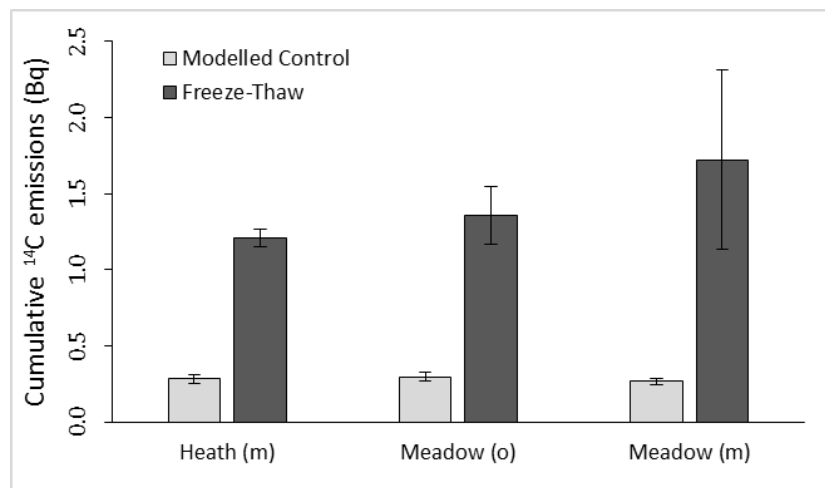


Fig. 5.1. Cumulative emissions of ^{14}C from soils 118-406 hours after freeze-thaw (FT) treatment and predicted emissions without FT calculated for each replicate by extrapolating the previous emissions using a triple first order decay equation in Heath mineral (m), Meadow organic (o) and Meadow mineral (m) soil. Values represent means \pm SEM ($n = 4$).

Measured emissions up to 118 hours after the FT treatment were generally below the machines lower limits of detection (30 decays per minute) and so were not analysed further. The emissions measured after 118 hours were taken over greater time periods and are above detection limits. These later emissions (between 118-406 hours) were significantly greater ($p < 0.001$) than predicted for the modelled controls by a factor of 4.4 ± 0.3 , 4.6 ± 0.3 and 6.5 ± 2.1 for the Heath mineral, the Meadow organic and the Meadow mineral soil, respectively (Fig 5.1). This suggests FT has increased the rate of mineralisation of the 18 month SOC. Measured values prior to the FTC treatment were not significantly different to modelled values which suggests that the model was a good fit. There was no significant difference between the relative increase between modelled and measured emissions between sites ($p = 0.432$).

5.3.2. $^{14}\text{CO}_2$ emissions after FT from the 6 month old SOC

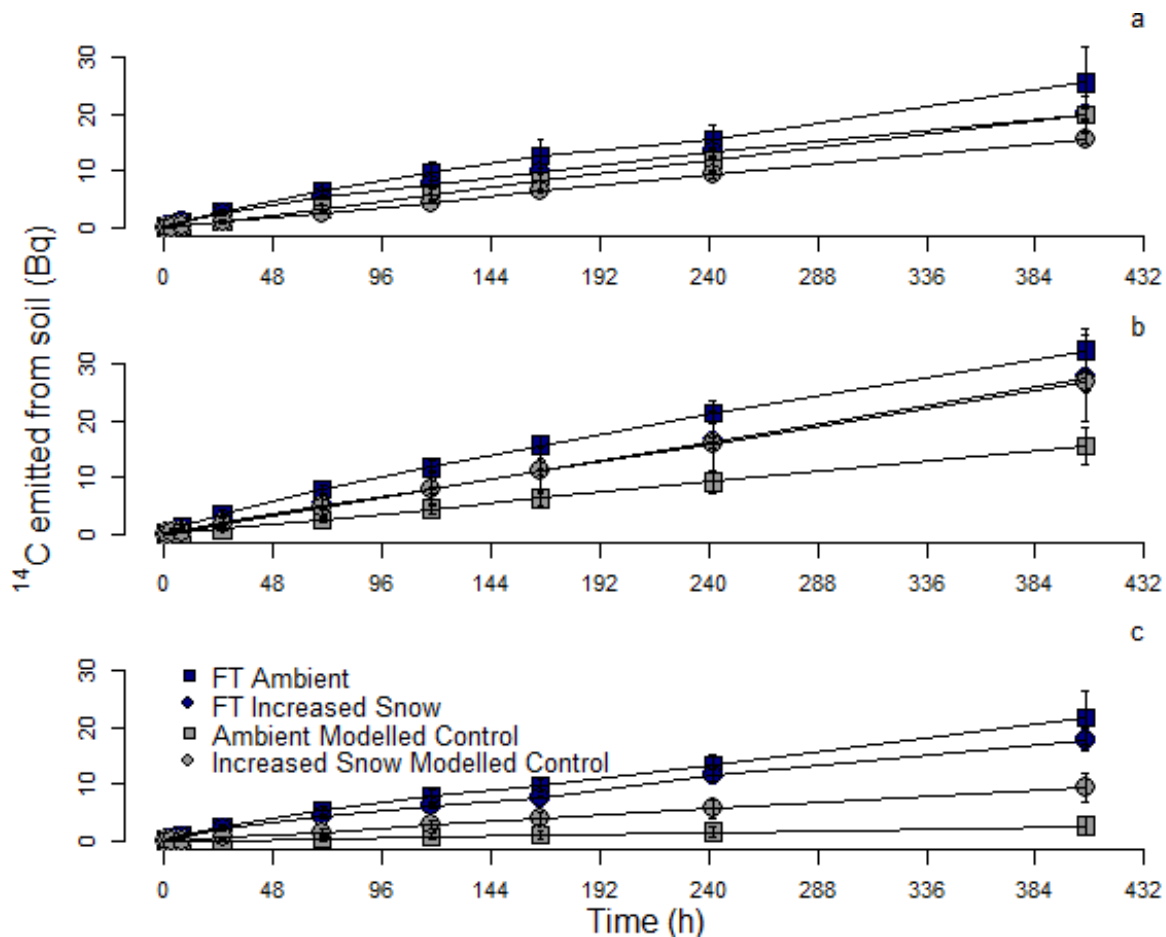


Fig. 5.2. Emissions of $^{14}\text{CO}_2$ from soil, labelled 6 months earlier, with time after FT, in soils experiencing a colder or warmer winter due to changes in snow accumulation a) Meadow mineral, b) Meadow organic, c) Heath mineral. Also shown are projected emissions for the soil without FT.

Emissions of $^{14}\text{CO}_2$ from the 6 month old labelled soils are shown in Fig. 5.2. The modelled controls had significantly lower $^{14}\text{CO}_2$ emissions than were measured after FT from the 6 month old labelled SOC ($p < 0.001$). There was a significant interaction between the modelled controls and the FT emissions over time ($p = 0.027$) with FT producing more rapid $^{14}\text{CO}_2$ emissions soil which decreased with time. The measured emissions at one hour time intervals immediately after the FT treatment tended to be below the LLD, but 4 – hours after FT the emissions are reliably quantifiable and significantly, or almost significantly, larger than modelled for emissions without FT for the Heath and Meadow mineral soils (36.2 ± 12.3 , 3.7 ± 0.8 , 2.5 ± 0.7 and 3.8 ± 0.7 times greater for ambient Heath mineral, increased snow Heath mineral, ambient Meadow mineral and increased snow Meadow mineral; $p = 0.004$, 0.028 , 0.099 and 0.025 , respectively). There were significant interactions between the difference in the emission due to FT and soil and snow ($p = 0.036$) although this was not significant when time was included as a factor. Emissions for the ambient Meadow mineral soil and the increased snow Heath mineral soil are significantly greater 26 hours after FT than predicted for no FT (1.9 ± 0.2 and 4.2 ± 1.2 times greater than modelled without FT, $p = 0.011$ and 0.005 , respectively). Enhanced emissions were maintained in the 6 month ambient Heath mineral and increased snow Meadow mineral soils for a week after the FT treatment (14.6 ± 6.2 and 1.2 ± 0.04 times greater, $p = 0.007$ and 0.016 , respectively). The organic Meadow soil did not show significantly different $^{14}\text{CO}_2$ emissions after FTs from those predicted by the model for no FT.

In some cases, significant differences between the modelled control and measured values for ^{14}C remaining in the soil occurred prior to the FT treatment. This is despite r^2 values for the model being > 0.994 . This occurred in the 6 months Meadow mineral and organic snow accumulation soil. The model also tended to overestimate the CO_2 emissions prior to the FT treatment. The model prior to FT was significantly greater than measured results in all the Meadow soils, although this was only by 2-3% ($p < 0.042$). As the $^{14}\text{CO}_2$ measurements used to generate the modelled controls were taken at increasing intervals over time, there were few measurements of soil CO_2 emissions in the later part of the data, which might decrease the model's accuracy. As the model predicted greater than measured emissions prior to FT, this suggests the predicted rates were faster than they should be and thus the significant differences measured in emissions post FT should still be true. The results are most likely

more conservative than they should be, i.e. not detecting a significant difference when there should be one.

5.4. Discussion

5.4.1. Do FTC induce a priming effect?

This study suffers from a lack of real control. Whilst predicted values are sample specific, they do not show if experimental artefacts, such as removing soils from the 5°C incubator to change vials, have increased the $^{14}\text{CO}_2$ emission rate. Also, as previously mentioned, the model used to generate the predicted values has few data points near the end which could decrease the model's accuracy. This makes conclusions from the data more tentative. However, some of the differences in the predicted and measured rates are quite large which makes it less likely that they could arise from model error or increasing temperature. The former is more likely to underestimate the difference, whilst the latter should cause a doubling of rate, assuming Q_{10} relationship, rather than the near order of magnitude increase observed in the ambient heath soil. It should be mentioned that Q_{10} could be higher at lower temperatures around 5°C which could increase the temperature affect (Farrar et al. 2012), but soils only spent a brief time out of the incubator which would reduce this effect.

The results from both the 6 and 18 month old labelled soil suggests that FTC could stimulate the breakdown of older SOC. However, 6 and even 18 months after labelling some of the ^{14}C could still be in the living biomass. It has been shown that FT damage can release microbial carbon to the soil making it available for degradation (Herrmann and Witter 2002). The effect observed here could be an apparent priming effect, i.e. an increase in the turnover of living material, rather than a real priming effect where dead OM is degraded (Derrien et al. 2014). The fastest emissions are observed immediately after FTC in the 6 month soil which is what would be expected from an apparent priming effect (Kuzyakov 2010). Yet, emission rates are faster than predicted over a week after FTC for the 6 month soil and at least 2 weeks later for the 18 month soil which Derrien et al. (2014) show is more indicative of a real priming effect. They also found that the older organic material was degraded at a later stage than the younger material, which appear to agree with the results here. However, how long a priming effect lasts is not known and could depend on the substrate. The later $^{14}\text{CO}_2$ emissions could be from the more complicated material from microbes damaged by the FTC. Also, Chowdhury et al. (2014) suggest that heightened emissions at this later stage could be due to an increase in internal metabolism. However, this suggestion was made due to low N concentrations at

the later period of their experiment and the true effect of N on the priming effect is still being discussed.

Low nutrient environments are more likely to experience a priming effect than high nutrient environments as the growth of microbial 'r' strategists is limited making 'k' strategists more competitive (Asmar et al. 1992; Fontaine et al. 2003). However, addition of nutrients can increase the priming effect in some cases (Chen et al. 2014). Added with sucrose, N can increase the amount of old C respired, but added with straw it had no effect and it also significantly decreases enzyme activity (Chen et al. 2014). Chowdhury et al. (2014) suggest that N addition is more likely to increase the apparent priming effect than the actual priming effect and Garcia-Pausas and Paterson (2011) found that it reduced the priming effect when added with glucose. Addition of N can reduce the need for N mining, thereby reducing the priming effect, but in very low N soils, like in the arctic tundra, microbes may need a source of N to produce the N needed for making enzymes (Allison et al. 2009; Wild et al. 2014). The FTC can produce organic and inorganic N sources. In the low nutrient arctic soils studied here, release of N sources during FT could make a priming effect more likely.

Wild et al. (2014) observed a positive priming effect after adding LMW-DOC, protein or cellulose to arctic mineral soils. However, they found that only protein had a positive priming effect on the arctic topsoil. The results in this chapter do not show a significant effect of FTC on the rate of mineralisation of the 6 month organic soil, but do on the mineral soils which agrees with the results of Wild et al. (2014). They suggest greater energy limitations exist in the mineral soil that is countered by the addition of fresh organic material and causes a greater priming effect. Blagodatskaya et al. (2011) and Derrien et al. (2014) suggest that the amount of fresh organic carbon added relative to the microbial biomass affects the priming effect. The meadow organic soil has a larger microbial biomass than the mineral soils (815 compared to 293 mg C kg FW, see chapter 6) so may need a greater input of fresh organic carbon to have a priming effect. Only the microbial biomass was labelled with ^{14}C not plant material so this experiment only shows if FTC stimulated the degradation of SOC of microbial origin. The organic soil is largely composed of partially degraded plant material, so a large priming effect could occur that is undetected by this experiment.

The amount, the availability and the quality of the added substrate affects the positive priming effect. For instance, more substrate increases the priming effect until it reaches a maximum value (Blagodatskaya et al. 2011; Paterson and Sim 2013). The Meadow soil has

generally shown to produce more LMW-DOC due to FTC than the Heath soil (Chapter 4), yet it does not appear to have a greater priming effect. Roots and vegetation were removed prior to this experiment which may have reduced the amount of LMW-DOC the FTC produced, particularly in the Meadow soil. Wheat and straw have a greater positive priming effect than glucose (Fontaine et al. 2003). More chemically complex DOC than glucose is produced during FTC, in both the Heath and Meadow soils, which could support a greater priming effect than glucose alone. The effect of snow cover on the soil varied with soil type with the ambient soil in the Heath site appearing to have a greater priming effect than the increased snow covered soil whilst the mineral soil from the Meadow site appeared to show the opposite effect. Chapter 8 shows that snow cover changes in the winter of 2012-2013 had little effect on soil characteristics and no significant effect on LMW-DOC mineralisation, but may have caused delayed plant growth on the snow accumulation side and potentially less microbial activity. How this affects the soil 6 months later is unclear. Subtle differences in microbial use of LMW-DOC that were not apparent over the initial 2 week monitoring phase, such as incorporation into more or less degradable compounds could be responsible for the difference in $^{14}\text{CO}_2$ emissions after FTC.

5.4.2. Suggested improvements to the method

In order to avoid microbial damage, one could extract soil solution from FTC treated soil and add this to labelled soil. However, with this approach, where inputs from FTC were located would change, which would change where hotspots of microbial activity occurred and affect how the C is degraded (Schmidt et al. 2011). Also, extracting soil solution would not extract all produced compounds, which could stick to the soil or be absorbed rapidly by microbes (Hill et al. 2008). For example, FTC in the Heath soil did not appear to cause a significant increase in glucose or amino acids in a previous experiment (Chapter 4), but in this experiment an increase in $^{14}\text{CO}_2$ from the Heath soil was observed. This could be because not all DOC was extracted when the soil solution was analysed, although after 6 months at 5°C the soil characteristics and microbial community could have changed, making them more susceptible to FTC. Adding extracted DOC could still stimulate microbial turnover, which would still make it difficult to separate apparent and real priming effects. Measuring the ^{14}C remaining in the microbial biomass prior to and post FTC would help estimate how much of the $^{14}\text{CO}_2$ produced due to FTC was of microbial origin.

Analysis of enzyme activities before and after FT could help establish if a real or apparent priming effect was observed. The former would cause an increase in extracellular enzyme

activity whilst the latter would not (Chen et al. 2014). Potentially, a release of internal enzymes due to microbial FT damage could occur. Degradation of old SOM this way could still be considered to be a real priming effect. Wang et al. (2014) observed a decrease in the activity of certain enzymes in soils due to FT whilst Yergeau and Kowalchuk (2008) found both a decrease and a potential increase. Further work is needed to discover how FTC affect enzyme activities.

As the emissions from the 18 month soil were too low to measure immediately after FT no quantitative comparison can be made with the 6 month emissions. The older soil should have had a greater amount of ^{14}C added so that emissions would be measurable over short time periods 18 months later. To get a better idea of the duration of the priming effect soils need to be monitored for longer than 2 weeks and at shorter intervals than 1 week as this does not provide very good resolution. Also, soils should be labelled as intact swards by $^{14}\text{CO}_2$ uptake by plants so as to include plant material in the old labelled SOC.

5.5. Conclusion

This study attempted to establish whether the DOC released by FTC could stimulate the degradation of older SOM, termed a positive priming effect. The results show an increase in $^{14}\text{CO}_2$ emissions after FTC from soil labelled 6 and 18 months prior. This suggests that FTC might have a positive priming effect. However, as this experiment lacks an accurate control, this result must be treated with caution. Also it is not possible to interpret whether the results show a real or apparent priming effect. Measuring changes to the microbial biomass and enzyme activities after FTC could help clarify if a real affect was occurring.

Chapter 6

Freeze-thaw cycles have minimal effect on the mineralisation of low molecular weight dissolved organic carbon in arctic soils

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6. Freeze-thaw cycles have minimal effect on the mineralisation of low molecular weight dissolved organic carbon in arctic soils

Abstract

Warmer winters in arctic regions may melt insulating snow cover and subject soils to more freeze-thaw cycles. The effect of freeze-thaw cycles on the microbial use of low molecular weight, dissolved organic carbon (LMW-DOC) is poorly understood. In this study, soils from the arctic heath tundra, arctic meadow tundra and a temperate grassland were frozen to -7.5°C and thawed once and three times. Subsequently, the mineralisation of 3 LMW-DOC substrate types (sugars, amino acids, peptides) was measured over an 8 day period and compared to controls which had not been frozen. This allowed the comparison of freeze-thaw effects between arctic and temperate soil and between different substrates. The results showed that freeze-thaw cycles had no significant effect on C mineralisation in the arctic heath tundra soils. In contrast, for the same intensity freeze-thaw cycles, a significant effect on mineralisation rate and microbial C use efficiency was observed for all substrate types in the temperate soil although the response was substrate-specific. Peptide and amino acid mineralisation were similarly affected by FT whilst glucose had a different response. Where a small effect of freeze-thaw on the arctic meadow tundra was observed, the changes were consistent with those found in the temperate soil. Further work is required to fully understand microbial use of LMW-DOC after freeze-thaw, yet these results suggest that relatively short freeze-thaw cycles have little effect on microbial use of LMW-DOC in arctic tundra soils.

Keywords Below-ground respiration • Carbon cycling • Climate change • Freezing temperature • Polar soils

6.1. Introduction

A conservative estimate of global soil organic C stocks is about 2,200 Pg in the top 1 m (Batjes 1996). It is estimated that between 14 and 44% of global soil C is in arctic regions depending on the soil depth used (Post 1982; Tarnocai et al. 2009). Future predictions suggest that the occurrence of freeze-thaw cycles (FTC), where soil temperatures drop below and then rise above 0°C , may increase at high latitudes as global temperatures increase (Henry

2008). Winter polar temperatures are expected to increase due to climate change which could make soils more prone experience temperatures above 0°C and potentially melt insulating snow cover. Enhanced emissions of greenhouse gases CO₂ and N₂O are sometimes associated with FTC, potentially caused by the mineralisation of the released DOC (Matzner and Borken 2008; Schimel and Clein 1996). As there is large uncertainty as to future soil C storage, it is important to identify what effect FTC may have on C turnover and allocation within arctic soils (Anisimov et al. 2007).

Most soil organic C (SOC) is not immediately available to heterotrophic microorganisms as it is composed of high MW, insoluble polymers that require extracellular enzymatic cleavage to low molecular weight (LMW) compounds prior to use (Farrar et al. 2012; van Hees et al. 2005). LMW dissolved organic carbon (DOC) is generally considered to contain molecules <1000 Da and much of it (<650 Da) can be taken up directly into the cell via specific transporters (Payne and Smith 1994). It is produced by the breakdown of larger organic matter, and directly from root and microbial exudates and from desorption of molecules precipitated/bound on particle surfaces. LMW-DOC is an important substrate for soil microorganisms (van Hees et al. 2005). It is comprised of a large variety of compounds including, but not limited to, amino acids, organic acids, amino sugars, mono and polysaccharides, peptides, lipids, sterols and phenolics (Kalbitz et al. 2003, van Hees et al. 2005). Certain compounds within this complex mixture are turned over rapidly by the microbial community and can be major contributors to total soil respiration (Boddy et al. 2007; Fujii et al. 2010).

Freeze-thaw cycles (FTC) have been shown to sometimes, though not always, increase the concentrations of DOC and to affect microbial activity and community structure (Stres et al. 2010; Yu et al. 2011). FTC can produce LMW DOC by causing damage and fatality to roots and microbes and by breaking up soil aggregates (Henry 2007; Herrmann and Witter 2002; Tierney et al. 2001). Some studies have shown that FTC can induce severe microbial mortality resulting in large changes in microbial community size and structure (Skogland et al. 1988; Stres et al. 2010; Wilson & Walker 2010). However, these laboratory studies used freezing temperatures and frequencies more extreme than those naturally experienced in soil. Where arctic or alpine tundra soils which are adapted to lower temperatures were used, less change to the microbial community structure was observed (Manisstö et al. 2009; Stres et al. 2010), although an increase in fungi relative to bacteria has been shown to occur during

frozen periods (Feng et al. 2007; Haei et al. 2011). Repeated, mild FTC decreased microbial biomass carbon in subarctic tundra (Grogan et al. 2004; Larsen et al. 2002).

How the microbes use the released DOC has not been studied in detail. Once produced, LMW compounds such as amino acids, peptides and glucose are rapidly consumed (within minutes) by the microbial community and the C used for both cell maintenance and growth (Hill et al. 2008; 2012). The subsequent partitioning of the LMW C inside the cell has previously been operationally split into two functional pools, one C pool is used immediately for respiration and the other C pool for biosynthesis before eventually being respired (Boddy et al. 2008; Glanville et al. 2012). A third pool has occasionally been assigned to represent more recalcitrant products of biosynthesis (Farrar et al. 2012). The proportion assigned to each pool depends on the substrate and can provide useful insights into C use efficiency and microbe responses to abiotic and biotic stresses (van Hees et al. 2005). Whether microbial use of LMW DOC is affected by FTC is less well known, but Lipson and Monson et al. (1998) showed no change in the respiration of glycine or glutamate after a FTC in alpine tundra. Degens et al. (2001) found less variability in the mineralisation of different LMW DOC compounds in temperate soils after 1FTC to -30°C, but the variability returned to normal after 4FTC. Further investigation will help illuminate the precise mechanisms that occur during FTC and provide more information on the fate of released DOC

The aim of this study was to identify how the mineralisation of LMW DOC was affected by FTC in both an arctic and temperate soil. We tested the hypotheses that FTC would cause a shift in microbial C use efficiency and mineralisation rate, that this change would be compound specific, that FTC would have a greater impact on the mineralisation of LMW DOC in the temperate soil than the arctic soil and that 3FTC would have less effect than 1FTC. To achieve this, soils from the arctic tundra and a temperate grassland were subjected to FTC. Subsequently ^{14}C labelled LMW DOC compounds were added and their evolution as $^{14}\text{CO}_2$ was monitored.

6.2. Methods

6.2.1. Soils

Individual replicate soil samples ($n=4$) were collected from 3 sites. Two contrasting arctic tundra vegetation sites in Svalbard were chosen, arctic Heath soil (*Dryas octopetala* and bare soil dominated) was sampled from Kolhaugen, Ny Ålesund (78°55.224'N; 11°52.439'E) and arctic Meadow soil (*Salix polaris* and lichen dominated) was sampled from Westbyelva, Ny Ålesund (78°55.4'N; 11° 54.4'E). Soil was also sampled from a temperate grassland (*Lolium perenne* and *Trifolium repens* dominated) in Abergwngregyn, UK (53°14.20'N; 04°01.03'W). The average winter temperature (1981-2009) in Ny-Ålesund, Svalbard, was -12°C (Førland et al. 2011), whereas the average winter temperature for the same period at the temperate site was 3.4°C (UK Met Office Statistics). Arctic soils are frozen from October – June and can experience variable snow cover from bare soil to >1m (Norwegian Meteorological Institute Statistics). The Heath site is more exposed than the Meadow site and experiences colder soil temperatures (Fig.1). The temperate soil seldom freezes or experiences snow cover (UK Met Office Statistics). The Heath site is approximately 50m a.s.l, whilst Westbyelva and Abergwngregyn are 25m a.s.l.

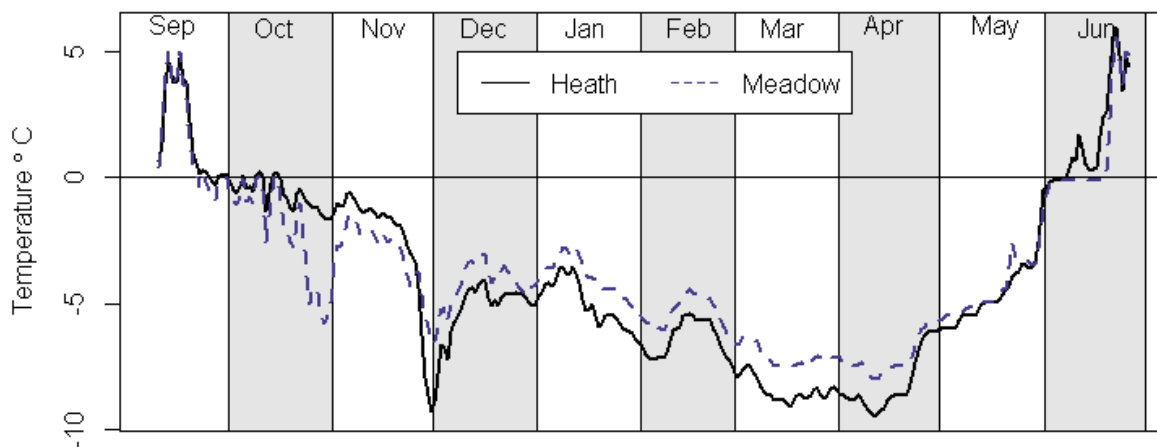


Fig. 6.1 Average daily soil temperatures in the winter of 2012-2013 measured by temperature data loggers at 1cm depth at the arctic sites

Both arctic soils are Cryosols, the arctic Heath soil is a sandy mineral soil beneath a very shallow/ absent organic layer, while the arctic Meadow tundra soil has an organic layer up to 10 cm thick overlying a gravel rich mineral layer. The temperate soil is a grazed grassland, Eutric Cambisol soil type. Samples were taken randomly over an area of half a hectare.

Soils were sampled at a depth of 5cm over a diameter of 10cm. The variable thickness of the organic layer at the meadow site meant that the soil sampled at 5cm was either mineral (Meadow mineral) or organic (Meadow organic). The organic layer at the heath site generally did not reach 5 cm so only the mineral soil (Heath mineral) was sampled. The overlying vegetation of the precise area sampled was similar for that of the entire site, save in the case of the Heath mineral soil which was taken from mostly bare soil areas as the *Dryas* cover was patchy. After sampling, soils were stored in oxygen permeable bags, stored at 5°C and transported to Bangor University and subsequently stored at 5°C.

6.2.2. Chemical analysis

Soil extractions (1:2.5 v/v soil: deionised water) were performed to assess soil nutrient status. Soil microbial biomass C was measured using the soil fumigation-extraction method of Vance et al. (1997). Briefly 10g of soil was fumigated with chloroform for 24 hours. Then DOC was extracted from fumigated and non-fumigated soil by shaking in deionised water and the difference in C content was assumed to represent the microbial biomass C. Fumigated and non-fumigated deionised water extracts were analysed for total organic C (TOC) and total dissolved N (TDN) on a TOC-V-TN analyser (Shimadzu Corp., Kyoto, Japan). Inorganic N (NO_3^- and NH_4^+) was measured using the methods outlined by Miranda et al. (2001) and Mulvaney (1996), whilst free amino acids (FAAs) were measured using the *o*-phthaldialdehyde- β -mercaptoethanol procedure of Jones et al. (2002). DON was estimated by subtraction of NO_3^- and NH_4^+ from the TDN value. Soil pH was measured in 1:2.5 (v/v soil: water) slurries, whilst moisture content was measured gravimetrically after heating to 105 °C overnight. Total Soil C and N were measured using a Carlo Erba NA 1500 Elemental Analyzer (Thermo Fisher Scientific, Milan, Italy). Soil characteristics are shown in Table 6.1.

6.2.3. Freeze thaw cycles

Treatment consisted of either 1 FTC or repeated (3) FTC and controls (i.e. no FTC) for each FTC treatment. A number of studies have shown the most damaging effects of FT to be observed within the first couple of cycles (Skogland et al. 1988; Yu et al. 2011) so some difference between 1 and 3FTC may be observed. Coarse roots and stones >2 mm were removed by sieving the sampled soils prior to treatment. Fresh soil (2 g) was taken from each replicate for each of the 4 treatments. Soils were frozen to -7.5°C at a rate of 1°C h⁻¹ and this temperature was maintained for 2 d. Soils were then thawed at a rate of 1°C h⁻¹ to 5°C. This freezing temperature is representative of winter soil temperatures at the arctic sites (minimum

of -7.5°C and -9.4°C at 1 cm depth for the Meadow and Heath site, respectively, during winter 2012-2013) and is also seen in other sites in Svalbard (Morgner et al. 2010; Semenchuk et al. 2013). Similarly, rapid changes in soil freezing temperature has also been observed (Semenchuk et al. 2013). In the repeated FTC this temperature was maintained for 2 d after which the freeze-thaw cycle was repeated a further 2 times. The mineralisation of the LMW C substrates in the soils were tested 12 h after the air temperature had returned to 5°C after either 1 or 3FTC. Controls soils were maintained at 5°C.

6.2.4. DON and DOC mineralisation

The mineralisation rates of LMW DON and DOC compounds were determined for three substrates: the oligopeptide trialanine (231 Da), an amino acid mixture (equimolar mix of L-isomeric alanine, arginine, aspartic acid, glutamic acid, glycine, histidine, isoleucine, leucine, lysine, phenylalanine, proline, serine, threonine, tyrosine and valine, 75-174 Da) and the sugar, glucose (180 Da). For the ¹⁴C-labelled glucose and amino acid substrates, 200 µl of a 100 µM solution (1.2 kBq ml⁻¹) was added to the soil surface. For trialanine, 200 µl of a 10 µM solution (1.4 kBq ml⁻¹) was added to the soil. The soils were incubated at 5°C in sealed 50 ml polypropylene tubes in which an alkali trap (1 ml of 1 M NaOH) was placed to trap the ¹⁴CO₂ produced. Traps were exchanged after 0.5, 1, 2, 4, 7, 21, 30, 48, 72 or 96 and 168 h. The ¹⁴C content of the NaOH traps was determined using a Wallac 1409 scintillation counter (PerkinElmer Corp., Waltham, MA) and Hi-Safe OptiPhase 3 scintillation cocktail (PerkinElmer Corp.). From this the percentage of the added ¹⁴C remaining in the soil at each sampling time was calculated by subtracting the % ¹⁴C emitted from 100.

A double first order decay model was fitted to the resulting data

$$Y = (a_1e^{-k_1t}) + (a_2e^{-k_2t}) \quad \text{Equation 1}$$

Where Y is the ¹⁴C remaining in the soil, a_1 and a_2 are the relative sizes of the quickly and more slowly turned over fractions of substrate respectively, k_1 and k_2 are their associated rate constants and t is time. As the substrates used have been shown to be taken up in minutes by soil microorganisms (Hill et al. 2008; 2012), the quickly turned over substrate fraction can be considered to represent substrate immediately respired by microorganisms, whilst the more slowly turned over substrate fraction characterises the substrate that is incorporated into biomass and subsequently respired. This model has been shown to be a good fit for the mineralisation over 2 weeks of the LMW DOC substrates used in this study and in a number

of environments including temperate grasslands and arctic tundra (Boddy et al. 2007; Farrell et al. 2011; Glanville et al. 2012).

6.2.5. Statistics

Significant effects of soil type, substrate type, FT treatment and cycle, plus significant interactions, were assessed using a weighted least squares full factorial ANOVA for each parameter derived from the fitted model. Within each soil type a weighted least squares full factorial ANOVA was applied to examine the effect of substrate type, FT treatment and cycle for that soil. Tukey's post hoc test was used to identify where significant differences occurred between FT treatment and cycle within each soil and substrate type. Differences between soil types were examined using weighted least squares one-way ANOVA and Tukey's post hoc test. Significant differences between soil chemical characteristics were identified using one-way ANOVA and Tukey's post hoc test. All statistics were performed in SPSS version 20 (SPSS Inc., Chicago, IL). Differences were considered significant where $p < 0.05$.

6.3. Results

6.3.1. Soil chemical properties

Soil type	pH	Total C (%)	Total N (%)	DOC (mg C /kg)	NO ₃ ⁻ (mg N/kg)	NH ₄ ⁺ (mg N /kg)	DON (mg N /kg)	TFAA (mg C /kg)	MBC (mg C /kg)
Meadow	6.28 ±	7.92 ±	0.67 ±	24.0 ±	<0.05	0.76 ±	4.18 ±	0.52 ±	293 ±
Mineral	0.12 a	2.43 b	0.19 b	2.3 b		0.4 a	0.67 a	0.12 a	58 c
Meadow	6.40 ±	24.0 ±	1.77 ±	36.7±	0.80 ±	1.08 ±	6.94 ±	0.63 ±	815 ±
Organic	0.16 ab	2.9 a	0.06 a	5.2 a	0.61 a	0.62 a	1.34 a	0.08 a	25 a
Heath	7.39 ±	5.51 ±	0.38 ±	23.2 ±	0.30 ±	<0.05	4.39 ±	0.37 ±	293 ±
Mineral	0.10 b	2.17 b	0.18 b	1.2 b	0.59 a		0.59 a	0.10 a	50 c
Temperate	6.10† ±	3.01† ±	0.23† ±	13.9 ±	10.5 ±	0.38 ±	5.03 ±	0.26 ±	524 ±
	0.10 a	0.16 b	0.01 b	1.2 c	0.9 b	0.05 a	0.90 a	0.17 a	37 b

Table 6.1. Soil chemical properties used in the freeze-thaw studies. Values are indicative of the mean ± SE ($n = 4$). MBC represents microbial biomass carbon and TFAA stands for total free amino acids. All values are expressed on a fresh weight basis where applicable. The letters indicate significant difference between soil types ($p < 0.05$). † indicates data from Roberts and Jones (2012).

The Heath mineral soil had a significantly higher ($p < 0.001$; Table 6.1) pH than the other soils. No other significant differences were observed in the chemical characteristics of the two arctic mineral soils ($p > 0.05$; Table 6.1), while the Meadow organic soil had a significantly higher soil C ($p < 0.001$; Table 6.1), N ($p < 0.05$; Table 6.1), DOC ($p < 0.022$; Table 6.1) and microbial biomass C ($p < 0.002$; Table 6.1) than the other soils. The temperate soil had the greatest ($p < 0.001$; Table 6.1) NO_3^- concentrations and the second largest ($p < 0.01$; Table 6.1) microbial biomass C (MBC). In the other measured characteristics, the temperate soil was similar to the arctic mineral soils ($p > 0.05$; Table 6.1). The pH, soil C and soil N of the temperate soil were not measured in this analysis, values from the same soil type, measured by Roberts and Jones (2012), have been provided for reference.

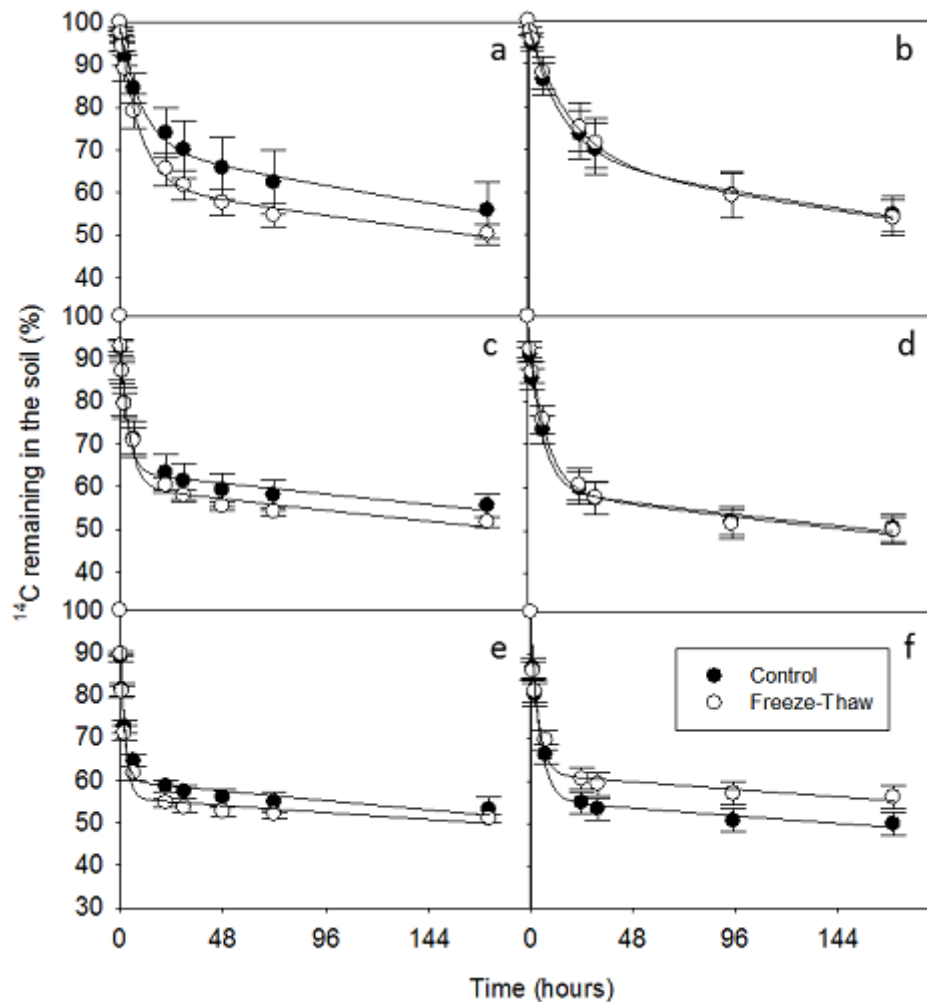


Fig. 6.2. Depletion of ^{14}C – added as Trialanine from arctic tundra soils: Heath mineral soil (a & b), Meadow mineral soil (c & d) and Meadow organic soil (e & f) after they had been subjected to 1 and 3 freeze thaw cycles (-7.5 to $+5^\circ\text{C}$). The results are fitted with a double first order exponential decay equation.

6.3.2. Soil mineralisation parameters

In all soils and for all substrates, a biphasic pattern of mineralisation was observed. A double exponential decay model was found to fit well to this experimental mineralisation data ($r^2 = 0.971$ to > 0.999) (Fig. 6.2-6.5). A significant effect of both soil and substrate was observed for all mineralisation parameters ($p < 0.001$ for all). There were significant differences in the modelled mineralisation parameters for the first cycle controls between soil types (Figs. 6.6-6.7; Tables 6.2-6.4). The first rate constant (k_1) describing the rate of trialanine turnover was significantly different between the soil types, with the temperate soil being the quickest, followed by the Meadow organic soil, then the Meadow mineral soil, with the Heath soil having the slowest turnover ($p < 0.05$; Table 6.2 k_1 control 1; Fig. 6.6).

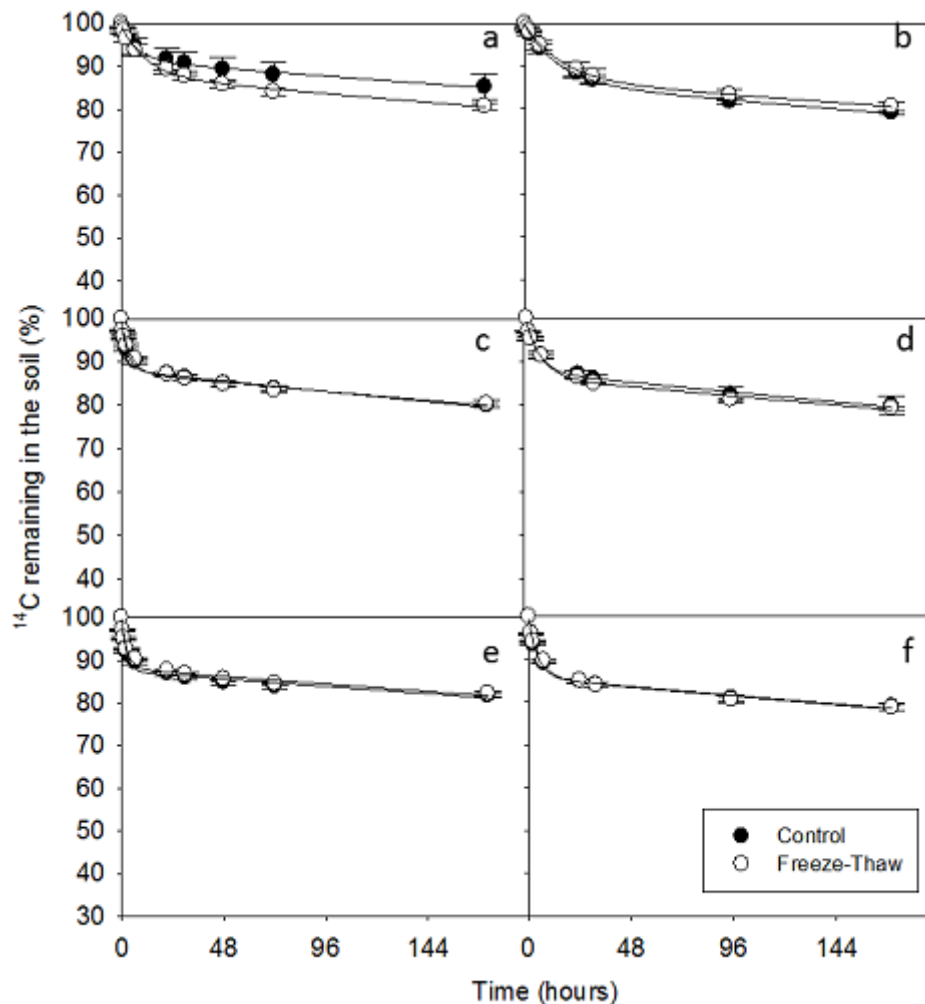


Fig. 6.3. Depletion of ^{14}C - added as Amino acids - from arctic tundra soils: Heath mineral soil (a & b), Meadow mineral soil (c & d) and Meadow organic soil (e & f) after they had been subjected to 1 and 3 freeze thaw cycles (-7.5 to $+5^\circ\text{C}$). The results are fitted with a double first order exponential decay equation.

In contrast, no significant difference in turnover rate was observed between soil types for the amino acid substrate ($p > 0.05$; Table 6.3 k_1 control 1; Fig. 6.6). The turnover of glucose showed some difference between soil types. Glucose turnover in the Heath soil was slower than in the Meadow soils, whilst the temperate soil had a significantly slower glucose turnover than the Meadow mineral soil ($p < 0.05$; Table 6.4 k_1 control 1; Fig. 6.6). The rate constant for the second slower C pool (k_2) was not significantly different in any soil type for amino acids, but trialanine showed the Heath mineral soil had a significantly greater k_2 than the Meadow mineral soil and the temperate soil ($p < 0.05$; Table 6.2 k_2 control 1; Fig. 6.6). For glucose, the turnover of the second C pool in the temperate soil was slower than in both Meadow soils ($p < 0.05$; Table 6.4 k_2 control 1; Fig. 6.6).

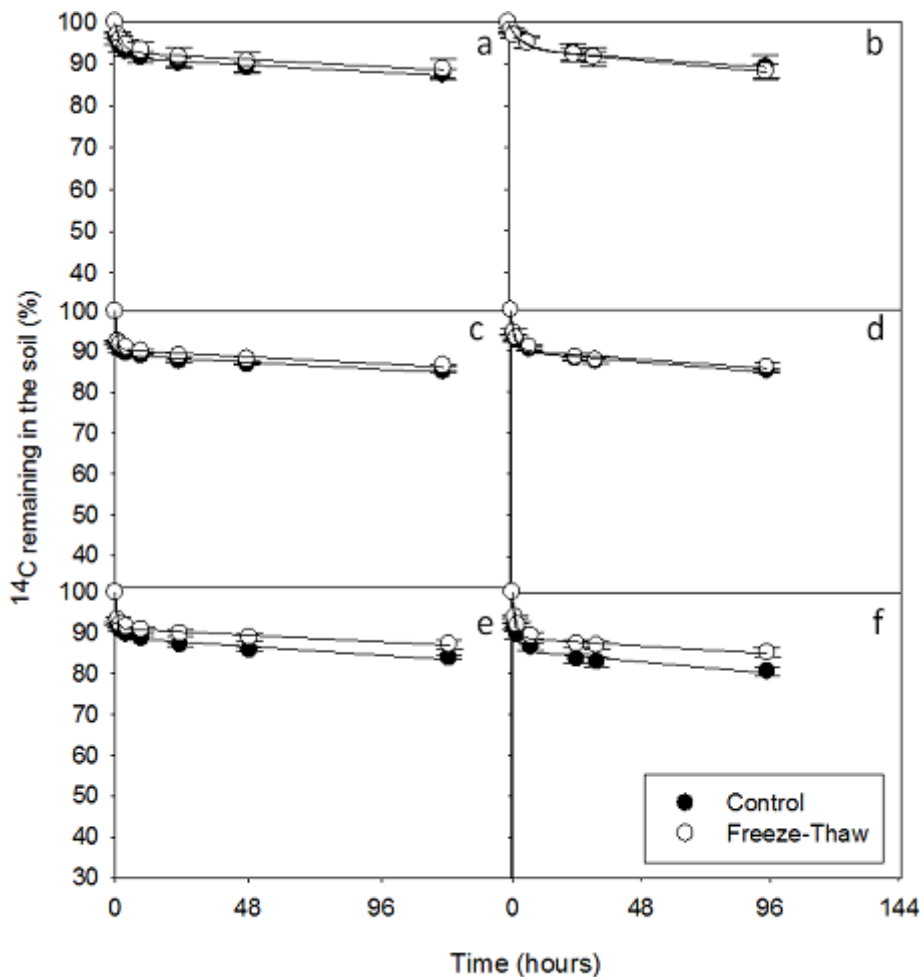


Fig. 6.4. Depletion of ^{14}C - added as Glucose - from arctic tundra soils: Heath mineral soil (a & b), Meadow mineral soil (c & d) and Meadow organic soil (e & f) after they had been subjected to 1 and 3 freeze thaw cycles (-7.5 to $+5^\circ\text{C}$). The results are fitted with a double first order exponential decay equation.

The internal microbial partitioning of glucose- and trialanine-derived C into either biosynthesis (immobilization - parameter a_2) or immediate respiration (mineralisation - parameter a_1) was different between the soil types. The temperate soil showed a lower proportion of glucose derived-C allocated to rapid respiration than soil from the Meadow site ($p < 0.05$; Table 6.4 a_1 control 1; Fig. 6.7). However, proportionally more trialanine was initially used for respiration in the temperate soil than in the Meadow organic soil ($p < 0.05$; Table 6.2 a_1 control 1; Fig. 6.7). The allocation of amino acid-derived C was not significantly different between soil types ($p > 0.05$; Table 6.3 a_1 control 1; Fig. 6.7).

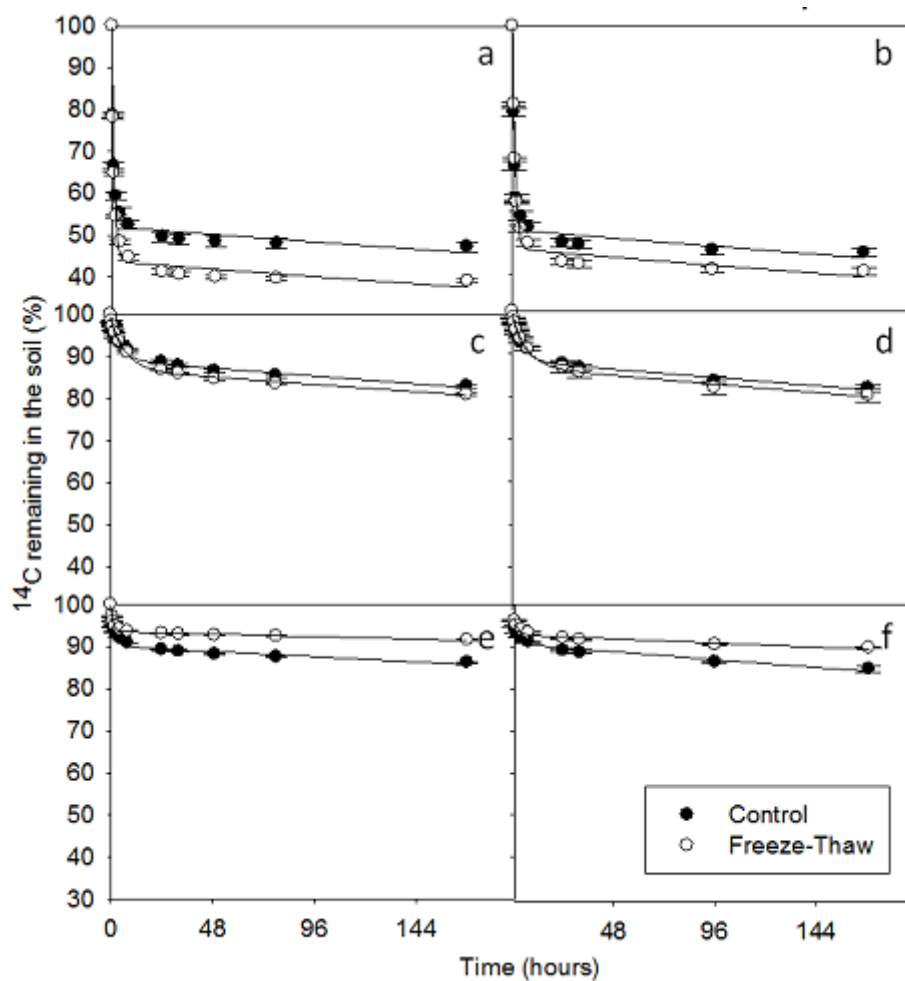


Fig. 6.5. Depletion of ^{14}C - added as ^{14}C -trialanine (a & b), ^{14}C -amino acids (c & d) and ^{14}C -glucose (e & f) from temperate grassland soils after they had been subjected to 1 or 3 freeze thaw cycles (-7.5 to $+5^\circ\text{C}$). The results are fitted with a double first order exponential decay equation.

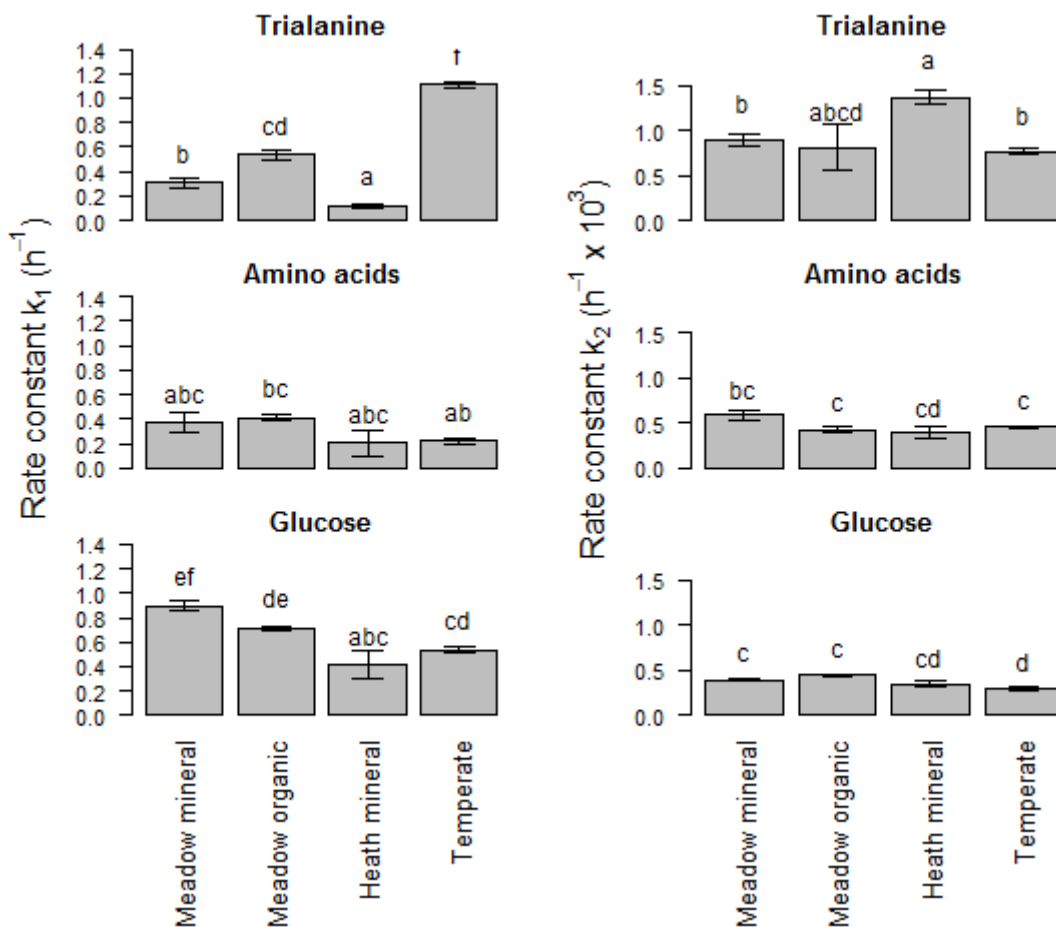


Fig. 6.6. Rate constants (left, parameter k_1 ; right, parameter k_2) of the control of each soil type after addition of ^{14}C substrate. Letters reveal significant difference within each parameter ($p < 0.05$)

6.3.3. Influence of freeze-thaw cycles on substrate mineralisation

Freeze-thaw treatment only had a significant effect on mineralisation parameter k_1 when comparing all soil and substrates ($p = 0.043$), but there were significant interactions between soil and FT treatment for all parameters except k_2 ($p = 0.001, 0.034, 0.013$ for parameters a_1, k_1 and a_2). The FT treatment had little effect on the mineralisation parameters in the arctic soils (Figs. 6.2-6.5, Tables 6.2-6.4, significant difference indicated by *). The Heath mineral soil showed no significant effect for any substrate after either 1 or 3 FT cycle ($p > 0.05$). However, it should be mentioned that there was relatively large variability between replicate results for the Heath mineral soil, which might have obscured any treatment effects. The Meadow mineral soil showed some effect of FT treatment on amino acid mineralisation, the

allocation of substrate-C into pools a_1 and a_2 changed after 3FTC with an increase in a_1 of $2.15 \pm 0.6\%$ ($p < 0.05$; Table 6.3, significant difference indicated by *). The Meadow organic soil only showed a significant treatment effect for glucose in k_1 after 1FTC (increase of 0.17 $p < 0.05$; Table 6.4, significant difference indicated by *).

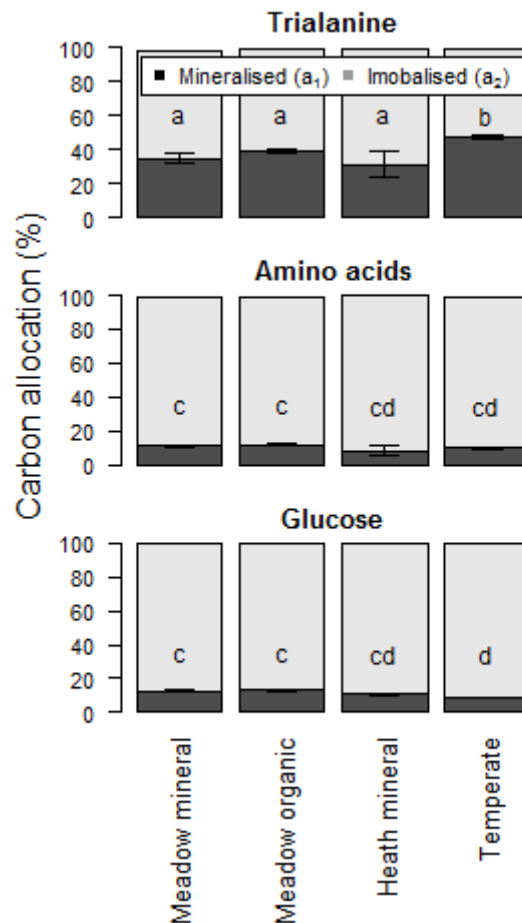


Fig. 6.7. Carbon allocation (parameters a_1 and a_2) in the control of each soil type after addition of ^{14}C substrate. Letters reveal significant difference ($p < 0.05$)

The temperate soil showed a greater response to FT treatment than the arctic soils (Fig. 6.2-6.5) with FT having a significant effect for a_1 , k_1 and a_2 ($p < 0.001$, = 0.013 and < 0.001 , respectively). There was also significant interaction between FT treatment and substrate for all mineralisation parameters ($p < 0.001$). For all three substrates after both 1 and 3FTC, except after 3FTC for glucose, the rate constant k_1 was significantly affected, decreasing for both trialanine and amino acids (by 0.20 ± 0.05 and $0.08 \pm 0.02 \text{ h}^{-1}$ after 1FTC and by 0.25 ± 0.06 and $0.06 \pm 0.01 \text{ h}^{-1}$ after 3FTC, for trialanine and amino acids, respectively). The

rate constant k_1 for glucose decreased after the first cycle, but analysis of the 3FTC was obscured by the variability in control 3. For glucose, the amount of substrate-C allocated to pools a_1 and a_2 changed after both 1 and 3FTC (a_1 decreased by 2.76 ± 0.03 and 1.85 ± 0.20 % after 1 and 3FTC, respectively).

Triananine					
Soil	Parameter	Control	FT	3 Control	3FT
Meadow Mineral	a_1 (%)	34.6 ± 3.7	37.8 ± 1.4	38.8 ± 3.4	39.9 ± 0.9
	k_1 (h^{-1})	$0.31 \pm 0.05\#$	0.24 ± 0.08	0.16 ± 0.03	$0.13 \pm 0.03\#$
	a_2 (%)	63.5 ± 4.0	60.1 ± 1.4	59.3 ± 3.7	58.2 ± 0.9
	k_2 ($\times 10^{-3} \text{ h}^{-1}$)	0.88 ± 0.06	1.02 ± 0.19	1.03 ± 0.07	0.98 ± 0.14
Meadow Organic	a_1 (%)	39.4 ± 1.2	41.8 ± 1.5	43.2 ± 1.5	38.8 ± 4.3
	k_1 (h^{-1})	$0.54 \pm 0.04!$	0.47 ± 0.03	$0.24 \pm 0.02!$	0.34 ± 0.07
	a_2 (%)	59.3 ± 1.1	57.0 ± 1.4	54.9 ± 2.7	59.7 ± 4.5
	k_2 ($\times 10^{-3} \text{ h}^{-1}$)	0.81 ± 0.26	0.68 ± 0.05	0.78 ± 0.12	0.66 ± 0.05
Heath Mineral	a_1 (%)	31.4 ± 7.4	29.6 ± 10.1	34.2 ± 5.8	34.1 ± 3.9
	k_1 (h^{-1})	0.12 ± 0.01	0.22 ± 0.15	0.08 ± 0.01	0.06 ± 0.01
	a_2 (%)	68.1 ± 7.7	69.6 ± 10.4	65.1 ± 5.9	65.3 ± 4.0
	k_2 ($\times 10^{-3} \text{ h}^{-1}$)	1.37 ± 0.09	1.00 ± 0.29	1.33 ± 0.11	1.34 ± 0.16
Temperate	a_1 (%)	$47.43 \pm 1.2\#\#$	$55.43 \pm 0.7^*$	48.87 ± 1.2	$53.16 \pm 0.7\#$
	k_1 (h^{-1})	$1.1 \pm 0.03\#\#$	$0.9 \pm 0.03^*$	$1.1 \pm 0.06^*$	$0.8 \pm 0.02\#\#$
	a_2 (%)	$52.01 \pm 1.2\#\#!$	$43.73 \pm 0.7^*$	$50.73 \pm 1.1!$	$46.17 \pm 0.8\#$
	k_2 ($\times 10^{-3} \text{ h}^{-1}$)	0.8 ± 0.04	0.9 ± 0.05	0.8 ± 0.01	1.0 ± 0.05

Table 6.2. Modelled double first order kinetic parameters describing the mineralisation of ^{14}C -labelled trialanine in arctic and temperate soils subjected to 1 or 3 successive freeze thaw (FT) cycles (-7.5 to $+5^\circ\text{C}$) and their respective non-frozen controls. * indicates a statistical difference between freeze-thaw treatment and the control, # indicates statistical difference between the freeze thaw treatment and the control of the other cycle, ! indicates statistical difference between cycles, ($P \leq 0.05$). Values represent means \pm SEM ($n = 4$).

For amino acids and trialanine, the amount of C allocated to pools a_1 and a_2 were only significantly affected by 1FTC (trialanine - a_1 increased by 8.00 ± 1.34 %, alanine - a_1 increases by 2.51 ± 0.53 %). A greater proportion of the trialanine and amino acid-derived C was allocated to initial respiration (pool a_1), whilst the opposite effect was observed in glucose. For glucose, the rate constant for the second C pool (k_2) was significantly lower after 1FTC (by $0.16 \pm 0.01 \times 10^{-3} \text{ h}^{-1}$), for the other substrates, k_2 was not significantly affected (p

> 0.05). There was significant interaction in the temperate soil between substrate and treatment and cycle for a_1 , k_1 and a_2 ($p = 0.037$, <0.001 and $= 0.013$, respectively). Less significant differences were observed between the 3FTC treatment and its associated control than between 1FTC and its control. However, the only significant differences observed between the 1FTC treatment and the 3FTC treatment, were in k_1 and k_2 for glucose.

Amino acids					
Soil	Parameter	Control	FT	3 Control	3FT
Meadow Mineral	a_1 (%)	11.2 ± 0.8	11.5 ± 0.7	10.8 ± 0.8*	12.9 ± 0.2*
	k_1 (h ⁻¹)	0.37 ± 0.08	0.24 ± 0.04	0.15 ± 0.03	0.14 ± 0.02
	a_2 (%)	88.1 ± 0.8	87.5 ± 0.7	88.4 ± 0.8*	86.2 ± 0.2*
	k_2 (x10 ⁻³ h ⁻¹)	0.59 ± 0.06	0.53 ± 0.07	0.47 ± 0.15	0.53 ± 0.03
Meadow Organic	a_1 (%)	12.0 ± 0.7	11.2 ± 0.3#!	13.3 ± 0.5#	13.7 ± 0.5!
	k_1 (h ⁻¹)	0.41 ± 0.03#!	0.37 ± 0.05!	0.22 ± 0.02!	0.19 ± 0.02#!
	a_2 (%)	87.4 ± 0.7	88.1 ± 0.4#!	85.9 ± 0.6#	85.5 ± 0.5!
	k_2 (x10 ⁻³ h ⁻¹)	0.43 ± 0.03	0.44 ± 0.02	0.54 ± 0.04	0.52 ± 0.03
Heath Mineral	a_1 (%)	8.6 ± 2.5	12.1 ± 0.5	13.5 ± 0.3	12.9 ± 1.7
	k_1 (h ⁻¹)	0.20 ± 0.11	0.10 ± 0.04	0.11 ± 0.04	0.06 ± 0.01
	a_2 (%)	90.9 ± 2.6	87.4 ± 0.4	86.0 ± 0.4	86.9 ± 1.6
	k_2 (x10 ⁻³ h ⁻¹)	0.39 ± 0.06	0.47 ± 0.04	0.55 ± 0.02	0.45 ± 0.04
Temperate	a_1 (%)	9.9 ± 0.5*	12.4 ± 0.3*	11.0 ± 0.6	12.5 ± 1.0
	k_1 (h ⁻¹)	0.22 ± 0.02*#	0.14 ± 0.00*#	0.21 ± 0.01*#	0.15 ± 0.01*#
	a_2 (%)	89.2 ± 0.5*	86.9 ± 0.3*	88.3 ± 0.6	86.9 ± 1.1
	k_2 (x10 ⁻³ h ⁻¹)	0.46 ± 0.01	0.44 ± 0.01	0.47 ± 0.03	0.50 ± 0.05

Table 6.3. Modelled double first order kinetic parameters that describe ¹⁴C labelled amino acid mineralisation in arctic and temperate soils subjected to 1 and 3 freeze thaw (FT) cycles of -7.5° C to +5° C. The parameters a_1 and a_2 are the proportion of substrate mineralised rapidly or slowly while k_1 and k_2 are their respective rate constants. * indicates a statistical difference between freeze thaw treatment and the control (not frozen), # indicates statistical difference between the freeze thaw treatment and the control of the other cycle, ! indicates statistical difference between cycles, ($P \leq 0.05$). Values represent means ± SEM ($n = 4$).

Glucose					
Soil	Parameter	1 Control	1 FT	3 Control	3 FT
Meadow Mineral	a_1 (%)	12.6 ± 0.3!#	11.3 ± 0.6	10.4 ± 0.3!	10.1 ± 0.6#
	k_1 (h ⁻¹)	0.89 ± 0.04#!	0.89 ± 0.06!	0.66 ± 0.05!	0.56 ± 0.06#!
	a_2 (%)	87.2 ± 0.3!#	88.1 ± 0.6	89.3 ± 0.3!	89.6 ± 0.6#
	k_2 (x10 ⁻³ h ⁻¹)	0.39 ± 0.01	0.38 ± 0.03	0.41 ± 0.04	0.34 ± 0.04
Meadow Organic	a_1 (%)	13.1 ± 0.5	10.7 ± 0.7#	14.0 ± 1.0#	11.1 ± 0.9
	k_1 (h ⁻¹)	0.72 ± 0.01*	0.89 ± 0.05*#	0.67 ± 0.03#	0.68 ± 0.05
	a_2 (%)	86.6 ± 0.5	89.1 ± 0.7#	85.8 ± 1.1#	88.8 ± 0.9
	k_2 (x10 ⁻³ h ⁻¹)	0.44 ± 0.02	0.36 ± 0.04	0.72 ± 0.01	0.46 ± 0.06
Heath Mineral	a_1 (%)	10.7 ± 0.9	9.1 ± 2.1	6.0 ± 1.9	5.9 ± 1.2
	k_1 (h ⁻¹)	0.41 ± 0.11	0.34 ± 0.07	0.19 ± 0.02	0.24 ± 0.04
	a_2 (%)	89.0 ± 1.0	90.3 ± 2.2	93.5 ± 2.0	93.8 ± 1.3
	k_2 (x10 ⁻³ h ⁻¹)	0.35 ± 0.03#	0.34 ± 0.03!	0.51 ± 0.1	0.66 ± 0.08#!
Temperate	a_1 (%)	8.9 ± 0.1*#	6.2 ± 0.1*#	8.6 ± 0.3*#	6.8 ± 0.2*#
	k_1 (h ⁻¹)	0.54 ± 0.03*	0.93 ± 0.08*!	0.70 ± 0.06	0.55 ± 0.03!
	a_2 (%)	90.2 ± 0.1*#	93.6 ± 0.1*#	90.7 ± 0.4*#	92.8 ± 0.2*#
	k_2 (x10 ⁻³ h ⁻¹)	0.30 ± 0.01*#	0.14 ± <0.01*#!	0.44 ± 0.07#	0.22 ± 0.01#!

Table 6.4. Modelled double first order kinetic parameters that describe the mineralisation of ¹⁴C labelled glucose in arctic and temperate soils subjected to 1 and 3 freeze thaw (FT) cycles (-7.5° C to +5° C). The parameters a_1 and a_2 are the proportion of substrate mineralised rapidly or slowly while k_1 and k_2 are their respective rate constants. * indicates a statistical difference between freeze thaw treatment and the control (not frozen), # indicates statistical difference between the freeze thaw treatment and the control of the other cycle, ! indicates statistical difference between cycles, ($P \leq 0.05$). Values represent means ± SEM ($n = 4$).

6.4. Discussion

6.4.1. Freeze-thaw effects on C mineralisation in different soils

Although the changes in C mineralisation we observed here in response to FTC were very small, in agreement with our initial hypothesis, temperate soils seem to be more susceptible to FTC than arctic soils. Freezing can reduce substrate and water supply to microbes, causing starvation and desiccation, whilst subsequent thawing can induce a rapid change in the

osmotic gradient, leading to cell lysis (Wilson and Walker 2010). Previous studies have suggested that FTC are most damaging when the microbial community is not adapted to them and where the freezing temperature is more extreme than they would naturally experience (Stres et al. 2010; Wilson and Walker 2010). The freezing temperatures used in this study are similar to minimum winter soil temperatures experienced at the arctic sites in 2012-2013, although they are colder than soil temperatures experienced in 2013-2014 (-3°C at the Meadow site and -4°C at the Heath site) which was a milder year with deeper snow cover (max - 131 cm, 45cm at Ny-Ålesund in 2013-2014 and 2012-2013 respectively (Norwegian Meteorological Institute Statistics)). The temperate soil rarely experiences freezing temperatures or snow cover. However, as some characteristics of the temperate soil were different to arctic soils (Table 1), namely nitrate, DOC, MBC and potentially others that have not been measured, there could be an alternative explanation for the greater effect shown by temperate soils. For example, different nutrient availability in the temperate soils could support a more active microbial community which is damaged more by FTC, as suggested by Schimel and Clein (1996).

There is some evidence that substrate mineralisation was less effected after 3FTC than after 1FTC as an interaction was observed between substrate, FT treatment and cycle. This is similar to some past studies where the adverse effects of FT have been show to decrease with repeated cycles (Goldberg *et al.* 2008; Larsen *et al.* 2002; Skogland et al. 1988; Yu et al. 2011), although Schimel and Clein (1996) suggested that up to 3 repeated FT might still have a damaging effect and other studies show maximum effect after 2FTC (Koponen et al. 2006; Morley et al. 1983; Yu et al. 2011). Whilst adverse effects decrease, significant recovery of microbial biomass during repeated FTC has not been observed (Morley et al. 1983; Skogland et al. 1988). There was no evidence that 3FTC has more of an effect than 1FTC in any of the soils.

Counter to the prediction of increased future FTC, projected increased precipitation could lead to increased snow depth, providing insulation from fluctuating air temperatures and thus warmer soils (Førland et al. 2011; Semenchuk et al. 2013). FTCs carried out over a narrow temperature range cause less damage than more intense freezing temperatures (Elliot and Henry 2009), so if a warmer freezing temperature had been used, we would have expected a smaller response. Whilst the temperatures used in this study were representative of air temperature fluctuations typically experienced at the arctic sites (Norwegian Meteorological Institute Statistics), it is unlikely that soils below the surface experience such extreme and

rapid temperature fluctuations. They are insulated by surrounding soil and vegetation (Henry et al. 2007). Furthermore, freezing and thawing soils require more energy than changing its temperature so a sustained period above freezing is required to fully thaw the soil even at the surface. These results suggest that an increase in air rapid temperature fluctuations around 0°C in the Arctic will have limited effect on the use of LMW DOC by soil microbes after thaw. It is possible that a longer freezing period than used in this experiment might have more effect on the microbial use of LMW DOC since longer freezing periods have been shown to be more damaging to the microbial community than shorter freezing periods (Haei et al. 2011). Such longer FTC would represent seasonal scale effects such as a winter warming event or thawing in spring rather than diurnal or weekly cycles.

6.4.2. Substrate specific freeze-thaw effects

The changes in mineralisation parameters for amino acids and trialanine were consistent with each other. The changes for glucose were different to the other compounds and were less consistent. Where the kinetic parameter k_1 was affected, it decreased for amino acids and trialanine. This suggests longer turnover times of the substrate. For glucose there was no trend in the change in k_1 . This was largely due to the change in the control between 1 and 3 FT events. It is unlikely that the change in k_1 was caused by any change in microbial community structure, size or activity as these have been shown to have little effect on LMW DOC mineralisation (Jones 1999; Rousk et al. 2011; Strickland et al. 2010). Strickland et al. (2010) found that P availability was important in predicting glucose mineralisation, in addition to land use and plant cover. FTC could potentially liberate organo-phosphates by cell lysis or soil bound phosphates by the break-up of soil aggregates (Freppaz et al. 2007). However, this would be likely to increase the mineralisation rate and that is not seen here. It is possible that the disintegration of soil aggregates could create fresh surfaces for phosphates to bind with, which could reduce the mineralisation rate (Özgül et al. 2012). Increases in the concentration of LMW DOC components due to FT could increase turnover time as there could be competition for uptake transporters (Jones and Hodge 1999). Uptake rate from soil has been considered to be equal to k_1 multiplied by the concentration of substrate in solution (Farrell et al. 2011). Thus an increase in soil substrate concentration caused by FTC could lead to an increase in microbial uptake rate despite a decrease in k_1 .

For amino acids and trialanine, a_1 increased due to FT whilst a_2 decreased. This suggests a decrease in C use efficiency as more substrate was used for respiration than growth and could be consistent with a lag phase after FT. Such a lag phase was shown in the results of Drotz et

al. (2010). The opposite result was observed for glucose making a lag phase less likely. However, glucose can be used by, or to make metabolites for, most bacterial metabolic systems (Cartledge et al. 1992). It is possible that opposing results could be caused by differences in internal substrate use. The parameter a_2 could also include substrate that was adsorbed to soil particles, however, the neutrally charged substrates used here all had low sorption potential. Haei et al. (2011) found changes in PLFA composition after FT, but they suggested this was due to changing membrane composition rather than changes to the microbial community structure. However, they also found that fungal growth rate increased relative to bacteria after FT. Jones et al. (2009, 2005) found the use of amino acids and peptides to be widespread amongst soil microorganisms so it is unlikely that a change in community would affect usage.

The k_2 parameter was only affected by FT for glucose. In the instances where this parameter was significantly affected it consistently decreased. The value of k_2 is dependent on the turnover of the microbial community and this is influenced by a number of factors including: grazing by organisms, such as protozoa, temperature, infection by viruses, heterolysis and substrate availability (Alexander 1981). It also depends on the biochemical pathway of the added substrate, for example, the allocation of the substrate-C into cell wall structures that tend to be slower to degrade compared to cytoplasm material, such as metabolites (Malik et al. 2013). The decrease in k_2 suggests a slower turnover time for biosynthesized glucose. If the microbial turnover had decreased it would be expected that the k_2 values of amino acids and trialanine would also decrease. This is not the case. It seems more likely that the glucose was used to produce less readily degradable material after FT. Further research is required to identify if this is the case.

6.4.3. C substrate turnover in soil

C cycling in arctic soils is typically dominated by the input and breakdown of plant residues (Bird et al. 2002). These residues are dominated by cellulose/hemicellulose and protein whose extracellular cleavage produces simple sugars, peptides and amino acids (Kögel-Knabner 2002). The results describing the mineralisation of these compounds in our arctic soil were similar to those presented for tundra heath by Boddy et al. (2008) and clearly showed a rapid turnover of this C within the soil solution. The amino acid half-life

($t_{1/2} = \frac{\ln 2}{k_1}$) for the temperate grassland calculated in our study (3.2 h) was similar to that measured by Jones et al. (2005) for the agricultural soil (2.9 h), but greater than that

measured for a similar soil from the same location (0.9 h) (Jones et al. 2005). This could be due to the soils being collected at different times (Glanville et al. 2012) or the much shorter mineralisation measurement period and warmer incubation temperature used by Jones et al, (2005). Farrar et al. (2012) measured longer turnover times for glucose in a similar temperate grassland soil than was found here. Few studies have looked at peptide mineralisation despite their importance in soil C and N cycling. Farrell et al. (2011) found a slightly slower turnover of trialanine ($k_1=0.77 \text{ h}^{-1}$ (Farrell et al. 2011) compared to 1.11 h^{-1}) in a similar temperate grassland soil to that used in our study. This is despite the fact that they used a higher incubation temperature (10°C compared to 5°C), which has been shown to increase k_1 in temperate soils (Jones 1999). The turnover of trialanine in the arctic soils appears to be slower than any of the soils analysed by Farrell et al. (2011, 2013). This could be due to the lower experimental temperature and less available nutrients in the arctic soils (Farrell et al. 2013; Strickland et al. 2010). Overall, we conclude our results describing substrate mineralisation in the control (non-FTC) soil are broadly similar to other studies providing confidence in this indicator to evaluate an FTC effect.

6.5. Conclusions

The aims of this study were to identify if FTC caused any change in the dynamics of LMW DOC mineralisation. The results described above indicate that short FTC induces a small change in LMW DOC mineralisation in soil from a temperate environment. However, FTC appeared to have little effect on the mineralisation of LMW DOC in arctic soils. Therefore, it seems unlikely that an increase in arctic FTC will directly affect the microbial utilisation of LMW DOC.

The changes in modelled mineralisation parameters due to FT, observed in the temperate soil and to some extent in the Meadow soils, varied with the LMW DOC compound. The changes observed for amino acids and trialanine were consistent with each other, showing longer turnover times and more C immediately allocated to respiration after FT. The response for glucose to FT was less clear and the change in C allocation was opposite to that of amino acids and trialanine, with a lower proportion of the C allocated to respiration due to FT. The reason for this difference requires further investigation.

Chapter 7

Liquid Water Content and Low Molecular Weight Dissolved Organic Carbon Mineralisation in Frozen Arctic Soils

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7. Liquid Water Content and Low Molecular Weight Dissolved Organic Carbon Mineralisation in Frozen Arctic Soils

Abstract

Arctic soils spend most, if not all, of the year frozen. Knowledge of dissolved organic carbon (DOC) mineralisation in frozen soil is vital for understanding the turnover of carbon and other nutrients in this ecosystem. The aim of this study was to investigate the microbial uptake and mineralisation of certain DOC substrates at different temperatures below 0°C in contrasting arctic tundra soils. Substrates labelled with ^{14}C were added to frozen soil to determine microbial substrate uptake and $^{14}\text{CO}_2$ mineralisation. As diffusion is an important factor limiting substrate use in frozen soils, the soil liquid water content below 0°C was also examined by alcohol calorimetry, the FREZCHEM model and a pre-melting model and the results were used to help model mineralisation rates below 0°C. Alcohol calorimetry showed the highest liquid water contents in frozen soils compared to the other models, but it lacked precision and possibly accuracy for the drier soils. FREZCHEM showed very low liquid water contents as this model does not include matrix effects. A pre-melting model showed higher liquid water contents than FREZCHEM, but was lower than derived from alcohol calorimetry, possibly due to hysteresis and an overly simple matrix model. Microbial uptake and mineralisation of all DOC substrates occurred at temperatures down to -6°C, but none was detected at -12°C. The increase in Q_{10} with decreasing temperature below 0°C was significantly less for glucose than for alanine or trialanine, whilst there was no clear difference between the latter two. There was no significant effect of soil type on Q_{10} , but the heath tundra soil had a lower substrate uptake rate at -3°C than the meadow tundra soil. A Dual Arrhenius Michaelis–Menten (DAMM) model gave a moderately good fit for the mineralisation rate of glucose at -3°C. These findings indicate that significant turnover of DOC can occur in frozen polar soils which has implications for modelling winter soil carbon loss in the Arctic.

7.1. Introduction

Although arctic soils can temporarily thaw in the summer, most spend much of the year at temperatures below 0°C. The soil's active layer can be frozen for up to 9 months. A number of previous studies have shown that the emissions of CO_2 during this period can be

substantial, up to 50% of the annual total, and can counteract the amount of carbon (C) sequestered over the growing season (Elberling and Brandt 2003; Grogan 2012; Sullivan et al. 2008). The origin of these emissions has been debated with suggestions that they could arise from either biologically-mediated processes, abiotic reactions, or the slow escape of CO₂ which was previously generated at a higher temperature (Elberling and Brandt 2003; Shanhun et al. 2012; 2014). Where the C mineralization is biologically mediated it remains unclear whether these reactions are occurring intra- or extra-cellularly or the extent to which this C is supporting cell growth or just maintenance. Microbial incorporation of substrate into biomass below freezing, however, has been shown in soils (Drotz et al. 2010; McMahon et al. 2009; Rakvina et al. 2000). In artificial media, microbial division has been observed down to -15°C (Mykytczuk et al. 2013). Cell maintenance has also been shown to occur at this temperature in frozen media (Dieser et al. 2013). Evidence for microbial metabolism exists as low as -39°C, however, the rates are extremely low (Panikov et al. 2006). Further investigation is required to gain a better understanding into the lower temperature limits to microbial life.

How changes in temperature affect the degradation of different C substrates in soil is still unclear. Some evidence suggests that microbes use more N-rich compounds under freezing conditions than when soils are warmer and it has been shown that the proportion of mineralised C which is of microbial origin increases (Mikan et al 2002; Schimel and Mikan 2005). Respiration in frozen soils correlates more with soil dissolved organic carbon (DOC) content than with total soil organic carbon (SOC) content whilst the reverse is true in unfrozen soils (Michaelson and Ping 2003). Ice growth confines substrate and enzyme transport to thin, viscous, water films. This could limit microbial access to the degradation products of plant detritus, forcing reliance on a more local C source. Alternatively, microbial death and damage due to freezing could increase the local availability of microbial C. The membrane composition of micro-organisms can change as temperature decreases and it has been suggested that this could alter a microbe's ability to take up substrate (Nedwell 1999). Low molecular weight dissolved organic carbon (LMW-DOC) can contain compounds which are produced and used rapidly by the soil microbial community above freezing. This could be an even more important C pool below 0°C (Michaelson and Ping 2003), but its functions and interactions has not been examined in much detail. As arctic temperatures are predicted to rise rapidly, particularly in winter, knowledge on how this will affect soil C dynamics is urgently needed (Anisimov et al. 2007).

Modelling microbial activity below 0°C has received more attention as the importance of winter greenhouse gas emissions has become apparent (Del Grosso et al. 2005). The Q_{10} relationship between temperature and microbial activity, which is used to model changes in CO₂ emissions with temperature, increases in value at temperatures around and below 0°C compared to those around 20°C (Mikan et al. 2002). Whilst models incorporate this change in Q_{10} at low temperatures, there is still debate as to how Q_{10} changes, with values from single figures to over 100 being reported (Del Grosso et al. 2005; Elberling and Brandt 2003; Tucker 2014). Another model has been proposed for respiration below 0°C. The Dual Arrhenius Michaelis–Menten (DAMM) model takes into account changes in liquid water content during soil freezing, enzyme activity, substrate supply and oxygen supply (Davidson et al. 2012; Tucker 2014).

Liquid water can exist in soils below 0°C due to; freezing point depression caused by solutes; thin films between ice crystals and mineral grains caused by repulsive forces between the surfaces; the instability of ice crystals of high curvature and a lack of ice crystal nuclei (Liu et al. 2007; Dash et al. 2006). A number of methods have been used to assess liquid water content below 0°C. These include nuclear magnetic resonance (NMR), chilled mirror hygrometry, time delay reflectometry (TDR), soil drying curves and FREZCHEM (Boike et al. 2008; Kurylyk and Watanabe 2013; Tilston et al. 2010; Watanabe 2012; Watanabe and Wake 2009). A model which calculates water content from forces between grains has also been proposed by Hansen-Goos and Wettlaufer (2010).

The activity of inorganic solutes can be modelled using FREZCHEM (Marion 1997; Marion et al. 2010). This model uses the Pitzer equations to find the activity of water and ions in solution, extrapolates below zero and has been calibrated using observations at high concentration natural systems and tertiary systems. However, this model was designed for sea and lake ice formation and does not take into account the soil matrix or organic compounds. In soils rich in organic material, ionic organic compounds, such as amino acids, could be a contributor to charge balance, but in most mineral soils the organic component should be relatively minor.

There were 4 main aims of this study. Firstly, ways to calculate soil water content at below 0°C were compared using alcohol calorimetry, FREZCHEM and the proposed equation for the pre-melting of ice in porous media (Hansen-Goos and Wettlaufer 2010). FREZCHEM was expected to produce the lowest soil liquid water contents. Secondly, to find out how

different freezing temperatures affect LMW DOC mineralisation in arctic tundra soil. We hypothesised that biological mineralisation of LMW DOC occurs below 0°C, albeit much more slowly than above 0°C and that this rate will continue to decrease with colder freezing temperature. That the mineralisation of N-containing compounds is less affected than the mineralisation of compounds without N. Also, that the mineralisation of larger MW compounds is more affected by freezing than for smaller compounds as they diffuse more slowly in thin films (Liu and Bruening 2004). For this purpose the microbial mineralisation of glucose, alanine and trialanine at different freezing temperatures was assessed. Thirdly, this study compared the mineralisation on N-containing (alanine) and non-N containing (glucose) compounds in different arctic tundra soils. It was expected that the organic rich soils would be less affected by freezing than the mineral soil of low carbon content. Finally, measured mineralisation rates at low temperature were compared with predictions made using the DAMM model.

7.2. Methods

7.2.1. Sample collection

Soil for the temperature-dependent mineralisation study was collected from Westbyelva near Ny-Ålesund, Svalbard (78°55.4 'N; 11°54.4'E) in July 2013 and consisted of a polar meadow mineral soil. Soils for assessing below-zero mineralisation rates were collected from Westbyelva and Kolhaugen in Svalbard (78°55.224'N; 11°52.439'E) in August 2014 and consisted of a polar meadow mineral and organic soil and a polar heath mineral soil. Properties of the soils are shown in Table 7.1. Soils were sampled from the top 5cm of the soil profile using a 5cm diameter steel core. The soils were transported to Bangor University and stored at 5°C in O₂ permeable bags. Stones, coarse roots and plant material >2 mm were removed by sieving or with tweezers for the mineral and organic soils respectively.

7.2.2. Soil characterisation

Soils were dried at 105°C prior to grain size analysis. To separate the sand, silt and clay fractions the soil was mixed with 3% sodium hexametaphosphate (w/v 1:6 soil:HMP), shaken for 15 minutes at 200 rpm and wet sieved between 2 mm and 63 µm. The between 63 µm and 2 µm, and < 2 µm size fragments were separated by leaving the < 63 µm suspension to settle for 5 hours. After this time, what was left in suspension was considered to be < 2 µm (Kettler

et al. 2001). Soil water content was measured gravimetrically by drying the < 2 mm soil at 105°C for 24 hours. Inorganic C concentrations in ground soil samples were measured gravimetrically by addition of 1 M phosphoric acid at 70°C using the method similar to that described by Skjemstad and Baldoc (2007). Total organic C content was measured using a Carlo Erba NA 1500 Elemental Analyzer (Thermo Fisher Scientific, Milan, Italy) and subtracting the total inorganic C content. Soil solution pH and electrical conductivity (EC) were measured in a soil-distilled water slurry (1:2.5, v/v soil:water ratio). Bulk density was calculated by dividing the dry weight of soil by its volume and soil particle density was measured by adding a known mass of soil to a 50 ml volumetric flask and dividing that weight by the volume of displaced solution. The ionic strength was estimated from the electrical conductivity according to Marion and Babcock (1976).

7.2.3. Below 0°C soil liquid water content

The liquid water content below 0°C was calculated using three methods namely: (1) FREZCHEM, (2) the equation suggested by Hansen- Goos and Wettlaufer (2010) for ice pre-melting in porous media, and (3) by alcohol calorimetry.

For FREZCHEM (version 5.2; Marion 2001)) the concentrations of the major anions and cations in the soil solution were required. Cations were extracted by shaking dry soil in 0.5 M ammonium acetate (1:10 w/v) for 1 hour, centrifuging for 8 minutes at 8000 rpm then filtering the supernatant through a 0.2 µm filter. Cation (Na, Ca, Mg, K, Fe and Al) concentrations were measured by inductively coupled plasma optical emission spectroscopy (ICP-OES Varian 720 ICP-OES, Varian Inc. Scientific Instruments, Palo Alto, USA). Anions (Cl⁻, SO₄²⁻, NO₃⁻) were extracted by the same method but using deionised water and were subsequently measured by ion chromatography (Metrohm 850 IC, Metrohm UK Ltd, Runcorn, UK). Carbonate alkalinity was assumed to be equal to the dissolved inorganic C content and was measured using a multi N/C 2100S (Analytic Jena AG, Jena, Germany). Measured concentrations were converted to mol kg⁻¹ soil solution at measured soil moisture contents and inputted into the model as described by Marion et al. (2010).

Hansen-Goos and Wettlaufer (2010) proposed a model for the liquid water content in thawing soils. They summed the water in thin films between soil grains and in areas of high curvature. Thin film thickness was estimated by the following equation:

$$0 = \frac{RT_m N_i}{d} + \frac{q_s^2}{\epsilon \epsilon_0} \left[1 - \frac{1}{\kappa d} \right] e^{-\kappa d} - \frac{A_H}{6\pi d^3} - \rho_l \mu_l \frac{\Delta T}{T_m}$$

Where the first term on the right hand side represents the Gibbs free energy of the ions in solution; the second and third are the interfacial free energy due to repulsion between grain surfaces, screened by the ions in solution, and the attraction from van der Waals interactions respectively; the final term shows the Gibbs free energy for the water to freeze. The symbols used represent the ideal gas constant ($R = 8.314 \text{ J K}^{-1} \text{ mol}^{-1}$); the melting temperature of ice in Kelvin ($T_m = 273\text{K}$); moles per unit area of solution (N_i) which is assumed to equal the number of moles of major ions per unit volume of soil divided by the calculated surface area, the molar density of water ($\rho_l = 55492 \text{ mol m}^{-3}$); film thickness (d), surface charge density of mineral (q_s) which is assumed to equal the charge density of ice and have a value between -0.5 and -1 C m^{-2} (Wang et al. 1997); the relative static permittivity or dielectric constant of water (ϵ) taken as 88, the vacuum permittivity ($\epsilon_0 = 8.854 \times 10^{-12} \text{ F m}^{-1}$); water's latent heat of fusion ($\mu_l = 6017 \text{ J mol}^{-1}$) and the difference between the melting temperature and the actual temperature (ΔT). The Debye length (κ^{-1}) is given by the equation

$$\kappa^{-1} = \sqrt{\frac{\epsilon \epsilon_0 k_B T_m d}{e^2 N_A N_i}}$$

Where $k_B = 1.3806488 \times 10^{-23} \text{ m}^2 \text{ kg s}^{-2} \text{ K}^{-1}$ and is the Boltzmann constant; e is the elementary charge ($1.60217657 \times 10^{-19} \text{ C}$), N_A is Avogadro's number (6.0221413×10^{23}).

An approximation for the Hamaker constant is given in Wilen et al. (1995) as:

$$A_{123} = -\frac{3}{2} k_B T \sum_{n=0}^{\infty} \left(\frac{\epsilon_1(i\xi_n) - \epsilon_3(i\xi_n)}{\epsilon_1(i\xi_n) + \epsilon_3(i\xi_n)} \right) \left(\frac{\epsilon_3(i\xi_n) - \epsilon_2(i\xi_n)}{\epsilon_3(i\xi_n) + \epsilon_2(i\xi_n)} \right) (1 + r_n) e^{-r_n}$$

Where the subscripts 1, 2 and 3 denote the mineral, ice and solution, respectively, the prime on the summation means that only half the $n=0$ value is used, n are integers, $r_n = \frac{2d\xi_n \sqrt{\epsilon_3(i\xi_n)}}{c}$, $i\xi_n = in \frac{2\pi kT}{h/2\pi}$ and is a range of imaginary frequencies, $\epsilon_j(i\xi_n)$ is the absolute permittivity or dielectric function of the mineral, ice or water. This approximation assumes that the dielectric function of all the materials is around 1. The dielectric function for each material approaches 1 at higher frequencies. The dielectric functions were calculated as described by Bonnefoy et al. (2005) and Hollertze et al. (2013). Parameters used to derive the dielectric function for

water, quartz, cellulose and ice were taken from Bergström et al. (1999), Hough and White (1980), Kislovskii (1959), Roth and Lenhoff (1996) and Seki et al. (1981) respectively. A_{123} gets smaller as d increases, but here it is assumed that A_{123} is non-retarded and it was calculated assuming d is a constant 1 nm.

In the model, Hansen-Goos and Wettlaufer (2010) use the random close packing of hard spheres model to calculate the surface area of soil such that $s_a = \frac{3 \times 0.634}{r_g}$, where r_g is the radius of the soil particles and 0.634 is the sphere packing fraction (Song et al. 2008). This is then multiplied by the film thickness from the first equation, calculated iteratively using the Newton–Raphson method, to give the volumetric liquid water content. Where this calculated liquid water content was greater than or equal to the above 0°C water content it was assumed that 100% of the water remained unfrozen below 0°C. The surface area does not include contact points between grains so this is likely an overestimate. Here a particle radius of 1 μm is used, corresponding to the clay grain size as the soils studied here contain these size fractions and it is the smaller fractions that control the water holding capacity (Botha and Eisenberg 1993). The pre-melting model also included liquid water in areas of high curvature and between ice grains, but at this grain size these were negligible. It should be mentioned that liquid water between ice grains is dependent on the size of the ice grains which is unknown (Hansen- Goos and Wettlaufer 2010). If the ice grains were smaller than assumed then this could become an important fraction of unfrozen water.

Alcohol calorimetry, adapted from Fisk (1986), was used to measure the weight of ice in frozen soil. Approximately 10 g of each soil ($n = 3$) was frozen at -3°C and the meadow mineral soil was also frozen at -6°C for 24 and 48 hours, respectively. The frozen soil was mixed with 50 ml of 99% ethanol in a steel calorimeter kept at 0°C in an insulated ice bath. The temperature of the soil-ethanol mixture was measured every 10 seconds using a temperature data logger (Maxim's iButton®, DS1922L). The temperature was plotted against time and the intercept of the tangent to the inflexion point of the increasing temperature curve (Fig. 7.1) was assumed to be equal to the total decrease in temperature if all the ice had melted simultaneously. This was converted to a weight of ice by calibrating with ice weighing between 0.5 g and 9 g. Dry soil was kept at the same temperature and used as a blank. It was considered that the presence of the soil matrix could delay the dissolution of the ice, which would cause a less sharp decrease in solution temperature, but also a longer duration decrease in solution temperature. This would cause a less steep increasing

temperature curve and could underestimate the ice content. Therefore, the area below 0°C of the time versus temperature graphs were also compared (Fig. 7.1).

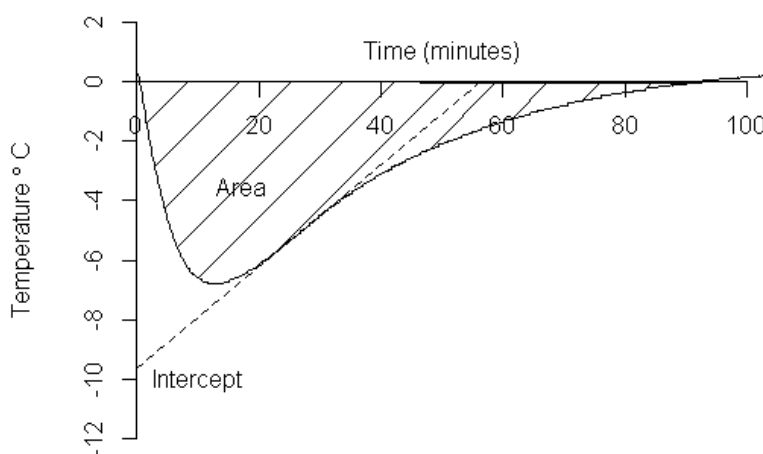


Fig. 7.1. Temperature of ethanol after addition of 8.4 g of ice. Illustrates methods of calibration for alcohol calorimetry.

7.2.4. ^{14}C -labelled sand

Acid washed, autoclaved silicon dioxide (50 g) was mixed with 5 ml of ^{14}C labelled glucose, alanine or trialanine (2 mM, 7.4 kBq ml^{-1}). The sand mixture was dried under a stream of air whilst being constantly agitated. The ^{14}C -labelled sand was stored at -20°C prior to use.

7.2.5. Mineralisation at different temperatures

Fresh soil and autoclaved soil (2 g) were weighed into sterile 50 cm^3 polypropylene centrifuge tubes. To investigate the effect of freezing temperature on the mineralisation of different substrates, polar meadow mineral soil was then left for 5 days at different temperatures (5°C , 0°C , -2°C , -6°C , -12°C , -35°C) in an incubator to acclimate. ^{14}C -substrate labelled sand (0.3 g) was added to the soil and mixed. The emitted $^{14}\text{CO}_2$ was captured by placing a vial containing 40 mg of solid $\text{Ba}(\text{OH})_2 \cdot 8\text{H}_2\text{O}$ inside the polypropylene tube containing the soil. The $^{14}\text{CO}_2$ traps were changed after 1, 2, 6, 24, 48, 72, 168, 336, 504 and 672 hours. To investigate the effect of freezing on different substrates between different soil types temperatures of 5°C , -1°C and -3°C were used and only alanine and glucose were investigated. The experiment proceeded as described above for the different soils except the measurements were stopped after 312 hours. To reduce potential temperature increases during $^{14}\text{CO}_2$ trap exchange and label addition, soils were placed on ice and only 3 vials were exchanged at a time. The temperature was monitored over the period using a temperature data

logger and only minor increases in temperature (1°C for 20 minutes) were observed during trap change.

After removing the $^{14}\text{CO}_2$ traps, deionised water (1 ml) was added to the vials and left over night for the $\text{Ba}(\text{OH})_2 \cdot 8\text{H}_2\text{O}$ to partially dissolve. The amount of ^{14}C in the barium hydroxide traps was quantified on a Wallac 1409 scintillation counter (PerkinElmer Corp., Waltham, MA) after mixing with Hi-Safe OptiPhase 3 scintillation cocktail (PerkinElmer Corp.). The percentage of the added ^{14}C remaining in the soil was calculated by subtracting the cumulative ^{14}C emissions up to each time point. The following double first order exponential decay model was fitted to the resulting data

$$Y = (a_1 e^{-k_1 t}) + (a_2 e^{-k_2 t})$$

Where Y is the percentage of ^{14}C remaining in the soil, a_1 and k_1 , are the relative size and rate constant, respectively, of the immediately mineralised substrate, a_2 and k_2 are the corresponding parameters for the more slowly mineralised substrate and t is time.

The ^{14}C remaining in solution after 672 or 312 hours was extracted using 5 ml ice cold 0.5 M Na_2SO_4 . The mixture was shaken on ice for 5 min at 200 rpm and centrifuged for 5 minutes at 5000 rpm. 1 ml of the supernatant was taken for ^{14}C quantification as described for the barium hydroxide traps.

7.2.6. Modelling the mineralisation rate below 0°C

A modified version of the DAMM model, proposed by Davidson et al. (2012) and modified for frozen soil by Tucker (2014) was used to calculate the mineralisation rate of glucose:

$$R_H = V_{max} \cdot \frac{[S_c]}{kM_s + [S_c]} \cdot \frac{[O_2]}{kM_{O_2} + [O_2]}$$

Where R_H is the mineralisation rate of glucose in soil. $V_{max} = \alpha e^{(-E_{as}/RT)}$ and describes the maximum mineralisation rate of the substrate when neither substrate nor oxygen is limiting, α is a pre-exponential constant, E_{as} is the activation energy of glucose and was taken to be 33 kJ mol^{-1} (Blagodatskaya et al. 2014), R is ideal gas constant and T is temperature. $[S_c]$ is the concentration of glucose available to soil microorganisms, $[O_2]$ is the oxygen concentration, kM_s and kM_{O_2} are the Michaelis-Menten half concentrations of glucose and oxygen respectively. kM_s was assumed to change with temperature and have a Q_{10} of 1.8 (section 7.3.6). kM_{O_2} was assumed to be unaffected by temperature.

The Michaelis-Menten parameters were determined for each soil by adding 200 µl of 8 concentrations (0.01, 0.05, 0.1, 0.5, 1, 10, 50, 100 mM) of ^{14}C -labelled glucose to the soil (2 g FW). The $^{14}\text{CO}_2$ released over 30 minutes was trapped and measured as described in section 2.4, but using 1 ml 1 M sodium hydroxide instead of barium hydroxide as the CO_2 trap. The results were used to determine kM_s and V_{max} by fitting regression models to the linear portion of the Eadie–Hofstee plot as described by Hill et al. (2008). The experiment was performed at 17°C and at 5°C allowing the calculation of α and thereby the calculation of V_{max} at different temperatures. It also allowed an assessment of the effect of temperature on kM_s . This temperature range was chosen to reflect the summer range of soil temperatures experienced by unfrozen arctic soils in the summer.

The concentration of glucose available to soil microorganisms $[S_c]$ is dependent on the diffusion of glucose through the soil. This is affected by decreasing water content as the tortuosity increases. Tucker (2014) take this into account using the following equation $[S_c] = [g] \cdot D_{liq} \cdot \theta$, where $[g]$ is the glucose concentration per gram of fresh soil, D_{liq} is a dimensionless diffusion coefficient and θ is the volumetric water content that remains unfrozen. Here D_{liq} is assumed to be the inverse of the water content at which the Michaelis-Menten parameters were calculated. The glucose concentration of the soils before the addition of ^{14}C glucose was measured by extracting soil solution by centrifugal-drainage (Gielsler and Lundström, 1993) and measuring glucose concentrations with an Amplex Red Glucose assay kit (Invitrogen™). It was found to be 1-3 nmol g⁻¹ DW which is an order of magnitude lower than the 30 nmol g⁻¹ FW of ^{14}C -labelled glucose added so we considered $[g]$ to be 30 nmol g⁻¹ FW.

The oxygen concentration was calculated by $[O_2] = D_{gas} \cdot 0.209 \cdot a^{4/3}$ where $D_{gas} = \frac{1}{a^{4/3}}$ and represents the dimensionless diffusion coefficient of O_2 in dry soil, 0.209 is its fractional abundance in air and $a = 1 - \frac{BD}{PD} - \theta - ice$ and is the air filled pore space of the soil. BD is the bulk density, PD is the soil particle density and $ice = (\theta_5 - \theta) \cdot \left(\frac{\rho_w}{\rho_i}\right)$, θ_5 is the water content at 5°C (i.e. above freezing), ρ_w is the density of water and ρ_i is the density of ice. kM_{O_2} is considered to be equal to $[O_2]$ at 5°C.

The calculated mineralisation rate was compared to the measured mineralisation rate given by the proportion of added ^{14}C mineralised in the first hour multiplied by $[g]$

7.2.7. Statistical analysis

Soil characteristics were analysed by one-way ANOVA. The effects of temperature, substrate and soil type on the mineralisation rate, Q_{10} and double first order exponential decay equation parameters were assessed using factorial ANOVA where homogenisation of variances could be achieved. Where this requirement could not be achieved a weighted least squares general linear model was used. TUKEY's post hoc tests were used to locate significant differences (SPSS version 22). The effect of temperature on the Michaelis-Menten parameters was assessed using a general linear model (Minitab version 17). Significance was taken where $p < 0.05$.

7.3. Results

7.3.1. Soil characteristics

Some clear differences exist between the properties of the soils used in the experiments (Table 7.1). The polar heath mineral soil is particularly low in organic carbon ($p > 0.003$), has a higher pH ($p < 0.001$) than the other soils and an electrical conductivity which is almost significantly greater than the other soils ($p = 0.102$ and 0.062 for the polar meadow mineral and organic soil, respectively). The polar meadow organic soil has the highest organic C content ($p > 0.029$) and has a low bulk density ($p < 0.007$) and particle density ($p < 0.002$). The moisture content was significantly different in all soils. The particle size distribution showed the polar meadow mineral soil had the greatest proportion in the sand particle size ($p < 0.001$), the polar heath mineral soil had a greater silt content ($p < 0.001$), whilst the polar meadow organic soil had a greater clay content ($p < 0.001$).

Soil	TOC (% DW)	pH	EC ($\mu\text{S cm}^{-1}$)	Initial Moisture (%)	Ionic strength ($\text{mol dm}^{-3} \times 10^{-3}$)
Heath mineral	1.1 ± 0.2 c	8.1 ± 0.1 a	181 ± 41 a	17.6 ± 0.4 c	2.6 ± 0.6 a
Meadow mineral	9.0 ± 2.3 b	5.8 ± 0.1 b	92 ± 24 a	26.8 ± 1.5 b	1.2 ± 0.3 a
Meadow organic	27.8 ± 5.8 a	6.0 ± 0.1 b	81 ± 17 a	57.6 ± 3.2 a	1.1 ± 0.2 a

Table 7.1. Summary of characteristics of the soils used in the experiments. Values represent mean ($n = 5$) \pm S.E.M, lower case letters indicate significant difference between soil types.

Soil	% sand 2 - 0.063 mm	% Silt 63 - 2 µm	% clay < 2 µm	Bulk density (g cm ⁻³)	Particle density (g cm ⁻³)
Heath mineral	49.1± 1.5 b	31.0± 1.1 a	19.9± 0.5 b	0.57± 0.03 a	2.39± 0.02 a
Meadow mineral	69.0± 1.6 a	12.1± 1.2 b	18.9± 0.5 b	0.44± 0.06 a	2.17± 0.12 a
Meadow organic	47.5± 1.3 b	9.5± 0.9 b	43.0± 0.7 a	0.16± 0.01 b	1.43± 0.04 b

Table 7.1. continued

The total extracted positive charge was greater than the negative charge for the major cations and anions. This is likely due to the cations being extracted from the negatively charged particle surfaces (i.e. the charge balance should also include the negative charge associated with the mineral surface). The ion concentrations were greatest from the meadow organic soil (Table 7.2 and 7.3). However, this soil had the lowest electrical conductance indicating that the ions were predominantly bound to organic matter cation exchange sites in this soil.

Soil	Na ⁺	K ⁺	Ca ²⁺	Mg ²⁺	Al ³⁺	Fe ²⁺
Heath mineral	12.3 ± 1.2 b	43.8 ± 0.9 b	537.2 ± 4.3 b	287.1 ± 6.3 b	1.0 ± 0.1 b	0.7 ± 0.1 b
Meadow mineral	33.4 ± 7.2 b	31.7 ± 3.7 c	469.3 ± 2.4 c	204.7 ± 10.0 c	3.8 ± 0.8 a	4.4 ± 1.1 a
Meadow organic	79.6 ± 10.1 a	336.3 ± 10.0 a	620.5 ± 8.3 a	429.9 ± 14.7 a	3.9 ± 0.4 a	5.2 ± 0.6 a

Table 7.2. Soil cation concentrations in mg kg⁻¹ dry weight. Values represent mean (n = 3) ± SEM..

Soil	Cl ⁻	NO ₃ ⁻	SO ₄ ²⁻
Heath mineral	26.6 ± 3.3 b	45.2 ± 2.6 a	35.9 ± 3.2 b
Meadow mineral	32.5 ± 2.0 ab	151.7 ± 14.4 a	33.9 ± 3.0 b
Meadow organic	54.5 ± 8.3 a	848.8 ± 413.6 a	97.6 ± 12.6 a

Table 7.3. Soil anion concentrations in mg kg⁻¹ dry weight. Values represent mean (n = 3) ± SEM..

7.3.2. Soil unfrozen water content

Use of FREZCHEM suggested a rapid decrease in water content below 0°C with 97% of available water freezing by -1°C. The differences observed in ion concentrations between the three soil types had only minor effects on the water content (Fig. 7.2). The FREZCHEM

version used (v5.2) experienced convergence problems at -16°C for a number of carbonate minerals. Namely dolomite, KHCO_3 , NaHCO_3 and CaCO_3 . This problem could be caused by the large charge imbalance from including the cations attached to the soil surface. The model would try and amend the charge balance by changing the carbonate chemistry. The polar meadow mineral solution could also have reached the eutectic point at -16°C where everything has precipitated.

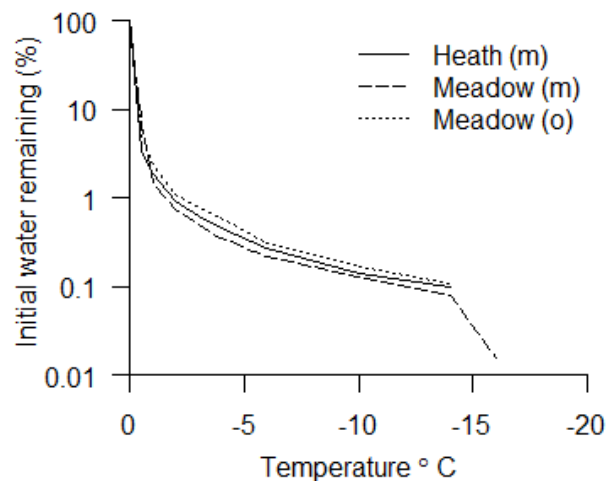


Fig. 7.2. Unfrozen water contents below 0°C modelled by FREZCHEM for the different soil types.

Calculating the Hamaker constant for the ice/water/quartz system gives a value of -2.74×10^{-21} J for a 1 nm film thickness. For an ice/water/cellulose system, the Hamaker constant at 1 nm separation was -8.18×10^{-22} J and for ice/water/lignin the Hamaker constant at 1 nm separation was -3.12×10^{-22} J. Wilen et al. (1995) obtained a positive Hamaker constant for the ice/water/quartz system compared to the negative one generated here. This is problematic as a positive Hamaker represents attractive van der Waal interaction whilst a negative indicates repulsion. This causes the collapse and preservation, respectively, of the thin water film at colder freezing temperatures and could be very important for assessing the thin film thickness. It has been shown that the dielectric function for water presented by Roth and Lenhoff (1996) is an overestimation at lower frequencies (Dagastine et al. 2000). This may be the cause of the different results. The value proposed by Wilen et al. (1995) of 3×10^{-23} J is therefore probably more accurate and was used for the calculation of film thickness. All the dielectric functions are derived from quite simple models and for full confidence in this

model at lower temperatures they need to be measured more accurately (Dagastine et al. 2000; Wilen et al. 1995).

The pre-melting model used here assumed that the soil solution phase behaves as an ideal solution. At high solute concentrations, however, this may not be the case. Assuming a surface charge of zero in the pre-melting model leaves only salt concentration to sustain liquid water so an assessment of using ideal solution parameters can be made. This produced an unfrozen (liquid) water content at least a factor of 10 greater than FREZCHEM (Fig. 7.3). With the pre-melting model using a particle radius of 1 μm the surface charge density only had a minor effect (Fig. 7.3b). Only at temperatures approaching -20°C was the water content produced by a surface charge density of 1 C m^{-2} (Coulombs per unit area) slightly higher than when the surface charge density was set to 0 C m^{-2} . Using a smaller particle radius of $0.1 \mu\text{m}$ rather than $1 \mu\text{m}$ increased the effect of surface charge relative to salt content. At the smaller grain size, and correspondingly larger surface area, the solute is more spread out so the film thickness decreases, bringing the surfaces closer together. Where surface charge was not included, no large effect of grain size was observed (Fig. 7.3a and b). For surface charge densities of 0.5 C m^{-2} and 1 C m^{-2} decreasing the particle size increased the unfrozen water content, particularly for 1 C m^{-2} (Fig. 3a). This suggests that surface charge is only an important factor at very small grain sizes. Despite the positive Hamaker constant, grain boundary collapse was not observed using these combinations of particle radius, concentration and surface charge density.

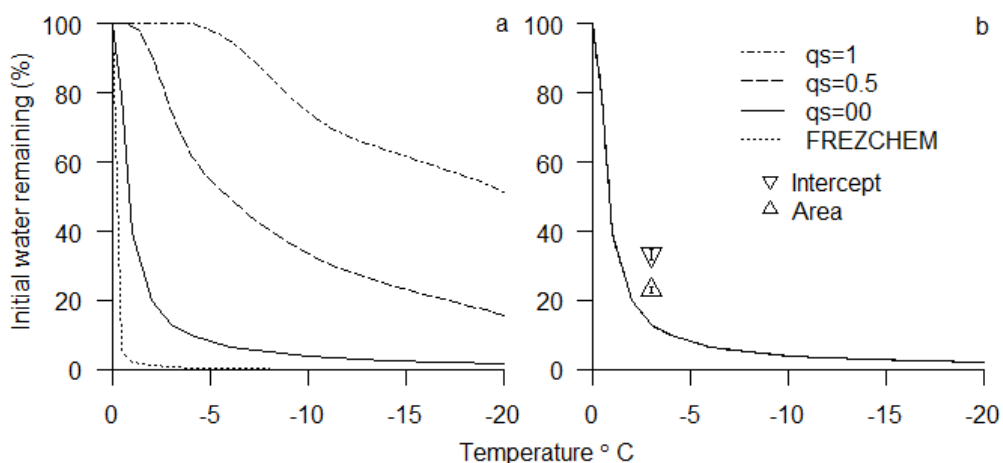


Fig. 7.3. Modelled liquid water contents below 0°C for the polar meadow organic soil for a particle radius of a) $0.1 \mu\text{m}$, or b) $1 \mu\text{m}$ for different surface charge densities (q_s in C m^{-2}). Surface charge density had no visible effect for $1 \mu\text{m}$ at the figures resolution. Also included are a) FREZCHEM and b) alcohol calorimetry results for the polar meadow organic soil.

The different salt contents of the 3 soils affected the pre-melting model results (Fig.7. 4). The polar heath soil had the greatest salt content per unit volume. This meant more of the initial water content was left unfrozen assuming a particle radius of 1 μm . At a lower grain size the salt content had the reverse effect, with the polar heath soil having the greater proportional decrease in water content. The ions in solution at the smaller grain size screen the effects of the surface charge and decrease film thickness and water content.

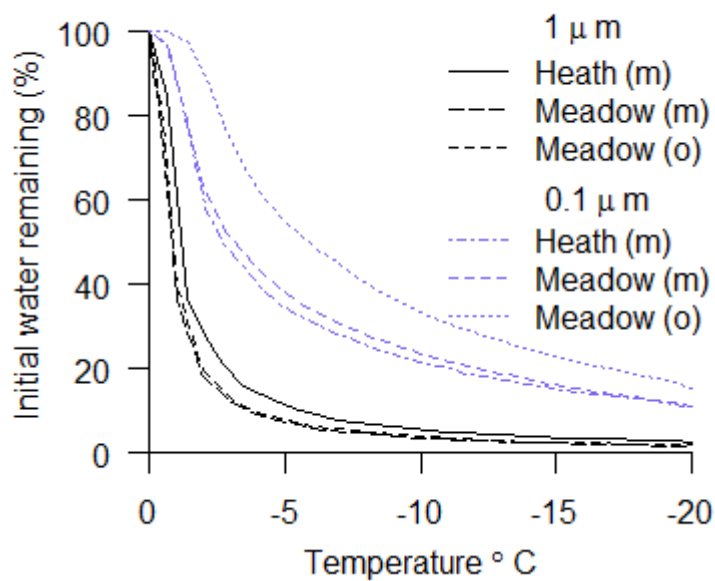


Fig. 7.4. Unfrozen water contents from pre-melting model results for the different soil types for a particle radius of 1 μm or 0.1 μm .

Alcohol calorimetry shows a decrease of water content with temperature, but this is only significant for the polar meadow organic soil (Table 7.4). The unfrozen water content in the mineral soils were more variable, each including one very low unfrozen water content. It is possible that ice could spill in to the calorimeter from the surrounding ice bath and thus may have affected the results. Using a larger amount of soil or wetting soils to a higher water content might produce better results. Using the area under the curve rather than an extrapolated intercept produced a significantly lower liquid water content in the meadow organic soil at -3°C (Fig.7.2b, Table 7.4). This suggests that using the intercept might underestimate the amount of ice formed when the ice is trapped in the soil matrix. The area

method is less accurate at lower ice contents (< 1 g) as small amounts of ice do not make the temperature of the ethanol drop below 0°C.

	> 0°C	-3°C	-3°C area	-6°C	-6°C area
Heath Mineral	17.4 ± 0.4	6.8 ± 3.5	9.8 ± 4.2		
Meadow Mineral	20.7 ± 5.5	9.6 ± 5.1	15.3 ± 3.9	6.0 ± 1.1	8.5 ± 2.8
Meadow Organic	57.3 ± 3.3 a	19.7 ± 2.3 b	13.5 ± 0.7 c		

Table 7.4. Mean (n = 3) liquid water content of fresh soil (w/w %) ± SEM. Letters indicate significant difference with temperature.

7.3.3. Changes in the mineralisation of LMW-DOC with temperature

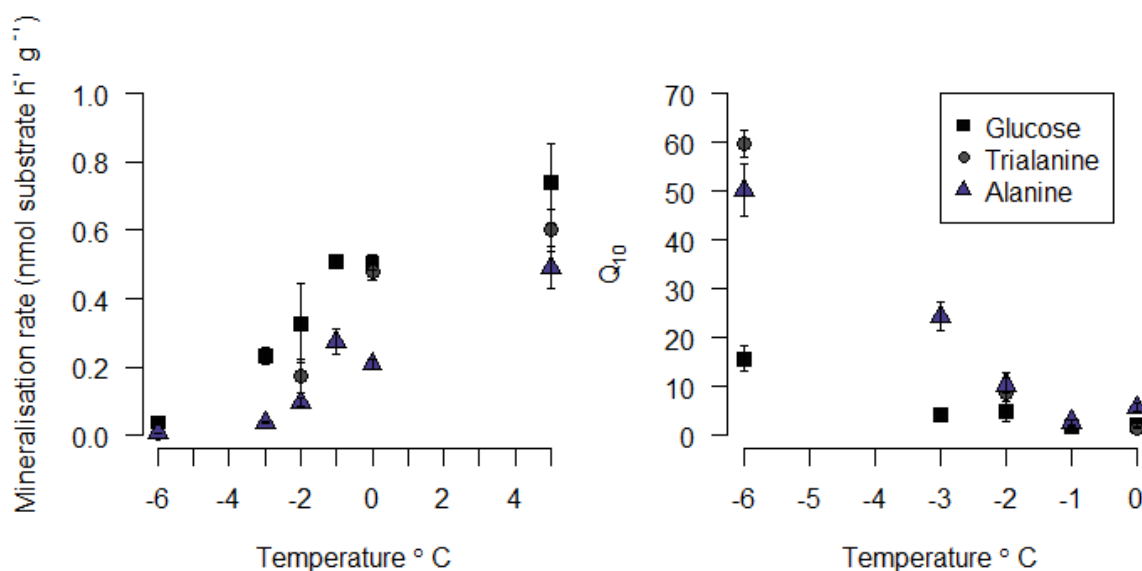


Fig. 7.5. The mineralisation rate of the added substrate in the first hour (left) and the Q₁₀ of this rate from 5°C. Values represent means ± SEM.

Emissions of ¹⁴CO₂ were detected at -6°C and above in the non-sterilised soil for all substrates (Fig. 7.5 and 7.6). Mineralisation rates in the first hour (Fig. 7.5) decrease significantly between 0°C and -2°C for trialanine (0.48 ± 0.03 to 0.17 ± 0.05 nmol trialanine h⁻¹; p > 0.001) and 0°C and -3°C for alanine (0.21 ± 0.01 to 0.04 ± 0.00 nmol alanine h⁻¹), glucose decreases significantly between 0°C and -6°C (0.51 ± 0.02 to 0.04 ± 0.01 nmol glucose h⁻¹; p > 0.001; Fig. 7.5). The mineralisation rate at -6°C is significantly lower than measured at any other temperature (p < 0.001). There is also a significant interaction between temperature and substrate with the mineralisation rate of glucose being significantly greater

than alanine or trialanine at -6°C . Correspondingly, the Q_{10} calculated from 5°C to the freezing temperature increased with decreasing temperature (Fig. 7.5). This increase was greater for alanine and trialanine than for glucose, but there was no significant difference observed between alanine and trialanine ($p > 0.05$). At -12°C , -35°C and in the sterile (autoclaved) soils, $^{14}\text{CO}_2$ emissions were not significantly greater than blank values. Therefore, these results were not fitted to the double first order exponential decay equation.

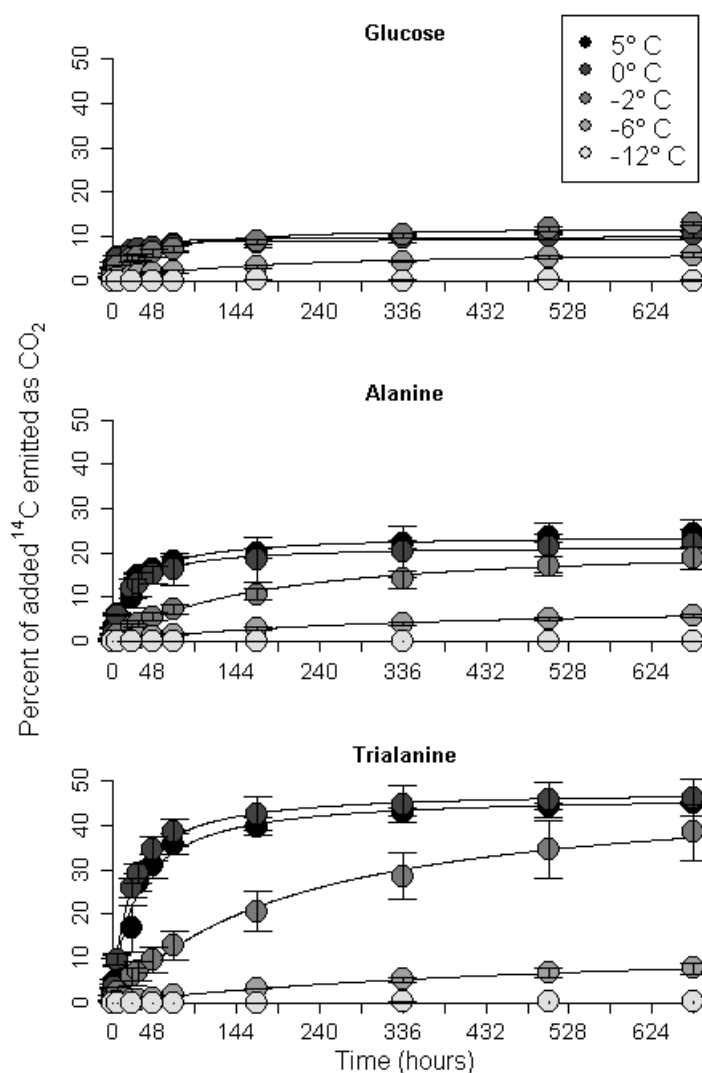


Fig. 7.6. Time-dependent mineralisation of LMW-DOC substrates in the polar meadow mineral soil at different temperatures. Values represent means \pm SEM ($n = 3$).

Significant effects of temperature ($p < 0.001$) and substrate ($p < 0.032$) were observed on all modelled mineralisation parameters (Table 7.5). A significant interaction between substrate and temperature ($p < 0.001$) was also observed for all mineralisation parameters. This interaction existed between all substrates except for the k_2 between alanine and trialanine.

The first rate constant (k_1 ; Table 7.5) decreased sharply between 0°C and -2°C ($0.230 \pm 0.013 \text{ h}^{-1}$ to $0.045 \pm 0.005 \text{ h}^{-1}$; $0.053 \pm 0.002 \text{ h}^{-1}$ to $0.018 \pm 0.004 \text{ h}^{-1}$; $0.041 \pm 0.002 \text{ h}^{-1}$ to $0.014 \pm 0.003 \text{ h}^{-1}$; for glucose, alanine and trialanine, respectively; $p < 0.003$ for all substrates) and to a lesser extent between -2°C and -6°C for all substrates ($0.010 \pm 0.004 \text{ h}^{-1}$; $0.004 \pm 0.001 \text{ h}^{-1}$; $0.003 \pm 0.001 \text{ h}^{-1}$, for glucose, alanine and trialanine, respectively; $p < 0.003$ for glucose, $p > 0.05$ for alanine and trialanine). There was no significant difference in the rate constants between 5°C and 0°C. The proportion of substrate assigned to the immediate mineralisation pool decreased significantly between 0°C and -2°C for both alanine and trialanine ($16.1 \pm 0.6\%$ to $8.5 \pm 0.8\%$ and $40.4 \pm 3.5\%$ to $15.8 \pm 4.6\%$, respectively $p < 0.007$), but not between -2°C and -6°C ($6.4 \pm 1.3\%$ and $9.0 \pm 2.1\%$ at -6°C for alanine and trialanine, respectively $p > 0.3$). The a_1 parameter for glucose also decreased below zero ($6.9 \pm 1.0\%$ to $6.3 \pm 0.6\%$ at 0°C and -2°C, respectively), but the difference did not prove significant ($p > 0.69$) until between 5°C and -6°C ($7.2 \pm 0.2\%$ to $3.8 \pm 0.3\%$, respectively; $p < 0.001$). The k_2 parameter did not decrease between 0°C and -2°C for any substrate (0.6 ± 0.03 to 1.0 ± 0.05 , 1.7 ± 0.03 to 4.8 ± 1.4 , 1.1 ± 0.01 to $1.8 \pm 0.04 \text{ h}^{-1} \times 10^4$, for glucose, alanine and trialanine, respectively), it actually increased significantly for glucose ($p < 0.001$). However, by -6°C k_2 values had decreased and were significantly lower than at 0°C for all substrates (0.03 ± 0.01 , <0.01 , $0.03 \pm 0.003 \text{ h}^{-1} \times 10^4$, for glucose, alanine and trialanine, respectively; $p < 0.001$).

Substrate	Temperature	a_1 (%)	k_1 ($\text{h}^{-1} \times 10$)	a_2 (%)	k_2 ($\text{h}^{-1} \times 10^4$)
Glucose	5°C	7.2 ± 0.2	2.49 ± 0.27	92.5 ± 0.2	0.86 ± 0.21
	0°C	6.9 ± 1.0	2.30 ± 0.13	92.9 ± 1.0	0.62 ± 0.03
	-2°C	6.3 ± 0.6	0.45 ± 0.05	93.2 ± 0.7	1.04 ± 0.05
	-6°C	3.9 ± 0.3	0.10 ± 0.04	96.0 ± 0.3	0.25 ± 0.10
Trialanine	5°C	38.6 ± 2.2	0.39 ± 0.04	60.4 ± 2.5	1.11 ± 0.20
	0°C	40.4 ± 3.5	0.41 ± 0.02	59.3 ± 3.5	1.69 ± 0.20
	-2°C	15.8 ± 4.6	0.14 ± 0.03	83.7 ± 4.9	4.81 ± 1.46
	-6°C	9.0 ± 2.1	0.03 ± 0.01	91.0 ± 2.1	0.00 ± 0.00
Alanine	5°C	19.4 ± 0.2	0.46 ± 0.01	79.7 ± 0.3	1.06 ± 0.07
	0°C	16.1 ± 0.6	0.53 ± 0.02	83.6 ± 0.5	1.13 ± 0.14
	-2°C	8.5 ± 0.8	0.18 ± 0.04	91.3 ± 0.8	1.83 ± 0.43
	-6°C	6.4 ± 1.3	0.04 ± 0.01	93.6 ± 1.3	0.03 ± 0.03

Table 7.5. Mineralisation parameters for the Meadow mineral soil at different temperatures measured over a 4 week period. Values equal mean ($n = 3$) \pm SEM.

7.3.4. Below 0°C mineralisation of LMW DOC in different Arctic soils

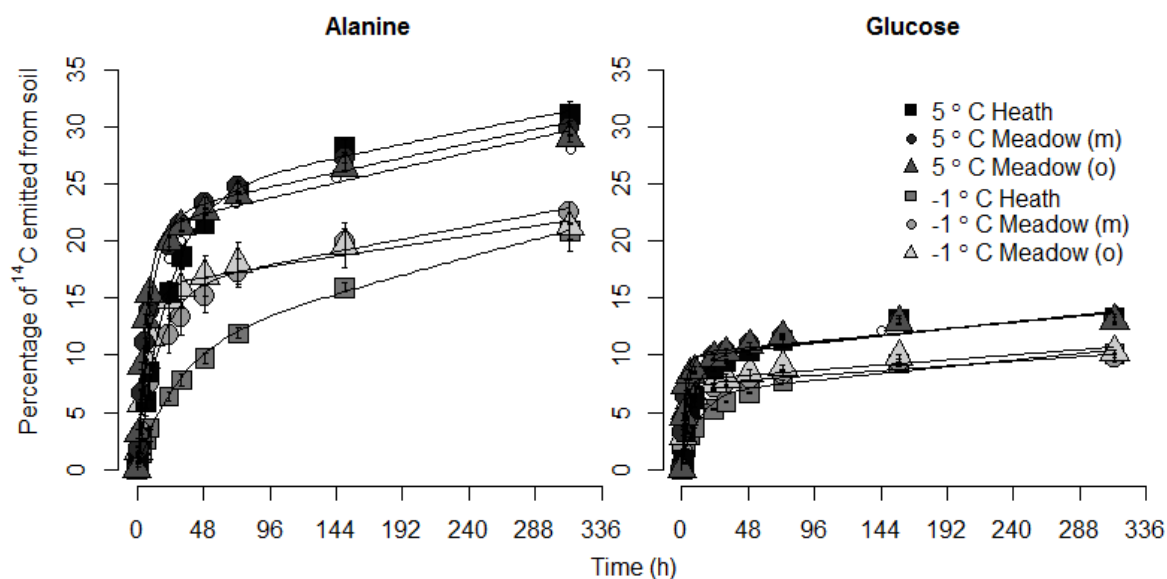


Fig. 7.7. Time-dependent mineralisation of alanine (left) and glucose (right) in different arctic tundra soil types +5°C or -1°C. Values equal mean ($n = 3$) \pm SEM (m) indicates mineral soil and (o) indicates organic soil.

Emissions of $^{14}\text{CO}_2$ were observed from all non-sterilised soils for both alanine and glucose at both -1°C and -3°C (Fig. 7.7 and Fig. 7.8). No emissions of $^{14}\text{CO}_2$ were detected from sterilised soils. Soil type had a significant effect on the mineralisation rate ($p < 0.001$) with the polar meadow organic soil having the greatest mineralisation rate in the first hour ($p < 0.031$), whilst the polar heath soil had the lowest mineralisation rate ($p < 0.001$) in the first hour for both substrates and temperatures. There was not a significant interaction between soil and temperature or between substrate and soil. There was a significant effect of soil, substrate and temperature on the rate constant k_1 ($p < 0.001$). The rate constant (k_1 ; Table 7.6 and 7.7) was consistently lower at -1°C compared to 5°C for alanine and glucose, although this decrease was not significant in any soil. A larger and statistically significant difference ($p < 0.005$) was observed between 5°C and -3°C for all soils and substrates. A significant interaction ($p < 0.001$) was observed between temperature and substrate with k_1 decreasing more for glucose than alanine. There was also a significant interaction between temperature and soil type ($p < 0.001$) with the decrease of k_1 being more pronounced in the meadow organic soil and least pronounced in the heath soil.

Temperature	Soil	a_1 (%)	k_1 ($h^{-1} \times 10$)	a_2 (%)	k_2 ($h^{-1} \times 10^4$)
5°C	Heath (m)	22.7 ± 0.7	0.45 ± 0.02	77.4 ± 0.6	3.33 ± 0.26
	Meadow (m)	21.0 ± 0.5	1.08 ± 0.03	78.9 ± 0.5	3.57 ± 0.19
	Meadow (o)	19.2 ± 0.7	1.64 ± 0.09	80.4 ± 0.7	3.41 ± 0.16
-1°C	Heath (m)	10.2 ± 1.5	0.33 ± 0.03	89.6 ± 1.4	3.98 ± 0.76
	Meadow (m)	15.3 ± 1.1	0.66 ± 0.17	84.4 ± 1.0	2.87 ± 0.09
	Meadow (o)	16.0 ± 1.5	1.42 ± 0.07	84.1 ± 1.6	2.31 ± 0.30
-3°C	Heath (m)	8.0 ± 5.7	0.09 ± 0.03	92.1 ± 5.7	1.47 ± 0.73
	Meadow (m)	9.6 ± 1.2	0.16 ± 0.02	90.4 ± 1.2	1.8 ± 0.61
	Meadow (o)	9.2 ± 0.1	0.20 ± 0.00	90.8 ± 0.1	3.15 ± 0.09

Table 7.6. Mineralisation parameters for alanine in different arctic tundra soils at different temperatures measured over a 2 week period. Values equal mean ($n = 3$) ± SEM. (m) indicates mineral soil and (o) indicates organic soil.

Soil, substrate and temperature all had a significant effect ($p < 0.001$) on the carbon allocation parameters a_1 and a_2 . A significant change in the amount of substrate-carbon immediately respired a_1 was observed between 5°C and -1°C, with values decreasing in all soils for both substrates, except the for alanine in the organic meadow soil, where this change was not significant. The a_1 parameter was also significantly lower at -3°C than -1°C for both substrates and all soil types, except in the meadow mineral soil for alanine. A significant interaction ($p < 0.001$) was observed between temperature and substrate with the substrate-carbon allocation of alanine changing more than glucose. This effect was observed to a greater extent in the heath soil (significant temperature and soil interaction, $p < 0.001$) Significant effects of temperature and substrate were observed on k_2 with significant interactions between temperature and soil and between temperature and substrate ($p < 0.05$). The k_2 values were usually less below zero, but further trends were not apparent.

Temperature	Soil	a ₁ (%)	k ₁ (h ⁻¹ x 10)	a ₂ (%)	k ₂ (h ⁻¹ x 10 ⁴)
5°C	Heath (m)	9.5 ± 0.3	0.99 ± 0.07	90.4 ± 0.3	1.88 ± 0.26
	Meadow (m)	9.2 ± 0.4	2.61 ± 0.15	90.5 ± 0.4	1.61 ± 0.14
	Meadow (o)	9.0 ± 0.2	3.97 ± 0.48	90.4 ± 0.1	1.89 ± 0.21
-1°C	Heath (m)	6.3 ± 0.3	0.80 ± 0.19	93.4 ± 0.3	1.35 ± 0.21
	Meadow (m)	7.3 ± 0.3	2.43 ± 0.08	92.6 ± 0.2	0.94 ± 0.08
	Meadow (o)	7.4 ± 0.3	3.69 ± 1.39	92.2 ± 0.2	1.03 ± 0.09
-3°C	Heath (m)	3.7 ± 0.2	0.25 ± 0.02	96.6 ± 0.3	1.31 ± 0.08
	Meadow (m)	5.1 ± 0.1	0.71 ± 0.11	94.5 ± 0.1	1.08 ± 0.13
	Meadow (o)	5.0 ± 0.2	1.01 ± 0.03	94.7 ± 0.2	1.26 ± 0.14

Table 7.7. Mineralisation parameters for glucose in different arctic tundra soils at different temperatures measured over a 2 week period. Values equal mean (n = 3) ± SEM. (m) indicates mineral soil and (o) indicates organic soil.

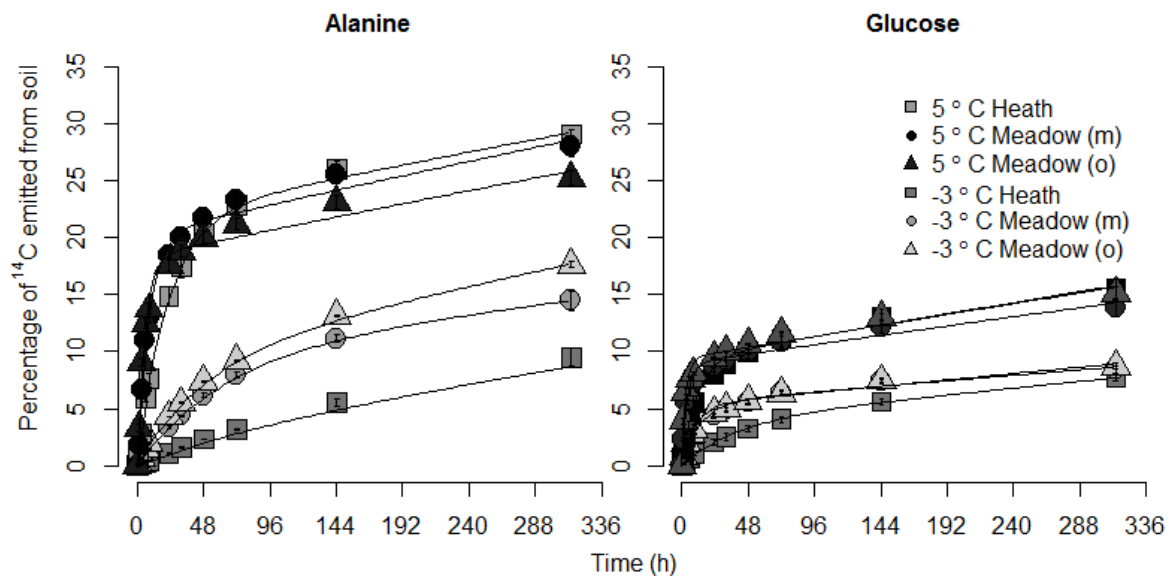


Fig. 7.8. The mineralisation of alanine (left) and glucose (right) in different arctic tundra soil types at either +5°C or -3°C. Values equal mean (n = 3) ± SEM. (m) indicates mineral soil and (o) indicates organic soil.

7.3.5 ^{14}C remaining in solution

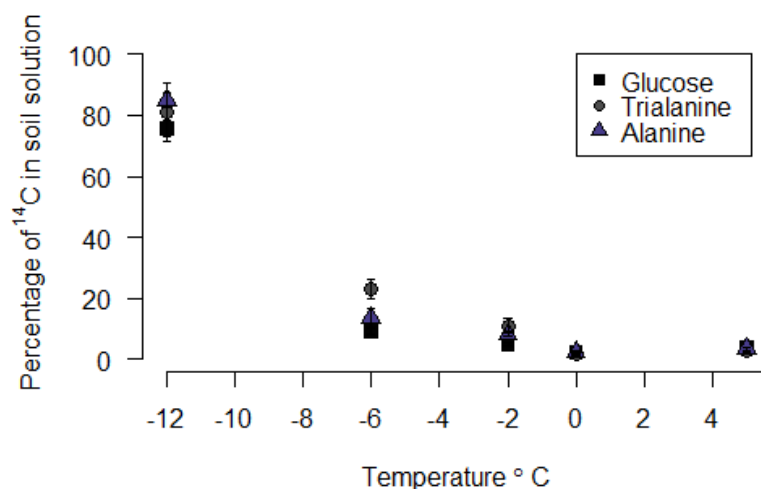


Fig. 7.9. Percent of added substrate remaining in the soil solution 4 weeks after addition to different arctic tundra soils. Values equal mean ($n = 3$) \pm SEM.

The amount of ^{14}C remaining in solution after 4 weeks increased with decreasing freezing temperatures, although differences between -2°C and -6°C were not significant (Fig. 7.9, $p > 0.05$). The ^{14}C remaining in solution at -12°C was not significantly different to the sterile soils (Fig. 7.9, $p > 0.05$). After 2 weeks there was significant interaction between substrate and temperature and between soil and temperature for the amount of ^{14}C remaining in solution (Fig. 7.10, $p < 0.001$). More ^{14}C tended to remain in solution for alanine than glucose at -3°C . The amount of alanine remaining in solution was significantly greater at -3°C than at 5°C . Whilst the alanine remaining in solution appears greater at -1°C than 5°C this difference was not significant ($p > 0.05$). For glucose the only significant difference in the ^{14}C remaining in solution soil was between the heath soil at -3°C and 5°C . There was also a large difference in the glucose 5°C controls between these experiments, just using the -3°C controls showed a difference between -3°C and 5°C in all soils. More ^{14}C remained in solution in the heath soil than in the meadow soils at -3°C .

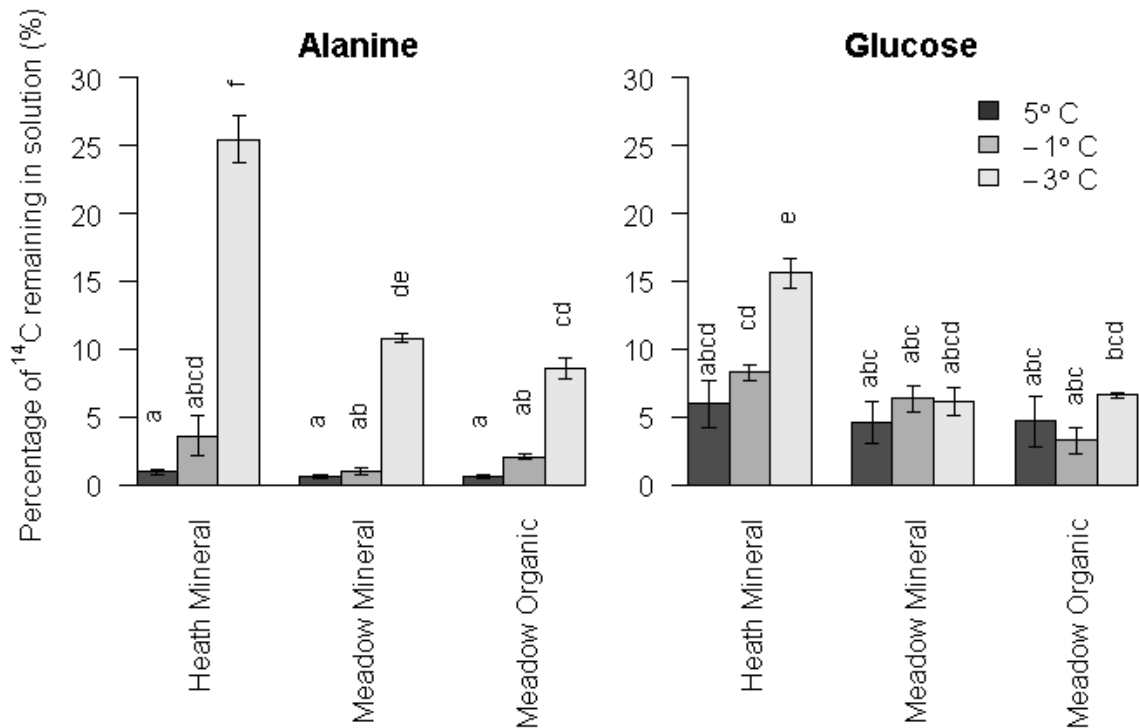


Fig. 7.10. Percent of added ^{14}C -substrate remaining in the soil solution 13 days after addition to different arctic tundra soils. Values equal mean ($n = 3$) \pm SEM. Letters indicate significant differences between treatments at the $p < 0.05$ level.

7.3.6. Kinetic parameters of mineralisation

Soil	17°C V_{max} (nmol glucose $\text{h}^{-1} \text{g}^{-1} \text{FW}$)	17°C kM_s (nmol $\text{g}^{-1} \text{FW}$)	5°C V_{max} (nmol glucose $\text{h}^{-1} \text{g}^{-1} \text{FW}$)	5°C kM_s (nmol $\text{g}^{-1} \text{FW}$)
Heath Mineral	2.2	34.7	1.6	19.6
Meadow Mineral	6.6	57.4	3.4	26.2
Meadow Organic	14.9	123.2	6.4	59.9

Table 7.8. Michaelis-Menten parameters for glucose mineralisation

The results of the kinetics of glucose mineralisation (Table 7.8) suggest a high affinity, saturable mechanism and a low affinity, non-saturable system. The results provided are those for the high affinity system. These values compare well to previous studies, although previously quite a large range of values have been found, possibly due to using different concentrations and only having a few points to calculate the parameters from (Rousk et al. 2014). Compared to the results of Boddy et al. (2008), who studied similar polar soil environments, the results presented here showed a similar V_{max} (0.039-0.007 here compared

to 0.027-0.007 $\mu\text{mol CO}_2 \text{ g}^{-1} \text{ h}^{-1}$) and a lower kM_s (1.2-3.6 mM C added compared to 2-5 mM C added). General linear modelling showed temperature had a significant effect on kM_s in the meadow mineral soil ($p = 0.029$) causing it to decrease with a Q_{10} of 1.8. The kM_s for Meadow organic soil and the Heath soil decreased with Q_{10} of 1.8 and 1.6 respectively but this was shown not to be significant ($p > 0.05$). This is a smaller Q_{10} than the 5.37 calculated by Blagodatskaya et al. (2014). V_{max} also tended to decrease with temperature. This was significant for the mineral soils (polar heath $p = 0.024$, polar meadow $p = 0.004$), but not for the organic soil ($p = 0.129$). Linear regression was significant ($p < 0.005$) for the Eadie–Hofstee plots of all soils, for added concentrations below 0.5 mM, and gave a reasonable fit for both mineral soils ($r^2 = 0.63$ -0.870), but the fit for the organic soil was less good ($r^2 = 0.45$ -0.48).

7.3.7. DAMM model fitting

The model produced glucose mineralisation rates within measured values at 5°C for all the soil types ($p > 0.17$). The model tended to overestimate the glucose mineralisation rate of all soils at -3°C when water contents measured by alcohol calorimetry using the intercept method. The difference was only significant for the meadow organic soil ($p = 0.002$) whilst the difference for the meadow mineral soil was almost significant ($p = 0.075$). The heath soil water content includes a negative soil water content which made the average rate lower than it should be. The modelled rate was improved by using the area under the curve to estimate water content in the meadow organic soil producing rates which were not significantly different to those measured, but they were still almost significantly higher ($p = 0.069$). This suggests that the liquid water content used was too high. This supports the suggestion that using the area under the curve is better for calculating water content in frozen soils. When unfrozen water contents generated by FREZCHEM were used the model severely underestimated the mineralisation rates (Fig. 7.11). Using the water content generated by the pre-melting model, for a particle radius of 1 μm , the DAMM model overestimated the Heath soil mineralisation, but underestimated the Meadow soil respiration (Fig. 7.11).

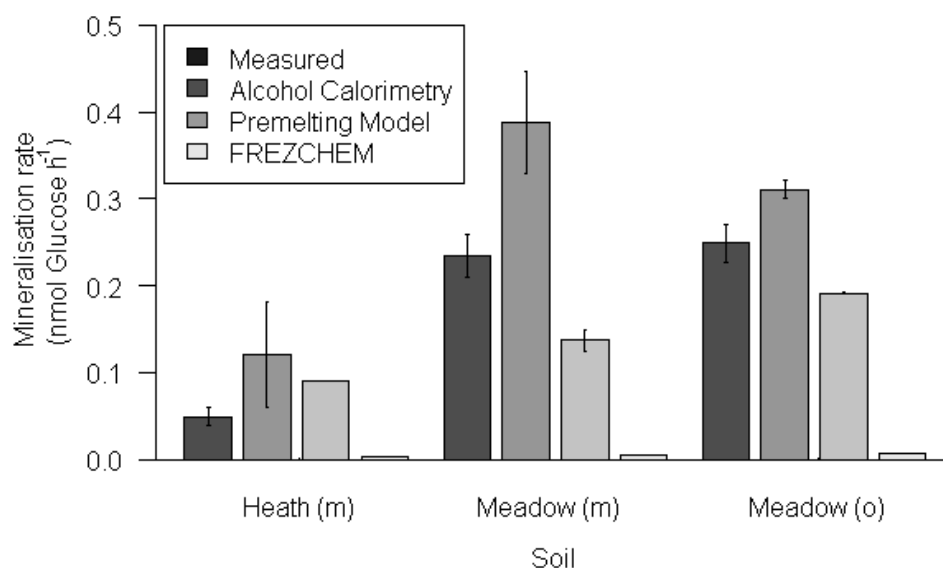


Fig. 7.11. Modelled versus measured mineralisation rates for glucose in three contrasting polar soils. Values represent means \pm SEM.

7.4. Discussion

7.4.1. Unfrozen water content

The liquid water content below zero was greatest when measured by alcohol calorimetry. Values measured were within the range of previously reported values. They were higher than those obtained by ^2H NMR by Tilston et al. (2010) at similar temperature which were between 5.3%-9.6% dry weight of soil compared to the 7-44% found here. They were lower than those reported by Öquist et al. (2009) which were 27-58% dry weight of soil. Compared to the volumetric unfrozen water contents calculated by Romanovsky and Ostercamp (2000) at -3°C (1.7-6.9%) the volumetric unfrozen water content calculated by alcohol calorimetry (area) for the Meadow organic soil (0.8%) was lower, but the other soils and the intercept method produced similar results (3-7.3%). Romanovsky and Ostercamp (2000) calculated liquid water content from measured in situ soil temperature using a heat flux model. Homogenising the soil samples likely decreased the bulk density of the soil samples used here and as a result decreased the volumetric water content. This might explain why the volumetric unfrozen water content of the Meadow organic soil was so low. It is possible that the lower initial water content in Tilston et al. (2010) could result in a lower water content after freezing. It has been shown that initial water content does not have an effect on the soil

water content after freezing, but this can only be true if all available particle surfaces are covered with water. If not then the maximum potential for liquid water below freezing is reduced. The water contents calculated by alcohol calorimetry predicted slightly too high mineralisation rates using the DAMM model. This suggests that the water contents were lower than predicted. It is possible that this could be due to the soils being frozen for 1 day the ice was measured, but 5 days before the mineralisation rate was measured so ice had less time to form. Therefore, the water contents calculated by alcohol calorimetry could be correct, but not necessarily the same as the water content when the mineralisation rates were determined.

Using FREZCHEM, for soil solution concentrations which include cations on soil exchange sites, suggested that between 0°C and -1°C more than 97% of the soil solution froze. This method produced the lowest predicted soil water contents in frozen soil. FREZCHEM predicted much lower water contents than was predicted using an ideal solution. Harrysson-Drotz et al. (2009) found that the solution in soil behaved similarly to an ideal solution even at high concentrations. It is possible that the soil particles separate the ions in the solution making them less likely to interact and making the solution behaviour more ideal. The very low predicted emissions from the DAMM model using water contents derived from FREZCHEM supports the use of the ideal solution for modelling the solute contribution to unfrozen water content below 0°C rather than using FREZCHEM.

The pre-melting model agreed with the results of Harrysson-Drotz et al. (2009) who found that salt concentration played a vital role in keeping water in liquid form. The model suggested that surface charge effects became relatively more important at colder temperatures as film thickness decreased, but only if the surface charge is great enough. Wang et al. 1997 showed that soils can have a permanent surface charge up to 0.48 C m^{-2} and that surface charge increases with both the ionic strength of the solution and the pH. The effect of surface is also more important for smaller particles as salt becomes spread out further and the liquid films became thinner. A particle size of $0.1 \mu\text{m}$ is possible for clay. Old degraded organic material can be rough, containing cracks and micropores so a particle radius smaller than $1 \mu\text{m}$ might not be inappropriate (De Gryze et al. 2006). The liquid water content from the pre-melting model in the DAMM equation predicted mineralisation rates which were close to measured rates. This suggests that the pre-melting model produced reasonable water contents when the particle radius was set to $1 \mu\text{m}$. Too great a mineralisation rate in the Heath soil, but too low in the Meadow soils was predicted. This could potentially be due to the greater

organic matter at the Mineral site which could have a greater surface area than predicted by the 1 μm particle radius as mentioned above.

The matrix model that the pre-melting model uses is quite simple, using one particle radius and one mineral type. These parameters of the pre-melting model need to be more accurately defined for the specific soil type before it can be used to estimate unfrozen water content. The soil surface area could be measured and used rather than using an approximate grain size. The surface charge density of both the mineral and ice would be affected by both the ionic strength of the solution and the proximity of the other surface (Behrens and Grier 2001; Thomson et al. 2013). Further work is required to fit this in to the model. The dielectric functions that are used to produce the Hamaker constant are also overly simple and need to be improved in order to make confident predictions of thin film thickness (Dagastine 2000; Wilen 1995). This might be more important in smaller grain size soils or sediments.

Freezing and thawing of soil exhibits hysteresis with thawing soils having a lower liquid water content than freezing soils at the same temperature (Sparman et al. 2004). Freezing in soil is not an equilibrium process, depending on ice crystal nuclei and geometry of ice crystal growth, slower reaction kinetics at cold temperatures and water flow, through soil towards the freezing front (Zachariassen and Kristiansen 2000; Zeng et al. 2015). Properly modelling the point where the bulk solution freezes during freezing is not possible with either the pre-melting model or FREZCHEM. The pre-melting model predicts the water content on thawing rather than freezing which partly explains why it is lower than the alcohol calorimetry method.

Pore sizes in soil tend to range from 10 nm to 1 μm in clay-rich soils (Diamond 1970), whilst sandy soils can have a larger proportion around 10 μm to 100 μm . Previous studies have shown that water in pores of 5 nm diameter do not freeze until -39°C (Morishige and Nobuoka 1997). This suggests that in clay rich soils a large proportion of liquid water available below 0°C could be held in these small pores. However, the compressibility of the soil can allow more ice growth and this is demonstrated by the soil polygons, frost boils and ice lenses (Dash et al. 2006). Ice could propagate from larger pores in to nanopores (Zeng et al. 2015). The smallest known bacteria are larger than 0.1 μm and most are larger than 1 μm so most microbes can only exist in the larger soil pores (Luef et al. 2015). Therefore, it is possible that only a proportion of the unfrozen water is microbially available. This is likely dependent on the connectivity of the water which could decrease if thin films collapse or in

unsaturated soil. In pores large enough to house microbes they can exist in small colonies or can form a biofilm depending on substrate availability. Microbes can exude extracellular polysaccharides which can help retain water through both increasing the concentration and by preventing the growth of large ice crystals which allows for the presence of liquid water between ice grains. However, smaller ice crystals could lead to slower substrate diffusion due to smaller liquid veins and greater tortuosity (Brown et al. 2012)

7.4.2. Substrate mineralisation below zero

The results presented clearly show microbial respiration at sub-zero temperatures. This is in agreement with numerous past studies (Bakermans and Skidmore 2011; Drotz et al. 2004; Panikov et al. 2006; Schimel and Mikan 2005). Shanhum et al. (2012) found that alkaline soils were more likely to produce CO₂ by inorganic than biological processes. The results for the low organic C, high pH heath soil show that these soils certainly have the capability to mineralise LMW substrates biotically. However, whether this could occur without the addition of substrate was not determined in this study.

This study adds the substrate as a sand after the soil has frozen. Therefore, these results show that microbes can take up LMW DOC and DON between 0°C and -6°C. At -12°C, however, microbial uptake either does not occur or it occurs too slowly to significantly change the amount of ¹⁴C remaining in solution after 4 weeks. Whilst it is not impossible that some substrate may have been taken up by microbes and returned to the solution either as extracellular compounds or by cell lysis, no detectable ¹⁴CO₂ emissions at this temperature supports the assumption that no microbial uptake occurred. Bacterial growth has been observed in *Planococcus halocryophilus* as low as -15°C (Mykytczuk et al. 2013). This occurred in growth media that does not freeze until -16°C. This suggests that microbes can take up substrate at temperatures below -12°C from which it can be inferred that limited diffusion is the cause of the observed lack of substrate uptake by microbes. The pre-melting equation suggests that the thin film of water that exists between ice crystals and quartz can collapse between -6°C and -12°C (Hanson-Goos and Wettlaufer 2010). This would limit liquid water existence to unconnected areas of high curvature or concentrated solution so transport of substrate would largely cease. This suggests that emissions observed from soil at -12°C and below come from within cell resources or nearby sources. However, Drotz et al. (2010) showed that microbes take a long time to adapt to temperature at -9°C so it is possible that microbial uptake could occur if the incubation time was increased. The model is also

overly simplistic using only quartz rather than the range of minerals potential in soil. Dielectric data for these minerals is required to make a better assessment of soil water availability at lower freezing temperature in soil.

Mineralisation rates decreased with temperature below 0°C or -1°C whilst the Q_{10} increased with decreasing temperature. This is consistent with past studies (Elberling and Brandt 2003; Mikan et al. 2002). Contrary to the stated hypothesis, the mineralisation rate of the N containing compounds, alanine and trialanine, was more affected by temperature than glucose. Also the uptake of alanine was significantly lower than glucose at -3°C. This suggests that the increase in N mineralisation observed in frozen soils is not due to a microbial preference for N containing compounds in frozen conditions, but due to the increased N content of available substrate on freezing (Schimel and Mikan 2005). However, it should be mentioned that the three substrates used only represent a small amount of the available substrate and other N containing compounds could be less affected by temperature.

The results also show some differences in mineralisation parameters below 0°C. The initial rate constant of glucose (k_1) decreases more than that of either alanine or trialanine. This is the opposite of the observed effect of temperature on mineralisation and seems to support the hypothesis that the use of compounds containing N is less affected by soils being frozen than compounds that do not contain N. However, the a_1 parameter, representing the proportion of rapidly mineralised substrate, of tri-alanine and, to a lesser extent, alanine decreased more than that of glucose with freezing temperature. This is counter to the above hypothesis. The mineralisation rate for trialanine and alanine appears to be decreasing due to a lack of available substrate rather than the speed the microbes could mineralise the substrate.

Usually the a_1 and a_2 parameters of the double first order exponential decay model can be used to stand for the proportion of C allocated to respiration or biomass. Here, this cannot be applied as decreased diffusion with temperature could decrease travel time and the uptake of substrate by microbes. Furthermore, the diffusion of CO₂ out of the soil would also decrease with temperature, although this should be the same for each substrate. Therefore, the proportion of substrate entering the biomass cannot be calculated or said to be increasing with decreasing temperature. However, as the a_2 parameter increased significantly more for trialanine and alanine than glucose, it can be suggested that either diffusion or microbial uptake of these substrates decreased more than glucose or more went to the biomass below freezing. That significantly more alanine than glucose was found in solution after 2 weeks at -

3°C supports the theory that alanine and trialanine mineralisation is either uptake or diffusion limited. Trialanine diffusion could be more limited than alanine due to its size hence the bigger increase in a_2 (Liu and Bruening 2004). Alanine is less soluble than glucose. This may retard its progress in thin films of high ionic strength which has less dissolving capacity than the bulk solution (Klein et al. 2004). Alternatively, microbial glucose uptake has a different active transport mechanism than alanine or trialanine, using group translocation as well as an ion driven or protein binding system. This could potentially make the membrane changes with cold temperature have less effect on glucose uptake (Nedwell 1999). It is also possible that more carbon is allocated to anabolic processes. Whilst the active microbial community has been shown to change with freezing, the mineralisation parameters of low molecular weight DOC has been shown to be little effected by changing microbial community composition (Buckeridge et al. 2013; McMahon et al. 2009; McMahon et al. 2011; Rousk et al. 2011).

The mineralisation rate in the different arctic soils was greatest both below and above 0 in the meadow organic soil which also has the highest organic C content and lowest in the low organic C content polar heath soil. No interacting effect of soil with temperature was observed on the mineralisation rate, but the organic meadow soil showed a much larger decrease in k_1 for glucose and the heath soil showed a larger decrease in a_1 for alanine. Less substrate was taken up by microbes in the Heath soil at -3°C than in the Meadow soils, this combined with the larger drop in a_1 for alanine in the Heath soil supports the hypothesis that the more organic rich soils would be less affected by freezing conditions (Harrysson-Drotz et al. 2010). This could be due to a greater unfrozen water content aiding substrate diffusion or possibly a larger microbial biomass with less distance between organisms would mean the substrate would not have to diffuse as far (Nunan et al. 2003). The larger decrease in k_1 for the organic soil is counter to that hypothesis and is also found for glucose. Possibly where diffusion is not the limiting factor, thanks to a shorter path length or less interference, the slower mineralisation rate is more apparent.

7.4.3. Modelling mineralisation below 0°C

The DAMM model was a moderately good fit for the mineralisation of glucose in frozen soil when the water content was measured by alcohol calorimetry and by the pre-melting model. However, without certainty in the accuracy of the used unfrozen water contents, a full validation of the model cannot be accomplished. Whether it would work as well for other

substrates would be useful to investigate. If charged, longer or less soluble compounds are more delayed in thin films than glucose it is possible that the change in the volume of the water will be insufficient for modelling the diffusion. This change takes into account the effect of increased tortuosity, but not increased viscosity due to more ordered water molecules, size exclusion or surface diffusion (Pignatello and Xing 1996).

7.5. Conclusion

FREZCHEM suggested very low water contents below 0°C. The model does not take into account the effects of the soil matrix and is therefore not preferred for use in soils without modification. The pre-melting model suggested that salt content can contribute a lot to unfrozen water content, whilst the effect of surface charge increases with decreasing temperature and grain size. The presence of degraded organic matter in soils could increase the surface area increasing the effect of surface forces. Alcohol calorimetry predicted higher water contents, but could potentially overestimate water contents depending on the calibration method used. This method still needs to be optimised, but could prove to be a cheap and easy method to determine unfrozen water content.

The mineralisation of glucose, trialanine and alanine decreased below 0°C. This effect was more pronounced for trialanine then for alanine and then for glucose. This suggests that larger and less soluble substrates are more affected by freezing temperature and does not support a microbial preference for N containing compounds. No microbial uptake of substrate was observed at -12°C or below. Less microbial uptake was observed in the Heath tundra soil and this effect was greater for alanine than glucose. The DAMM model estimates of the mineralisation of glucose were not significantly different from measured values, but they tended to be greater and the difference was close to being significant. Whether this is due to the model or the water content used is unclear. The DAMM model therefore requires further testing and validation in a wider range of soils.

Chapter 8

Snow cover changes alter C and N cycling in high arctic tundra soils

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8 Snow cover changes alter C and N cycling in high arctic tundra soils

Abstract

Changes to the arctic climate are predicted for the next century, particularly in winter with warmer temperatures and greater precipitation predicted. How this will affect soil temperatures and biogeochemical cycles is unclear. Here soil C and N parameters were monitored early in the growing season after 2 winters in high arctic where tundra soils were subjected to snow depth manipulation. Soils were much warmer over the second winter which appears to have increased carbon limitation, amino acid turnover and vastly increased nitrification in the tundra soil. The microbial community composition may also have been affected by a warmer winter. However, minor warming from snow manipulation did not have a significant effect on any of the above processes. An earlier thaw caused more respiration, potentially greater plant growth and increased susceptibility to freeze-thaw damage. Differences in carbon allocation were observed between deciduous and evergreen shrub types. Also the fate of N taken up by plants appeared to depend on the substrate type, with more nitrate-N remaining in the plant after 1 year than amino acid-N. These results suggest that warmer winters and an earlier thaw will enhance plant N availability stimulating plant growth and possibly affecting the plant community. More N loss could also occur from nitrate transport in to rivers and N₂O emissions. Warmer and shorter winters could also make soils and plants more vulnerable to freeze-thaw (FT) damage and greater nitrate availability could make N₂O emissions after FT possible.

8.1 Introduction

Freezing temperatures in the arctic can last for 8 - 9 months of the year, from late September to June, with sunlight being absent for 3 of these months. A number of studies have shown that this frozen period has an important impact on both carbon and nitrogen cycling in the terrestrial ecosystem. For example, studies have suggested that CO₂ emissions over the frozen period can, on some years, exceed those in the growing period (Elberling and Brandt 2003; Morgner et al. 2010). This shows that soil processes during the arctic winter can be annually important. Climate change is expected to cause a large increase in arctic winter temperatures (c.a. +7°C predicted for Longyearbyen by 2100) and also increase precipitation (12% for Longyearbyen by 2100) in the coming century (Førland et al. 2011). There is some

uncertainty as to how these changes in climate will affect soil temperature (Cooper 2014). Warmer winters and increased snow could increase soil temperature, both from greater ambient temperature and from insulation from the coldest winter air temperatures. However, the timing of the snow cover could alter this effect. Snow fall after soil has frozen could have little warming effect and snow cover at the end of the frozen period could delay soil warming and thaw (Grogan 2012; Lafreniere et al. 2013). Warmer temperatures could also completely melt the snow cover which could cause an earlier thaw, warmer soils and a longer growing season. This could also increase the potential for soils to experience freeze-thaw cycles as they would be exposed to fluctuating air temperatures which occur before the 3 months of 24 hour daylight (Henry 2008).

Uncertainty also exists in how changes to winter temperature will affect soil carbon cycling. Variability between tundra ecosystems in winter CO₂ emissions has been observed (Fahnestock et al. 1998). Arctic soil, in general, is currently an important carbon sink, but even at present some tundra ecosystems could be a net source of CO₂ to the atmosphere and rising temperatures will likely enhance this (Fahnestock et al. 1999; Morgner et al. 2010). Arctic soils contain a large proportion of global carbon. How susceptible this stored soil organic carbon (SOC) is to degradation in frozen soil is unclear. Grogan et al. (2001) found older carbon made only a minor contribution to winter CO₂ emissions. Degradation is dependent on microbial activity, enzyme activity and diffusion. Low oxidative enzyme activity is observed in winter, thus little degradation of lignin occurs (Sistla and Schimel, 2013). Diffusion is limited in frozen soil due not only to temperature, but also to only narrow films of water being available for enzyme transport (Tucker 2014). How well warmer freezing temperatures ease these problems is unclear. Microbes in frozen soil could only have access to dissolved organic carbon forms and microbially produced compounds (Michaelson and Ping 2003; Schimel and Mikan 2005). If SOC degradation requires unfrozen soil then an earlier thaw and deeper active layer might have more effect on arctic carbon storage than winter temperature. This could partially be offset by more primary production in a longer growing season.

Less direct changes could also affect carbon degradation. Freezing can have a large effect on the microbial community and microbial function. A different set of bacteria is active in the winter than during the growing season (McMahon et al. 2011). Fungi appear to be more capable of adapting to freezing conditions and maintaining their activity over winter than prokaryotes as they have wider access to nutrients and potentially more freedom to modify

their membrane (Hall et al. 2008; McMahon et al. 2009). They may benefit from warmer, but still freezing temperatures. Fungi are more associated with the decomposition of lignin so changes to this group could affect the efficiency of SOC degradation, but as mentioned above, oxidative enzyme activities are low during winter so the fungi may not be degrading lignin during this period. Also, fungi maybe more susceptible than bacteria to damage during freeze-thaw cycles, either due to damage to hyphae, greater size or their greater activity in winter (Feng et al. 2007; Haei et al. 2011). Freeze thaw cycles may increase with warmer temperatures. Furthermore, if winter temperatures get warm enough bacteria may benefit more than fungi. Functional genes associated with nitrogen cycling have also been shown to be affected by FT (Sharma et al. 2006).

Changes in the nitrogen cycle can affect the carbon cycle. Arctic ecosystems are particularly low in nitrogen. N deposition and warming could increase plant and microbial N availability. This could have an effect on the carbon cycle as a reduction in N limitation could both increase degradation and plant growth. Changes in N have also been noted to change plant community composition, which could change the type of carbon input to the soil which might affect CO₂ emissions (Grogan et al. 2012; Sturm et al. 2005). Net nitrogen mineralisation has been shown to occur over winter whilst immobilisation occurs over the growing season (Hobbie and Chapin 1996), but Schimel et al. (2004) only observed N mineralisation when freezing temperatures were mild (< -4°C) rather than intense (< -20°C). Changes to nitrogen mineralisation over winter could potentially influence plant available N in the growing season. Whilst mineral N forms have traditionally been considered the main source of nitrogen for plants, a growing number of studies have shown the importance of amino acids and peptides as plant N sources with plants preferring organic N sources over nitrate (Hill et al. 2011). This is particularly the case for plants growing in low N environments such as tundra ecosystems (Schimel and Bennet 2004). Whilst these compounds may have a low concentration in soil solution this represents rapid turnover and continuous renewal and they could be an important and available N source for plants (Inselsbacher and Näsholm 2012). Therefore, not only N mineralisation but also the production of low molecular weight dissolved organic nitrogen (LMW-DON) could be important for plant nutrition. The effect of changing winter conditions on LMW-DON availability requires further investigation.

The aim of this project was to study carbon and nitrogen turnover and partitioning in high arctic tundra soils which experienced different snow cover and soil temperatures. These were manipulated over two winters using snow fences. It was hypothesised that snow would

accumulate on the side sheltered from the prevailing wind causing warmer soil temperatures, less freeze-thaw and a later thaw than the soil on the windward side. The two winters also had different snow regimes and temperatures. This project investigated the hypotheses that warmer winters caused a faster turnover of carbon and nitrogen; that the fate of the different types of bioavailable N and plant fixed carbon would be affected by the snow fence treatments; that changes to the microbial community occurred over the two years and from the snow fence treatments. Potential effects of the thaw date and winter temperature on FT were examined with greater DOC concentration being expected from the soils which experienced a warmer winter.

8.2 Methods

8.2.1 Snow Fences

Field sites were chosen to represent two ecosystem types, meadow (78°55.4'N; 11° 54.4'E) and heath (78°55.224'N; 11°52.439'E) tundra. Three snow fences were erected at each site in July 2012. The fences were 1.5 m high by 4 m wide and ran from north to south. This is perpendicular to the predominant Easterly and South Easterly wind direction (Norwegian Meteorological Institute). The fences were built about 20 m from each other and north or south of each other to prevent interference between snow fences. To monitor soil temperature, data loggers (Maxim's iButton®, DS1922L) were buried 1cm beneath the soil surface, 1 m from each snow fence side and set to record the temperature every 3 hours. The data loggers were collected and redeployed in early July 2013 and late July 2014.

8.2.2 Soil characteristics – TC/TN MBC/MBN Moisture

Soils were collected in early July 2013 and late July 2014. Intact cores 5×5 cm (diameter × depth) were collected for further analysis. Soil moisture was assessed gravimetrically by drying soil cores (n = 6) at 105°C. Coarse roots and shoots > 4mm were removed using tweezers. Dried soils for TC/TN were ground in a ball mill. The prepared soils were analysed by Carlo Erba NA 1500 Elemental Analyzer (Thermo Fisher Scientific, Milan, Italy). Fresh soil cores (n = 6) were divided into organic and mineral layers. Microbial biomass C and N was measured using the chloroform fumigation method as described in Chapter 6.

8.2.3 Soil Solution characteristics

Soil solution was collected non-destructively in July 2013 and 2014 by inserting Rhizon filters (Rhizosphere research products) into the soil on both sides of the snow fences and extracting the soil solution into an evacuated tube. Nitrate was measured according to the VCl_3 method of Miranda et al. (2001). Ammonium was analysed by sodium nitropruside method described by Mulvaney (1996). Absorbance was measured by Biotek PowerWave XS. Amino acids were analysed by a fluorescence method of Jones et al. (2002) using a leucine standard. Dissolved organic carbon and total N were measured by multi N/C 2100S (Analytic Jena AG, Jena, Germany). DON was calculated by subtracting inorganic N from total N.

8.2.4 Microdialysis

Soil nutrient diffusion was measured using a microdialysis method similar to Inselbacher and Näsholm (2012). Two soil cores (10×5) were taken from each plot in July 2013 and 2014. These were kept in the dark at ambient temperature (3-10°C). 2-3 microdialysis probes were inserted in to the surface soil of each core and deionised water was pumped through them at a rate of $2 \mu\text{l min}^{-1}$ for 3 hours. Calibrations were run each day. This allowed any temperature effects to be monitored and the relative recovery (RR) of the microdialysis probes to be calculated as follows:

$$RR (\%) = \frac{[\text{microdialysis solution}]}{[\text{calibration solution}]} \times 100 \quad \text{Eq. 8.1}$$

This meant that the flux of substrate diffusing in the soil could be calculated from the concentration in the recovered microdialysis solution

$$\text{Diffusive flux (nmol cm}^{-2}\text{h}^{-1}) = \frac{[\text{microdialysis solution}]}{\frac{RR}{100} \times \text{Surface Area}} \times \text{flow rate} \quad \text{Eq. 8.2}$$

Samples were run in a random order to avoid bias. Obtained solutions were transported frozen to Bangor University, UK where the solutions were concentrated by a factor of 3 under a stream of N_2 gas. The solutions were then analysed for nitrate, ammonium and amino acids as described in section 8.2.3

8.2.5 Respiration

This was measured in the field using a PPsystems EGM-4 IRGA attached to a SRC-1 respiration chamber which was pressed into the ground. Three measurements were taken per plot on the 8th of July 2013 and the 3rd of August 2014.

8.2.6 LMW DOC mineralisation

Radiolabelled substrates (glucose, amino acids (equimolar mix of L-isomeric alanine, arginine, aspartic acid, glutamic acid, glycine, histidine, isoleucine, leucine, lysine, phenylalanine, proline, serine, threonine, tyrosine and valine) and trialanine; 0.2 ml; 100 μ M; 10 kBq ml⁻¹) were added to 2g of fresh soil from snow accumulation and control plots (n = 4) collected in July 2013. The soils were kept at 5°C and the emitted ¹⁴CO₂ was trapped using 1M NaOH vials exchanged after 1, 2, 4 and 8 hours, 1, 2, 3, 4 and 7 days. This was quantified as described in chapter 6. A double first order decay model was fitted to the percentage of added ¹⁴C remaining in the soil (*Y*) such that:

$$Y = (a_1e^{(-k_1t)}) + (a_2e^{(-k_2t)}) \quad \text{Eq. 8.3}$$

(see chapter 6 for detailed description of the equation and parameters).

8.2.7 Freeze-thaw

Intact cores (5 x 5 cm) were taken from each plot (2 per plot) in July 2013. These were subjected to a freezing rate of 1°C/hour until -7.5°C was reached. This temperature was maintained for 46 hours and then allowed to increase at the above rate to 5°C. Another set of cores were kept at 5°C as a control. Soil solutions were extracted by centrifugation and analysed for amino acids and glucose as described in chapter 3.

8.2.8 DNA extraction and Analysis

DNA was extracted using a PowerSoil® DNA isolation kit according to the manufacturer's (MO BIO Laboratories, Inc) instructions. The DNA concentration was calculated using a ND-1000 Spectrophotometer (NanoDrop Technologies, Inc., Wilmington, USA). Prior to PCR a portion of each DNA extract was diluted to a concentration of 1 mg L⁻¹. For 2013 bacterial DNA analysis the primers used were ITSF and ITSReub. The PCR and Ribosomal intergenic spacer analysis (RISA) were performed as described by Gertler et al. (2009). For 2013 and 2014 fungal and bacterial analysis automated RISA (ARISA) was performed. DNA was mixed with 9.45 μ l H₂O, 12.5 PCR mastermix (PROMEGA, Wisconsin, U.S.A.), 1 μ l

forward and reverse primers (ITSWH and ITS4, respectively for fungi and as above for bacteria) and 0.05µl BSA. The PCR sequence started with 2 minutes at 95°C, then 30 cycles of 1 minute at 95°C, 45 seconds at 54.2°C and 1.5 minutes at 72°C, this was followed by 5 minutes at 72°C and 4 minutes at 15°C. The PCR product was analysed by an ABI Prism 3100 Genetic Analyzer (Applied Biosystems, California, U.S.A)

8.2.9 Stable isotope ¹³C labelling

Vegetated plots (20x35cm), of *Dryas octopetala* at the Heath site and *Salix polaris* at the Meadow site, were chosen either side of the snow fences. Solutions of citric acid (1M) and ¹³C labelled sodium carbonate (1M) were added to a vial suspended from the top a transparent plastic cover 10x20x35cm which was placed over the plot. The ¹³CO₂ produced was dispersed throughout the area by a battery operated fan. Each plot was labelled for 30 minutes a day over 6 days in mid-July 2012. Green leaves (30 per plot) were collected at the end of the labelling period to quantify how much of the label the plants had obtained.

In 2013 and 2014 soil solutions were extracted from each plot using Rhizon filters (Rhizosphere research products). Soil and vegetation samples were also collected (2x2x4cm) and separated into leaves, root/shoot, litter and soil. All fractions were dried at 70°C. Samples were ground using a ball mill and sent for analysis to the NERC Life Sciences Mass Spectrometry Facility, where they were analysed by Elemental Analysis - Isotope Ratio Mass Spectrometry.

Isotope enrichment was calculated as follows:

The δ¹³C values were converted to R_{sample} by,

$$R_{\text{sample}} = [(\delta^{13}\text{C} / 1000) + 1] \times 0.0112372, \quad \text{Eq. 8.4}$$

where 0.0112372 is the international standard value of PDB. R_{sample} was used to calculate the fractional abundance (A) of ¹³C relative to total C (disregarding ¹⁴C) by,

$$A = R_{\text{sample}} / (R_{\text{sample}} + 1) \quad \text{Eq 8.5}$$

Natural abundance calculated from unlabelled samples (as in Eq. 8.5) was subtracted from the fractional abundance. This was multiplied by the carbon content of the sample to give the ¹³C enrichment per g sample.

8.2.10 Stable isotope ^{15}N labelling

Two plots 10×10 cm, vegetated with *Salix* and *Saxifrage*, on each side of the snow fences at both sites were labelled with a solution containing either ^{15}N labelled nitrate, ^{15}N labelled ammonium or ^{15}N labelled amino acids (Algal amino acid mix, Sigma Aldrich) in early July 2013. Another two plots on either side of the fence were used as a blank having only ^{14}N compounds added. Solutions contained an equimolar mixture of nitrate, ammonium and amino acids such that the total N addition equalled 4 kg ha⁻¹. A vegetation and soil sample (2×2×4cm) was collected from each plot 1 and 3 days and 1 year after labelling. This was divided in to soil and vascular vegetation then dried at 70°C. Samples for the two plots were composited to obtain enough sample for analysis. Samples were then ground and analysed as in section 8.2.9. Enrichment was calculated as in section 8.2.9 except the international standard value was 0.3663033

8.2.11 Statistics

Significant differences and interactions between site, years and snow fence treatments were determined by univariate linear modelling performed in SPSS (version 12). Homogeneity of variances was achieved by square root or log₁₀ transformation. Where homogeneity of variance could not be achieved a weighted least squares linear model was applied using the inverse of the squared standard deviation as the weight. ARISA results were binned in base pairs of 5 and a distance matrix was created using Bray Curtis similarity equation. Non metric multidimensional scaling (NMDS), followed by pairwise ANOSIM was performed in PRIMER (version 6) used to assess differences in bacterial and fungal diversity. Values were considered significantly different if $P < 0.05$.

8.3 Results

8.3.1 Soil Temperature

Between the 20th of October 2012 and June the 1st 2013 the average soil temperatures were -5.52°C and -4.81°C in the ambient and the snow accumulation Heath plots and -4.97°C and -4.72°C in the ambient and the snow accumulation Meadow plots. Snow accumulation significantly increased the average temperature over the 2012-2013 winter by 0.71°C at the Heath site, but only 0.25°C at the Meadow site. Soil temperatures (Fig. 8.1) were warmer in the 2013-2014 winter than in the previous winter with an average of -2.62°C and -2.26°C in

the ambient and snow accumulation Heath plots and -1.29°C and -1.48°C in the Meadow plots. Snow depth, measured at the nearby Norwegian Meteorological Institute weather station, was much greater in 2013-2014, with little difference in snow depth being observed between either side of the snow fence at the Meadow site. Average air temperatures were -7.84°C and -6.62°C for 2012-2013 and 2013-2014, respectively, for the same time period. 3 to 4 mild freeze thaw cycles were observed in September-October 2012, but soil temperatures fluctuated only slightly above and below zero with a minimum temperature of -1.31°C and maximum of 0.24°C . Such mild temperatures may not actually cause freezing. A more intense cycle was observed in September 2013 with temperatures at the meadow site reaching -3.82°C before rising to 2.35°C . This cycle may have caused some FT effect although it is still quite a mild cycle that would be unlikely to penetrate to any great depth in the soil.

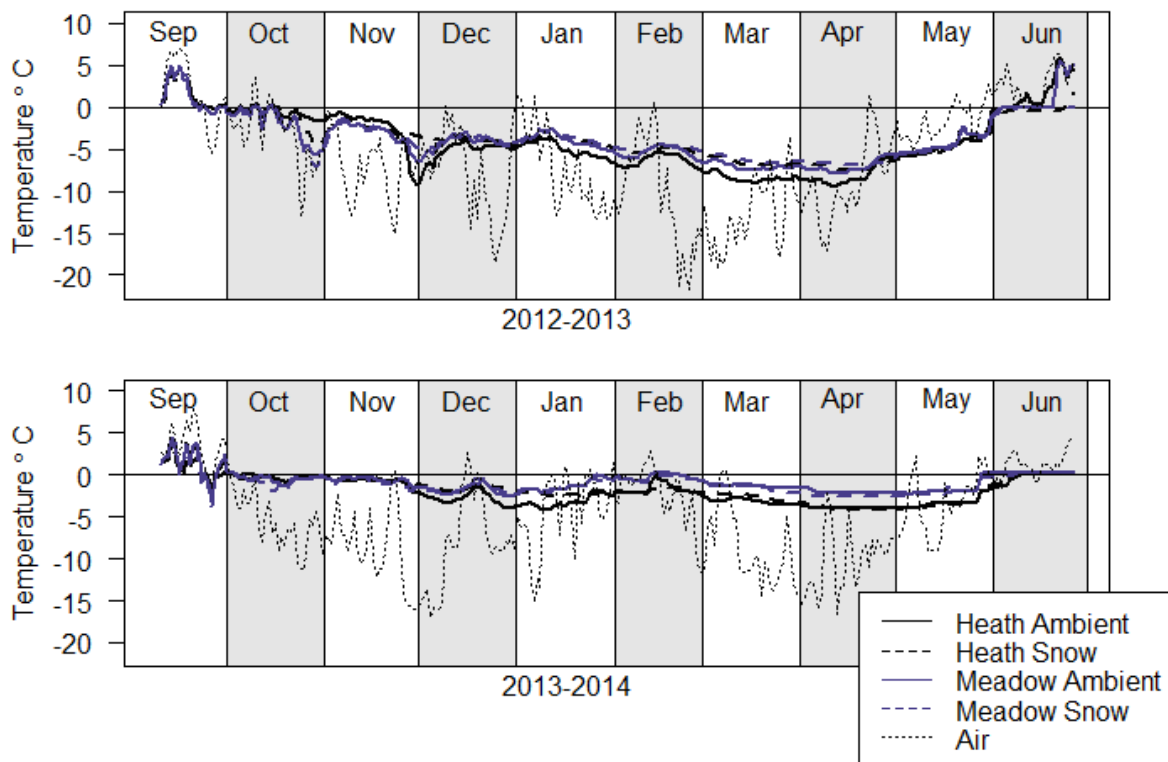


Fig. 8.1. Average daily winter soil temperatures over the period 2012-2014 at the Heath and Meadow sites with soils subjected to increased and ambient snow cover. Air temperature from Ny-Ålesund are also presented and were provided by Dr Marion Maturilli of the Alfred Wegener Institute for Polar and Marine Research

8.3.2 Soil and soil solution characteristics

Site	Snow	Carbon (%)	Nitrogen (%)	Moisture (% FW)	DOC (mg C L ⁻¹)	DON (mg N L ⁻¹)
Heath	2013 E	6.8 ± 3.5	0.44 ± 0.26	26.9 ± 2.4	45.5 ± 13.4	0.97 ± 0.19
	2013 W	13.5 ± 9.1	0.70 ± 0.52	31.6 ± 5.2	34.9 ± 7.7	1.12 ± 0.27
	2014 E	7.2 ± 3.1	0.36 ± 0.20	32.3 ± 8.1	29.0 ± 3.7	0.93 ± 0.22
	2014 W	17.3 ± 7.2	0.92 ± 0.43	17.2 ± 1.3	32.2 ± 3.0	1.33 ± 0.16
Meadow	2013 E	16.9 ± 3.5	0.69 ± 0.16	45.5 ± 2.0	35.4 ± 9.1	1.20 ± 0.38
	2013 W	18.4 ± 4.7	0.98 ± 0.31	36.9 ± 1.6	19.0 ± 3.9	0.67 ± 0.20
	2014 E	17.9 ± 3.8	0.66 ± 0.11	37.7 ± 4.1	27.1 ± 4.1	0.99 ± 0.21
	2014 W	13.3 ± 3.7	0.51 ± 0.08	32.1 ± 7.0	26.2 ± 1.7	1.15 ± 0.16

Table 8.1. The soil characteristics for each site in July 2013 and 2014 for soils on the East (E) side of the snow fence which experienced more ambient conditions and those on the West (W) side where snow tended to accumulate. Values represent mean ± 1 standard error (n = 6 or 9 for soil solution parameters) for soil carbon and nitrogen concentrations, soil solution organic carbon and nitrogen concentrations and moisture content in percentage of fresh weight of soil.

No significant difference was measured in soil moisture content (Table 8.1) between treatments or year, but the Meadow site tended to have a greater water content than the Heath site (37.8 ± 2.9 compared to 27.3 ± 3.6 % FW, $p = 0.021$). For DOC there was an almost significant effect of year ($p = 0.087$) with 2013 having greater concentrations (35.4 ± 4.0 compared to 26.9 ± 2.7 mg C L⁻¹ in 2014) and this became significant when values were square root transformed ($p = 0.033$) which was performed as the variances were close to being significantly different ($p = 0.058$). No significant difference was observed in soil C or N or in DON for year, site or snow treatment.

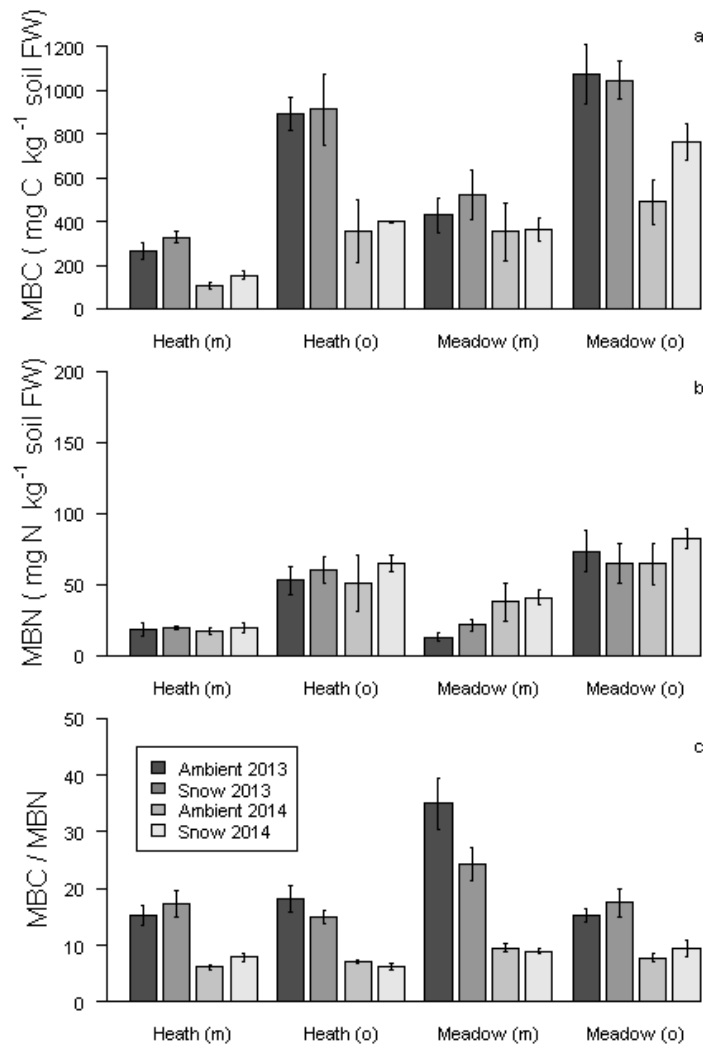


Fig. 8.2. Microbial biomass carbon (a), microbial biomass nitrogen (b) and the microbial biomass C/N ratio (c) in the mineral and organic soils from the Heath and Meadow sites from either side of the snow fence, (data represents mean \pm SEM, n = 4)

There was a significant effect of year on MBC ($p < 0.001$) with less MBC (approximately half) being measured in 2014 compared to 2013 (Fig. 8.2 a). A significant interaction between soil type and year was also observed ($p = 0.004$) with the Heath mineral and the organic soils showing a greater decrease in MBC between 2013 and 2014 (by a factor between 2.4 – 1.7) than the Meadow mineral soil (decrease by a factor of 1.3). No effect of snow fence treatment was observed. No change in MBN (Fig. 8.2 b) was observed due to either snow fence or year except in the Meadow mineral soil where MBN increased by a factor of 2.4 between 2013 and 2014 ($p = 0.035$). MBC and MBN were greater in the organic rather than mineral soils ($p = 0.003$) by a factor of 3 at the Heath site and a factor of 2 at the Meadow site save for the MBN in 2013 where the organic soil contained 3 or 5.7 times more

MBN than the mineral soil for the increased snow and ambient soils, respectively. The MBC/MBN ratio (Fig 8.2 c) tended to be greater in the Meadow soil. A decrease in the MBC/MBN ratio was observed between 2013 and 2014 ($p = 0.035$) and significant interaction was observed between soil type and year ($p < 0.001$) and between soil type and snow fence treatment. The meadow mineral soil showed the steepest decline in MBC/MBN between 2013 and 2014. Snow accumulation showed slightly higher MBC/MBN in the Heath mineral and Meadow organic soil, but the opposite effect in the other 2 soils.

Snow fences treatment had no significant effect on the concentration of any measured N form in the soil solution (Fig. 8.3). Significant interaction was observed between N form, site and year ($p = 0.01$). A 1.8 times lower ammonium concentration was observed in 2014 than 2013 at the Meadow site. Higher nitrate concentrations (by a factor of 5.6) were found in 2014 than 2013 at the Heath site. In 2013 high nitrate concentrations were measured by one of the snow fences at the Meadow site which may obscure underlying trends. Where these values were excluded nitrate increased between 2013 and 2014 at both sites, but tended to be greater at the Heath site. Amino acids tended to be slightly higher in 2014 than 2013 (by 2.0-68.2 $\mu\text{g N L}^{-1}$), but individual plots were not significantly different.

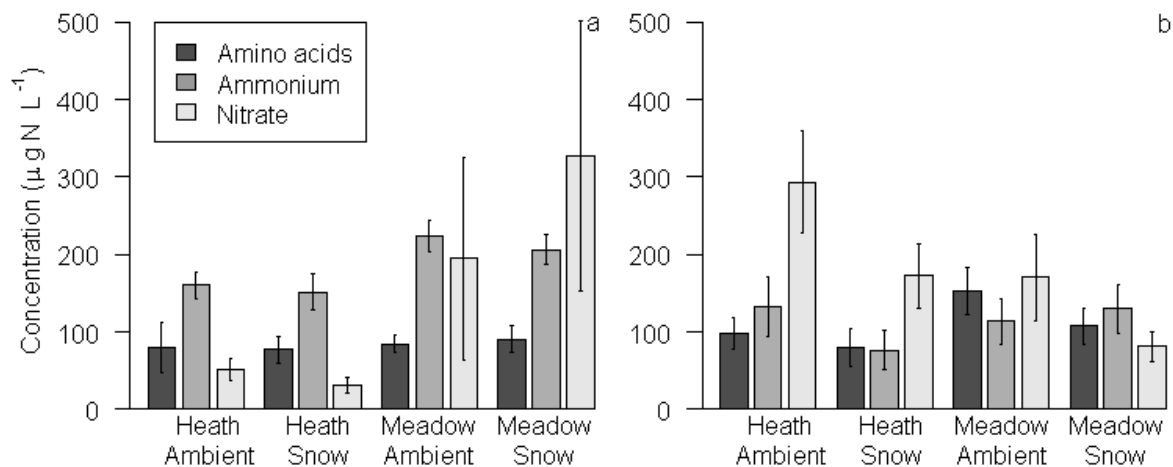


Fig. 8.3. Concentrations of N forms in soil solution extracted by Rhizon samplers in a) 2013 and b) 2014.

8.3.3 Microdialysis

Differences were observed in the relative recovery of ammonium and amino acids over time. Ammonium recovery started at 100% and decreased to 68% in the first year. In the second

year the relative recovery of ammonium fell from 86% to 50%. Amino acid recovery was on average 21% in the first year. In the second year the relative recovery started at 60% and fell to 30%. Nitrate recovery was 31% in the first year and 29% in the second. The average recovery was used for each substrate for each year. Temperature was on average 8.7°C, over the sampling period in 2013, but it fell to 5°C on the last day. In 2014 the temperature started at 3°C but rose during the sampling period to 10.9°C by the end, with a similar average to 2013.

No significant differences in the availability of any substrate were observed due to site or snow fence treatment (Fig. 8.4). A significant effect of year was observed ($p < 0.001$) with significant interaction between N form and year ($p = 0.005$). An increase in amino acid diffusion (by a factor of 2.1) in 2014 compared to 2013 was observed ($p = 0.003$). This was also observed for nitrate, but on average the values increased by a factor of 7.2 ($p < 0.001$). Ammonium fluxes were similar in both years. Nitrate was significantly more available than any other substrate in 2014 ($p < 0.001$), having a flux more than double either amino acids or ammonium, whilst there was no difference in availability between the N substrates in 2013.

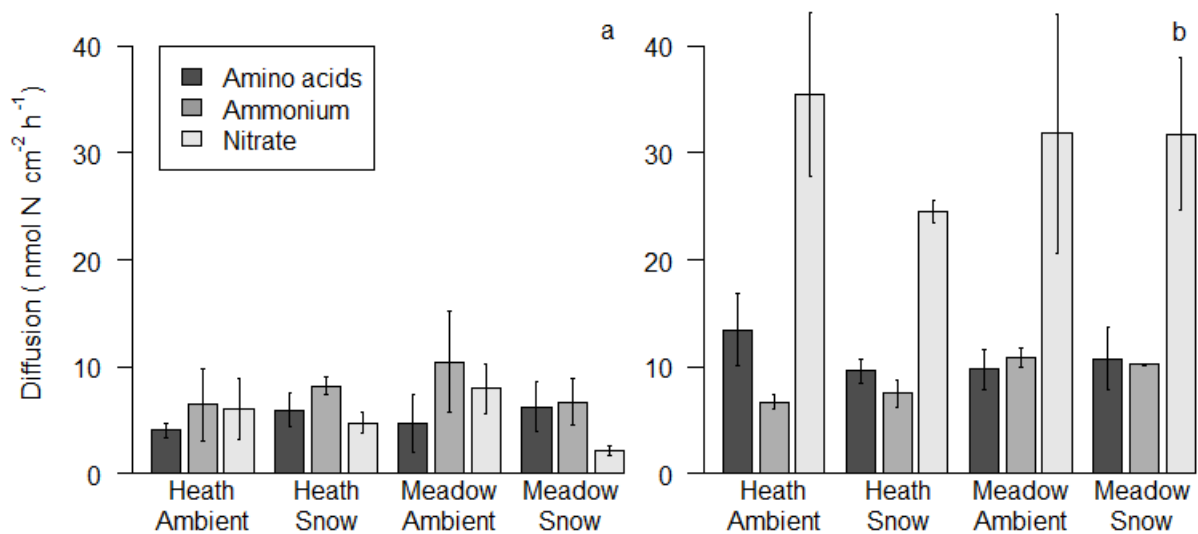


Fig. 8.4. Diffusive flux of N compounds in soils measured by microdialysis in a) 2013 or b) 2014

The relative concentrations of amino acids, ammonium and nitrate varied according to whether they were extracted with Rhizon samplers (Fig. 8.3) or microdialysis probes (Fig. 8.4) ($p < 0.001$) and depending on year ($p < 0.001$) and there was interaction between extraction method, substrate and year ($p = 0.005$). Nitrate was proportionately more

important in the microdialysis solutions than in the Rhizon solutions (35.4-55.9% of calculated N flux compared to 19.1-44.3% measured N, for Microdialysis and Rhizon solutions, respectively) whilst ammonium tended to be greater in the Rhizon solutions in 2013 (34.6-44.0% in the microdialysis extraction compared to 52.9-61.7% in the Rhizon solutions). In 2013 ammonium was the main N form extracted (40.5-56.6%) whilst in 2014 nitrate was the main form (44.3-55.9%). Amino acids were not consistently greater in either extraction technique. Rhizon samplers extract substrate at the concentrations they occur in soil solution, whilst microdialysis probes extract compounds over time. The differences between the extraction techniques may indicate whether compounds of low concentration are low because they are being continuously used and produced or not. This can show if the compound is bioavailable despite a low concentration.

8.3.4 Respiration

A significant effect of both year ($p < 0.001$) and snow treatment ($p = 0.005$) was observed on measured CO_2 emissions (Fig. 8.5), with 2014 having lower CO_2 emissions than 2013 (1.2-2.0 times lower) and the increased snow sites having less CO_2 emissions than the ambient snow sites (by a factor between 1.2 and 2.6). Interaction was observed between snow treatment and year ($p = 0.001$) and between snow, site and year ($p = 0.011$). The decrease in respiration due to increased snow cover was 3.2 times less in 2014 than 2013 and 4 times greater at the Heath site than the Meadow site.

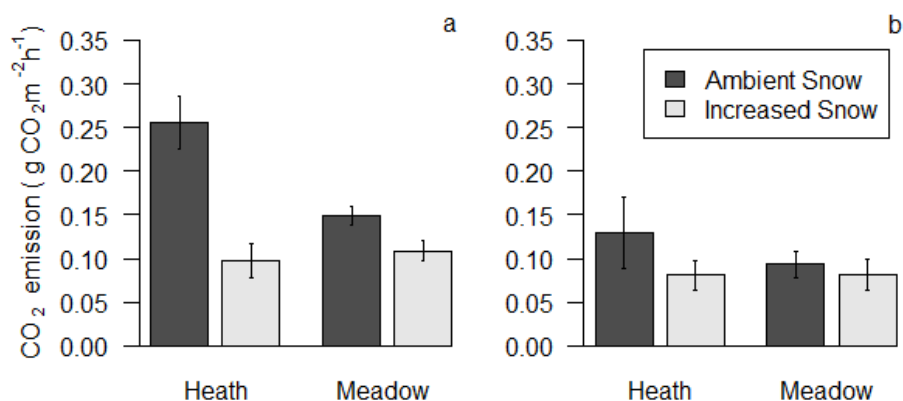


Fig. 8.5. Measured CO_2 emissions at the field sites in a) 2013 or b) 2014

8.3.5 Mineralisation of LMW DOC and DON

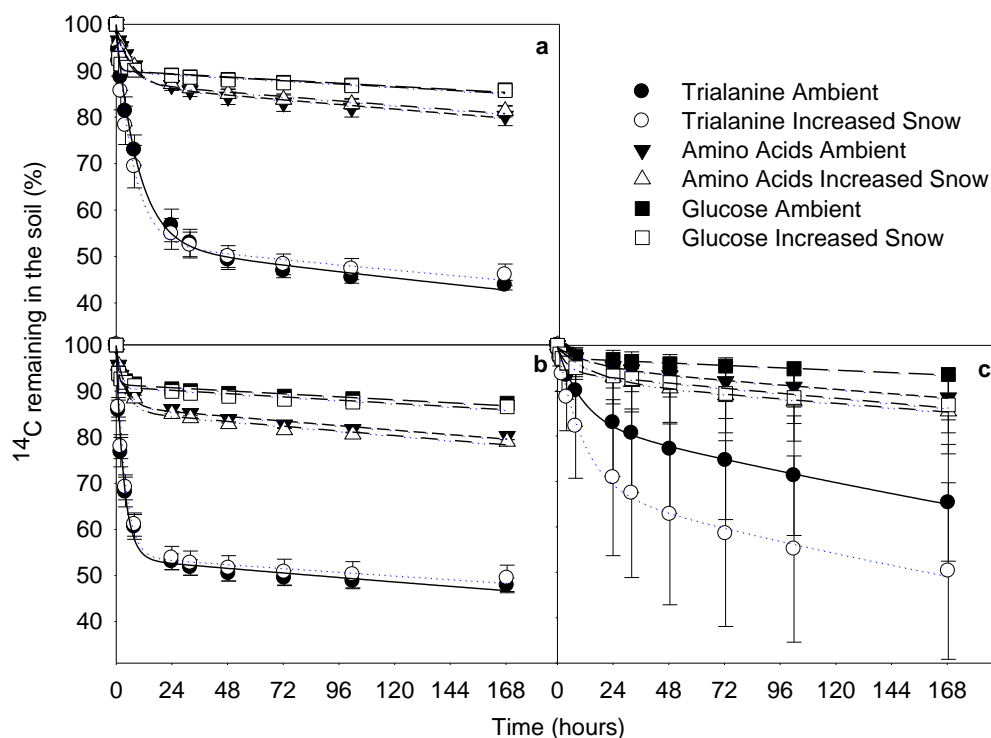


Fig. 8.6. The decrease of ^{14}C in a) Meadow mineral, b) Meadow organic or c) Heath mineral soil with time after addition as ^{14}C labelled trialanine, amino acids or glucose. Soils were sampled in July 2013 from plots covered by ambient or increased snow depth. Values represent mean error bars show ± 1 standard error ($n = 4$)

Changes to snow cover caused no significant difference to any of the double first order exponential decay parameters for glucose, amino acids or trialanine calculated from ^{14}C depletion curves shown in Fig 8.6 (Table 8.2; $p > 0.05$). This was the case for all the soil types.

			a_1	k_1	a_2	k_2
Trialanine	Meadow mineral	E	46.43 ± 2.31	1.04 ± 0.23	52.26 ± 2.83	11.71 ± 3.12
		W	45.63 ± 1.65	1.39 ± 0.40	52.78 ± 2.35	9.26 ± 1.91
	Meadow organic	E	44.96 ± 1.39	2.95 ± 0.58	53.60 ± 1.31	8.18 ± 0.94
		W	44.49 ± 2.49	2.85 ± 0.52	54.21 ± 2.56	6.70 ± 0.82
	Heath mineral	E	16.63 ± 13.28	0.81 ± 0.34	82.97 ± 13.50	14.73 ± 4.51
		W	30.33 ± 18.90	0.80 ± 0.23	69.06 ± 18.96	21.87 ± 7.35
Amino acids	Meadow mineral	E	12.35 ± 1.40	1.44 ± 0.62	86.57 ± 1.72	4.80 ± 0.43
		W	11.69 ± 0.88	1.75 ± 0.54	87.25 ± 0.96	4.60 ± 0.41
	Meadow organic	E	12.62 ± 0.97	2.38 ± 0.24	86.53 ± 0.90	4.98 ± 0.27
		W	13.68 ± 0.43	2.41 ± 0.41	85.52 ± 0.41	5.23 ± 0.24
	Heath mineral	E	5.54 ± 2.47	0.61 ± 0.20	93.92 ± 2.56	4.70 ± 2.08
		W	19.31 ± 18.56	0.43 ± 0.35	80.31 ± 18.36	4.61 ± 2.32
Glucose	Meadow mineral	E	9.93 ± 0.33	11.76 ± 1.33	90.00 ± 0.34	3.34 ± 0.25
		W	9.98 ± 0.18	11.50 ± 0.51	89.95 ± 0.18	3.28 ± 0.35
	Meadow organic	E	9.53 ± 2.02	8.18 ± 3.80	90.15 ± 2.45	3.71 ± 1.11
		W	8.98 ± 0.24	9.34 ± 1.00	90.88 ± 0.25	3.32 ± 0.23
	Heath mineral	E	2.65 ± 0.67	3.34 ± 1.26	97.17 ± 0.69	2.32 ± 0.74
		W	8.95 ± 8.06	1.84 ± 1.12	90.18 ± 9.07	2.57 ± 1.87

Table 8.2 Modelled double first order kinetic parameters describing the mineralisation of ¹⁴C-labelled trialanine, amino acids or glucose in arctic tundra soils that had underlain snow manipulation plots on the East (E) side (ambient) of a snow fence or the West (W) side (increased snow) Values represent mean ± 1 standard error (n = 4)

8.3.6 Microbial DNA Analysis

ARISA of bacterial DNA showed no peaks above background. Fig. 8.7 shows the NMDS results for the fungal DNA ARISA which shows the similarity between the communities of each sample as ranked distances. ANOSIM analysis of the same data produces R values which typically range between -1 and 1, with a value of 1 showing clear differences in the microbial communities and near 0 or less suggesting little separation is present. For fungi, ANOSIM revealed that only low R (-0.025, p = 0.840; Table 8.3) values existed between increased snow and ambient treatment plots suggesting no treatment effect. Overall site and year also had low R values (0.058 and 0.326, p = 0.039 and 0.001, respectively). However,

pairwise ANOSIM analysis revealed both quite high and significant R values between the Meadow 2013 results and all the 2014 results (0.62-0.42 $p < 0.015$). This suggests that there might be a difference in the fungal community between the Meadow 2013 sites and all the 2014 sites.

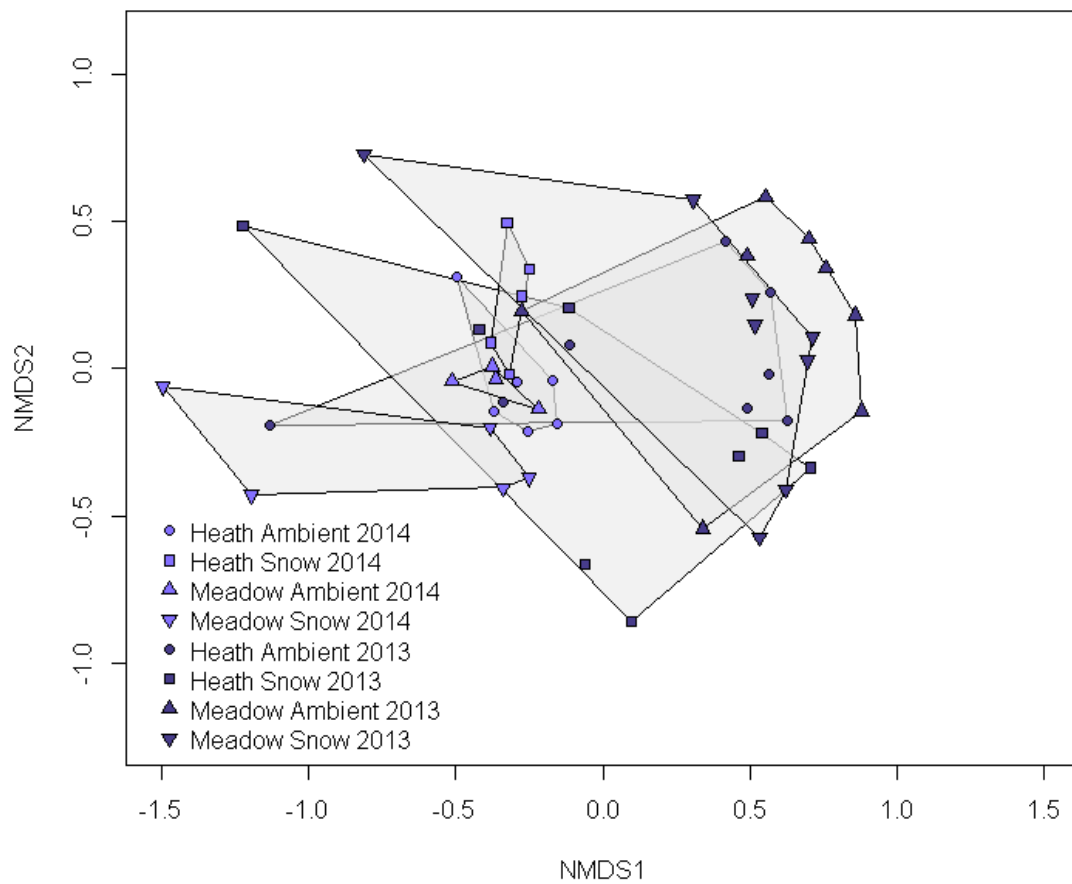


Fig. 8.7. Non Metric Dimensional Scaling analysis for ARISA results of fungal DNA, extracted from soil taken from ambient and increased snow plots for the Heath and Meadow sites in 2013 and 2014

R\P	Heath Ambient 2014	Heath Snow 2014	Meadow Ambient 2014	Meadow Snow 2014	Heath Ambient 2013	Heath Snow 2013	Meadow Ambient 2013	Meadow Snow 2013
Heath Ambient 2014		0.249	0.733	0.158	0.032	0.684	0.004	0.001
Heath Snow 2014	0.076		0.071	0.008	0.045	0.339	0.003	0.014
Meadow Ambient 2014	-0.087	0.313		0.73	0.097	0.838	0.002	0.008
Meadow Snow 2014	0.091	0.266	-0.078		0.022	0.143	0.003	0.009
Heath Ambient 2013	0.308	0.274	0.188	0.355		0.802	0.146	0.627
Heath Snow 2013	-0.048	0.039	-0.159	0.136	-0.070		0.04	0.329
Meadow Ambient 2013	0.622	0.611	0.617	0.621	0.08	0.170		0.585
Meadow Snow 2013	0.516	0.422	0.499	0.582	-0.034	0.027	-0.023	

Table 8.3. ANOSIM R (bottom left) and P (top right) values for 2014 and 2013 fungal DNA ARISA analysis results. Significant ($p < 0.05$) R values are in bold.

RISA analysis of the 2013 bacterial DNA results show lots of faint bands (Fig. 8.8) which may not have shown up above the background on the ARISA. This suggests relatively high bacterial diversity at all sites. Only subtle differences are observed between samples (Fig. 8.9). ANOSIM and NMDS reveals some significant differences (R c.a. 0.6, $p < 0.05$; Table 8.4) between sites. This analysis included samples below *Dryas* plants at the Heath site as well as bare soil. Quite large R values were observed between these sites suggesting a different community. This was not observed for the fungi (data not shown).

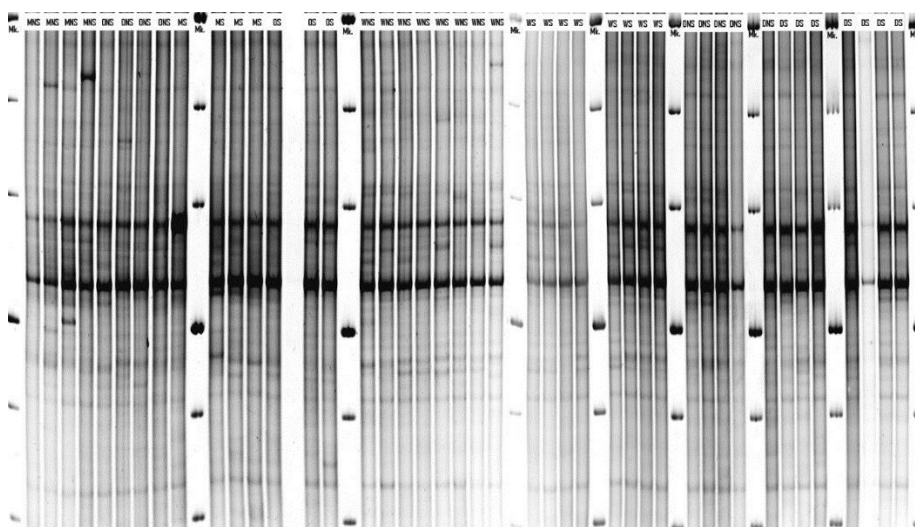


Fig. 8.8 Acrylamide gel for RISA of bacterial DNA in 2013

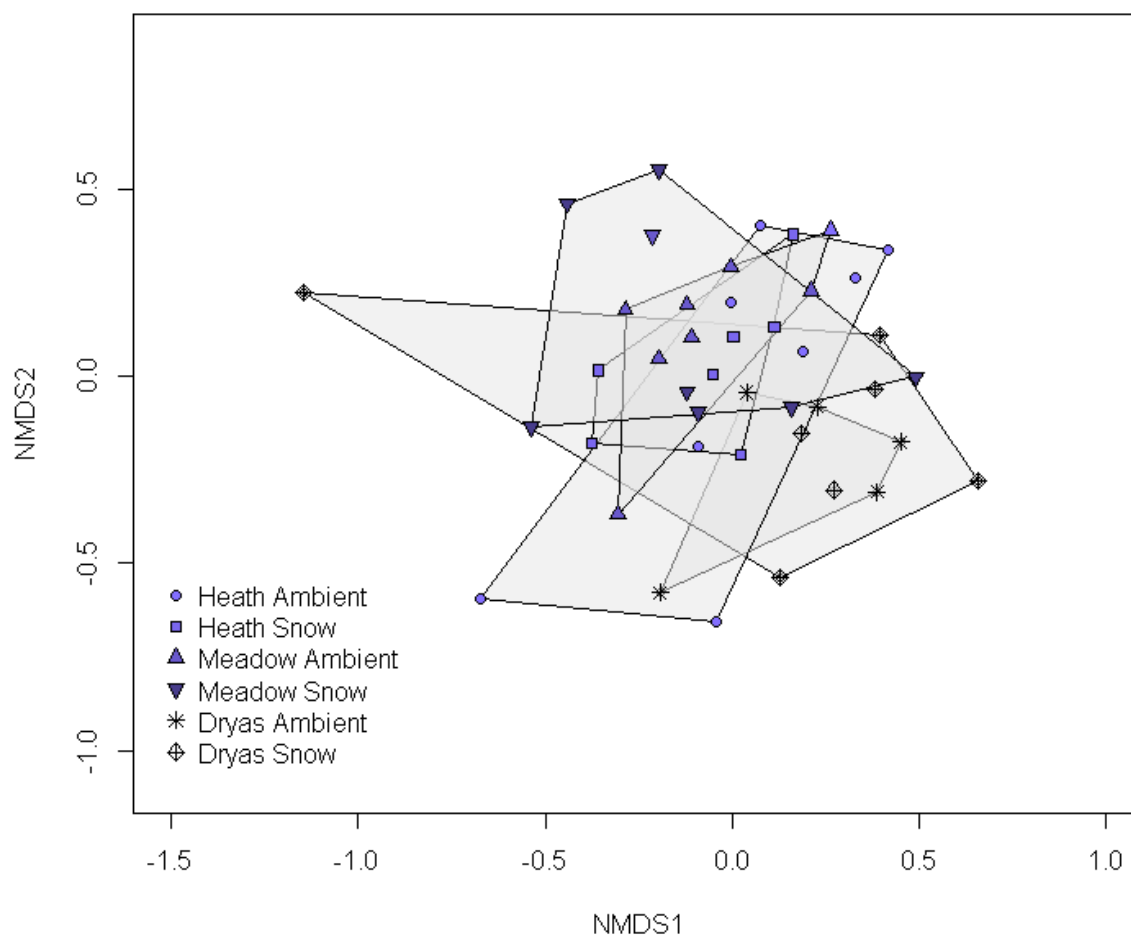


Fig. 8.9. Non Metric Dimensional Scaling analysis for RISA results of bacterial DNA, extracted from soil taken from ambient and increased snow plots for the Heath, Meadow and underneath *Dryas* sites in 2013

R/P	Heath Ambient	Heath Snow	Meadow Ambient	Meadow Snow	Dryas Ambient	Dryas Snow
Heath Ambient		0.225	0.016	0.001	0.001	0.002
Heath Snow	0.065		0.02	0.001	0.001	0.001
Meadow Ambient	0.205	0.238		0.001	0.001	0.001
Meadow Snow	0.448	0.493	0.310		0.017	0.002
Dryas Ambient	0.532	0.758	0.674	0.342		0.02
Dryas Snow	0.292	0.555	0.411	0.323	0.265	

Table 8.4. ANOSIM R (bottom left) and P (top right) values for 2013 bacterial DNA RISA analysis results. Significant ($p < 0.05$) R values are in bold.

8.3.7 Stable isotope analysis

Only in half the plots at the Heath site were plants successfully enriched with ^{13}C . At the Meadow site 5 out of 6 plots were successfully labelled. The most likely reason for this failure would be a poor air tight seal between the ground and the transparent cover. This was difficult to achieve at the Heath site due to the uneven ground surface including rocks and woody plants, but could perhaps be improved by using a larger plastic cover and removing stones. Labelling was not uniform between plots. About $11 \mu\text{g } ^{13}\text{C g}^{-1}$ leaf was incorporated in to the *Dryas octopetala* leaves at the Heath plots and $18\text{-}19 \mu\text{g } ^{13}\text{C g}^{-1}$ leaf was taken up by *Salix polaris* in the Meadow plots. As there is approximately double the mass of leaves at the Heath site than at the Meadow site this means slightly more was added at the Heath site. As labelling failed at the Heath site, particularly on the increased snow side, the analysis is unbalanced with respect to site and snow. Therefore the statistics could fail to recognise significant effects involving these factors on the amount of ^{13}C in each substance type, shown in Fig. 8.10 (Shaw and Mitchell-Olds 1993). Less ^{13}C enrichment tended to be observed after 2 years than after 1 year ($p < 0.001$) and less ^{13}C enrichment per gram substance was observed at the Heath site than at the Meadow site ($p < 0.001$). A significant effect of substance ($p < 0.001$) was observed with roots-shoots being almost always enriched in ^{13}C (by $+2.7\text{-}+5.0 \mu\text{g } ^{13}\text{C g}^{-1}$ except for 2013 Heath ambient which was $-0.5 \pm 0.7 \mu\text{g } ^{13}\text{C g}^{-1}$), soil showed no enrichment in ^{13}C , litter showed occasional enrichment, whilst leaves also showed variation in ^{13}C content. Significant interaction was observed between substance, year and site ($p < 0.001$). After 1 year at the Meadow site the leaves were enriched with ^{13}C (11.7 ± 2.0 and $12.1 \pm 1.6 \mu\text{g } ^{13}\text{C g}^{-1}$ in the ambient and increased snow plots, respectively) whilst after 2 years no leaf enrichment was observed (0.5 ± 0.8 and $-1.5 \pm 0.9 \mu\text{g } ^{13}\text{C g}^{-1}$ in the ambient and increased snow plots, respectively). In contrast, the leaves at the Heath site were isotopically lighter than the blanks after both years (-6.0 ± 1.8 , -6.5 ± 1.8 , -7.7 ± 2.5 and $-8.4 \pm 0.2 \mu\text{g } ^{13}\text{C g}^{-1}$ in the ambient and increased snow Heath plots, from 2013 and 2014, respectively). Interaction was observed between snow treatment, year and site ($p = 0.022$). In the increased snow Heath samples, enrichment is observed in the root-shoot and litter samples, but no overall enrichment was observed after one year in the ambient Heath sites (-0.5 ± 0.7 and $-1.7 \pm 0.19 \mu\text{g } ^{13}\text{C g}^{-1}$ for root-shoot and litter respectively) However, as enrichment is observed at the ambient Heath site in the subsequent year in both root-shoot and litter samples (3.5 ± 0.5 and $3.1 \pm 1.5 \mu\text{g } ^{13}\text{C g}^{-1}$, respectively) this sheds some doubt on

the veracity of this result. After two years only the root-shoot samples are significantly enriched for all sites.

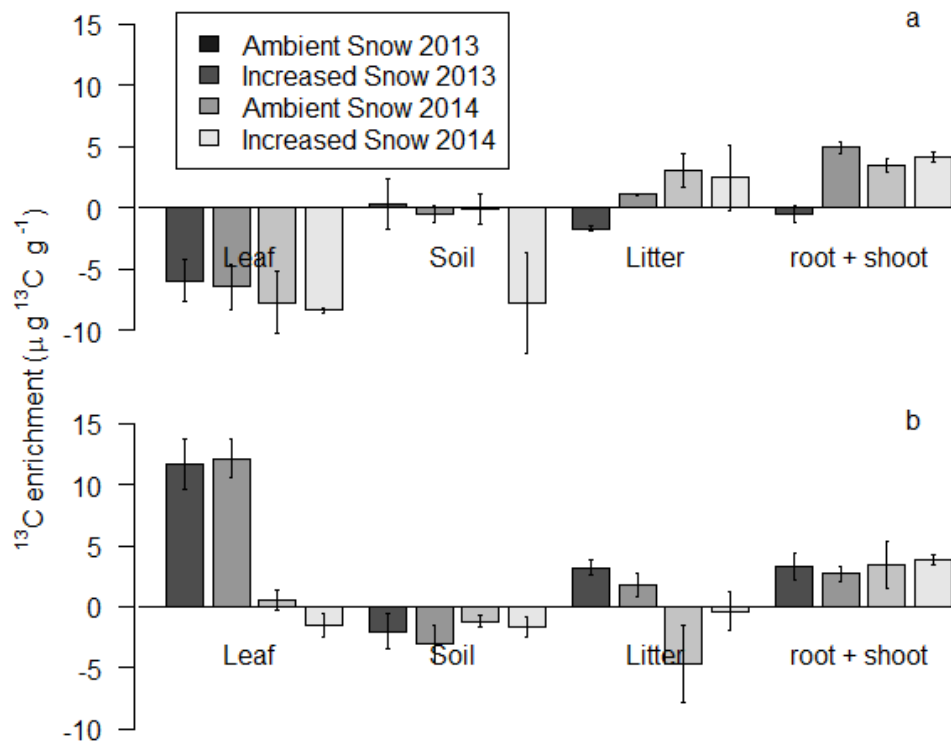


Fig. 8.10. ^{13}C enrichment in a) Heath and b) Meadow leaf, soil, litter and root/shoot material 1 year (2013) and 2 years (2014) after labelling in plots subjected to manipulated snow depth.

The day after ^{15}N labelling (Fig. 8.11 a) a significant effect of site ($p = 0.001$), material ($p < 0.001$) and substrate ($p = 0.009$) was observed. There was generally more ^{15}N enrichment in the vascular plant and soil at the Heath site (1.5 times more) than the Meadow site, in the vascular plant material (2.2 times more) than the soil and generally more ^{15}N that was added as amino acid per gram material (1.6 times more) than was added as nitrate or ammonium. Significant interaction was observed between site and material type (vascular plant or soil), with more N in the vascular plants at the Heath site than at the Meadow site by a factor of between 1.6 and 6.3. There was also interaction between site, snow and substrate ($p = 0.029$, with no clear trend being shown). Three days after labelling (Fig. 8.11 b) there was twice as much ^{15}N enrichment per gram material in the vascular plant than soil ($p < 0.001$), but no other significant effect. After 1 year (Fig. 8.11 c) a significant effect of site ($p = 0.001$), material ($p = 0.013$) and substrate ($p < 0.001$) was observed with more ^{15}N at the Meadow site ($1.3 \pm 0.2 \mu\text{g } ^{15}\text{N g}^{-1}$ compared to $0.7 \pm 0.1 \mu\text{g } ^{15}\text{N g}^{-1}$ at the Heath site), vascular plants

showing 3 times greater ^{15}N enrichment per gram than soil and more ^{15}N from nitrate recovered ($1.4 \pm 0.3 \mu\text{g } ^{15}\text{N g}^{-1}$) than ^{15}N added as amino acids ($0.7 \pm 0.1 \mu\text{g } ^{15}\text{N g}^{-1}$). Significant interaction is observed between site and substrate ($p = 0.015$), site and material ($p = 0.003$), snow and substrate ($p = 0.032$) and between material and substrate ($p < 0.001$). ^{15}N originally from ammonium and nitrate appear higher at the Meadow site than the Heath site (by a factor of 1.8 and 2.6, respectively) whilst amino acid does not. Vascular plants seem to have double the ^{15}N at the Meadow site than at the Heath site. ^{15}N from ammonium appears to be greater in the snow accumulation plots ($1.2 \pm 0.3 \mu\text{g } ^{15}\text{N g}^{-1}$) than in the ambient plots ($0.6 \pm 0.1 \mu\text{g } ^{15}\text{N g}^{-1}$) whilst amino acids showed the opposite trend (0.7 ± 0.1 and $0.9 \pm 0.2 \mu\text{g } ^{15}\text{N g}^{-1}$ in the increased snow and ambient plots, respectively) although the differences in themselves are not significant, the interaction is. There does not appear to be any difference after 1 year in the plant or soil location of ^{15}N originally from amino acids whilst plants have more ^{15}N originally from nitrate and ammonium (2.5 ± 0.4 and $1.4 \pm 0.2 \mu\text{g } ^{15}\text{N g}^{-1}$, respectively) than soil (0.3 ± 0.1 and $0.4 \pm 0.1 \mu\text{g } ^{15}\text{N g}^{-1}$, respectively).

The $^{13}\text{C}/^{15}\text{N}$ ratio (g/g) on day 1 was on average 0.04 and did not significantly vary between plant and soil. As ^{13}C in the amino acid is in excess compared to ^{15}N this low value suggests that much of the ^{13}C has been respired, otherwise removed from the plot area or retained in the moss/lichen layer. Either way the $^{13}\text{C}/^{15}\text{N}$ ratio suggests that the amino acids are no longer intact.

As samples from days 1 and 3 were not weighed total ^{15}N recovered cannot be calculated. A greater mass of plant or soil could have an isotope dilution effect. Also the moss and lichen layer was not sampled, this layer could have intercepted a good deal of the ^{15}N label. Sampling or N addition may have damaged plants, after 1 year (2014) in some of the plots the vascular plants appeared dead.

8.3.8 Changes to DOC on Freeze-thaw

Freeze thaw significantly increased glucose (Fig. 8.12) and amino acid (Fig. 8.13) concentrations in soil from the Meadow site under ambient snow cover. No significant increase due to FT was observed in soil from the Heath site or from the increased snow Meadow soil.

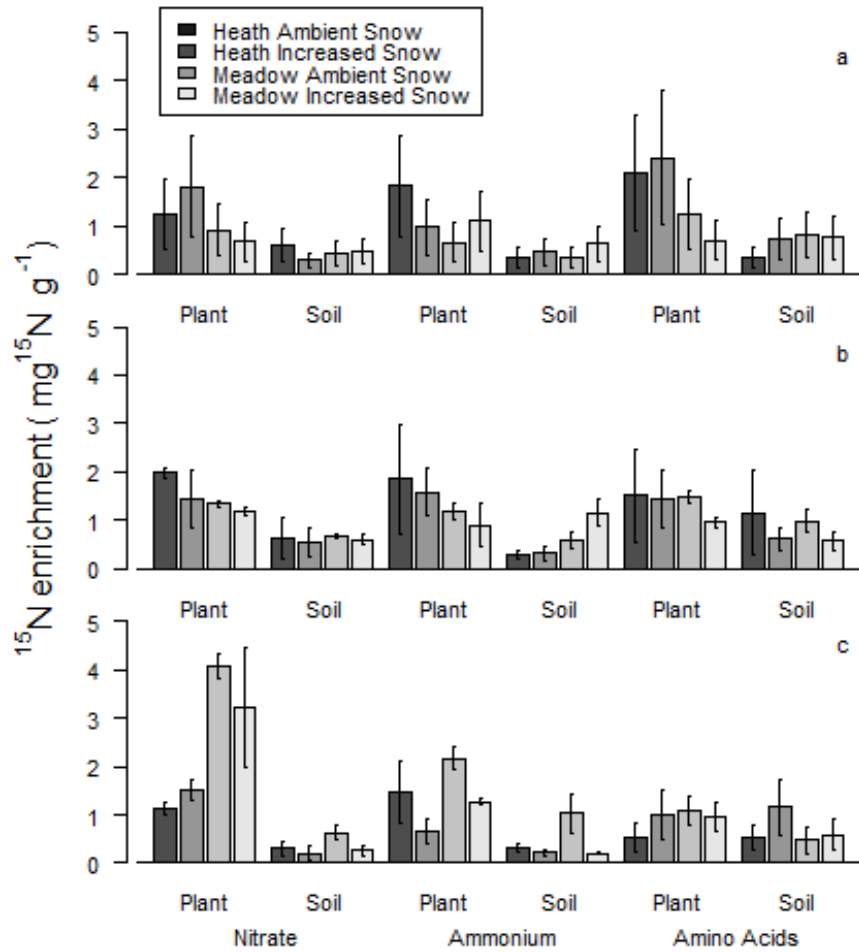


Fig. 8.11. ^{15}N enrichment in vascular plant and soil a) 1 day, b) 3 days and c) 1 year after labelling in plots subjected to manipulated snow depth.

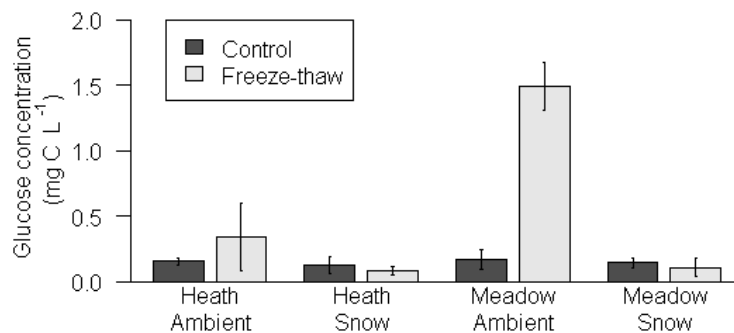


Fig. 8.12 Glucose concentrations in soil solution subjected to 1 Freeze-thaw cycle to -7.5°C or kept at 5°C as a control from soils from both ambient and increased snow plots at the Heath and Meadow sites in 2013

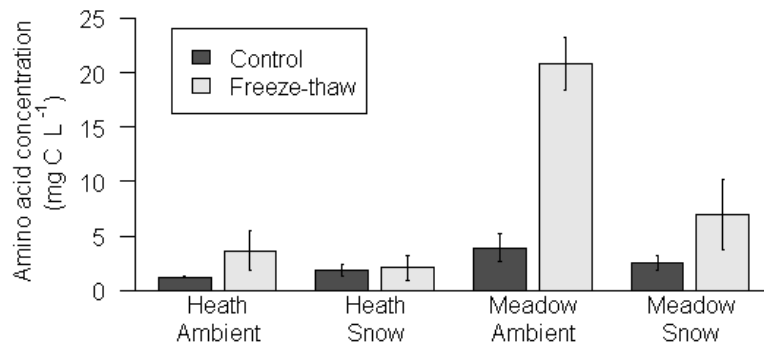


Fig. 8.13 Amino acid concentrations in soil solution subjected to 1 Freeze-thaw cycle to -7.5°C or kept at 5°C as a control from soils from both ambient and increased snow plots at the Heath and Meadow sites in 2013

8.4 Discussion

8.4.1 Snow fence treatment

The snow fence was successful in increasing the average winter temperature in the snow accumulation plots except in the Meadow site 2013-2014. The temperature increase was small but larger at the Heath site which is less sheltered than the Meadow site. The snow pack was exceptionally thick in winter 2013-2014 and completely buried the snow fences (1.5m deep) at the Meadow site making them ineffective. This snow depth also insulated soils from the air temperature and is partially responsible for the warmer soils and later thaw in 2013-2014 than in 2012-2013. In late October 2012 the soils at the ambient sites were warmer than in the snow accumulation plots. This is likely due to the strong North Westerly winds on the 23rd and 24th October (Norwegian Meteorological Institute). This wind direction was opposite to the prevailing wind direction and potentially caused snow accumulation on the wrong side of the snow fence. The cold air temperatures during this period (-14°C) would then have most affected the soils on the western side of the snow fence. Snow accumulation after soil has frozen has less of a warming effect on soil (Lafreniere et al. 2013). Snow cover started almost a month earlier in 2013 than 2012, at the start of October rather than near the end, which kept the soil warmer. The increased snow treatment caused a later thaw with soils in the Heath snow accumulation plots thawing 2 weeks later than the ambient plots and Meadow snow accumulation plots thawing 1 week after the ambient plots in 2013. Thaw was later in 2014 (8th July) than 2013 (8th to 26th June), but was at around the

same time in both sites and snow treatments. Snow fences did not cause a greater number of freeze thaw cycles on the ambient snow side compared to the increased snow side.

8.4.2 The effect of winter climate on C cycling

Microbial biomass carbon was much lower in 2014 than 2013 (Fig. 8.2). However, no decrease in microbial biomass nitrogen occurred. This suggests that the decrease in MBC was not due to lysis, as this would likely decrease both. Edwards et al. (2006) found lower MBC/MBN in July rather than earlier in the year, but they also found a decrease in MBN which was not observed here. There is the potential that microbes with relatively greater N content prevailed over the warmer 2013-2014 winter. Bacteria have a greater N content which could go some way to explaining the decrease in MBC/MBN (Strickland and Rousk 2010). Fungi generally dominate in frozen soils, if more bacteria survived due to the warmer winter this could cause the relative decrease. Alternatively bacteria become more abundant through spring into summer (Buckeridge et al. 2013), as 2014 soils were sampled later in the year and were warmer after thaw (by 3°C-6°C in the 2 weeks prior to sampling) they may be more bacterial dominant for this reason. An assessment of fungal to bacterial biomass was not made in this study so it cannot be shown whether more fungal to bacterial biomass was present in 2014 than 2013. Changes to the fungal community were observed between 2013 and 2014 at the Meadow site (Fig 8.7). This could be due to the warmer winter. However, no significant change was observed at the Heath site which is where the largest MBC decrease was observed. An alternative theory would be that carbon availability was lower in 2014 than 2013. The warmer winter would allow faster use of available carbon, but the presence of ice would limit diffusion so microbes would have to rely on a nearby carbon source. This would likely be of microbial origin and therefore be higher in N than a degraded litter source. The results for the microbial biomass are consistent with Buckeridge and Grogan (2008) with a lower C/N ratio caused by a warmer winter. Higher carbon limitation in the warm winter of 2013-2014 would suggest that even at warm freezing temperatures SOM degradation is limited. However, the longer freezing duration over 2013-2014 may also have contributed as well as the temperature.

Soil respiration was greater in soils which had experienced a colder winter with the ambient plots and 2013 showing greater CO₂ emissions (Fig. 8.5). The air temperature when respiration measurements occurred was similar on both occasions (10°C) so could not have caused the difference. It is possible that a warmer temperature over winter causes more

degradation of labile carbon leaving less available in spring (Grogan 2012). However, a later thaw can delay plant growth early in the growing season (Sutinen et al. 2015). This could result in less root exudation and rhizosphere respiration. Morgner et al. (2010) observed less CO₂ emissions due to a later thaw and this maybe what is actually occurring particularly in the increased snow plots in 2013. Less available carbon could be the cause of less soil respiration in 2014 which perhaps supports the theory that the soil is carbon limited. The lower DOC concentrations in 2014 compared to 2013 also support this theory.

No difference in LMW DOC mineralisation was observed due to site or snow treatment (Fig. 8.6). This test was done in the laboratory after sieving to homogenise. This likely had more of an effect on the soil than the snow treatment. Differences between *in-situ* mineralisation measurements and laboratory measurements have been observed previously using this method (Glanville et al. 2012)

The fate of photosynthesised C appears to be affected by the type of vegetation. The *Salix polaris* used much of the incorporated ¹³C in new leaves the year after labelling, whilst in the *Dryas octopetala* it was only found in the root/shoot (Fig. 8.10). This suggests that some of the freshly photosynthesised ¹³C is stored in *Salix polaris* to produce new leaves the year after. Deciduous plants have been shown to store carbohydrates in their roots for use for growth in spring (Tomlinson et al. 2013). No ¹³C enrichment was observed in the *Salix polaris* litter in 2014, but litter can be transported by the wind and may not be from the labelled plant. Alternatively plants can remove nutrients from leaves prior to senescence. The leaves of *Dryas octopetala* were more depleted in ¹³C in the years after labelling than prior to labelling. This could be due to natural variation in the plants $\delta^{13}\text{C}$ which could occur due to changes in stomatal conductance or photosynthesis rate. The labelling year was drier at the Heath site (moisture content 14.6% \pm 1.6 FW) than the following years (see Table 1) which could have caused reduced stomatal conductance, producing higher $\delta^{13}\text{C}$ values by reducing CO₂ exchange with the atmosphere (Blok et al. 2015)

Winter warming in the arctic may affect plant communities due to an earlier thaw, changing N availability and water content (Robinson et al. 1998; Semenchuk et al. 2013; Sturm et al. 2005; Wahren et al. 2005). Shrubs, particularly deciduous shrubs, and graminoids are fast growing and can best take advantage of N availability and warming, which benefits depends on soil moisture and ambient temperature (Elmendorf et al. 2012; Wahren et al. 2005). Deciduous shrubs may store more photosynthate as carbohydrates in their roots than

evergreen shrubs which convert it to structural material. Therefore, an increase in deciduous shrubs could lead to more labile C being released on FT which might stimulate CO₂ and N₂O emissions. Taller plants themselves can affect snow cover and soil temperature by providing a wind break and snow accumulation (Sturm et al. 2005). This could be countered by an increase in shading and decreased soil temperatures during the summer and greater soil carbon storage (Sistla et al. 2013)

8.4.3 The effect of winter climate on N cycling

The warmer winter in 2013-2014 possibly increased amino acid availability (Fig 8.4) though not necessarily its concentration (Fig. 8.3) and possibly decreased ammonium concentrations (Fig. 8.3) but not availability (Fig 8.4). Large increases in nitrate concentration (Fig. 8.3) and availability (Fig 8.4) were observed in 2014 compared to 2013. This suggests that the warmer winter increases the availability of both amino acids and nitrate with ammonium may have been turned over more quickly, reducing its concentration. This is consistent with carbon limitation with organic N being used as a carbon source and excess N being mineralised (Geisseler et al. 2010). These results are consistent with many past studies (Freppaz et al. 2008; Natali et al. 2012; Schimel et al. 2004) who found that N was mineralised in snow accumulation plots over winter. Other studies have shown that more FT can cause more nitrate mineralisation due to release of DON from root and microbial damage (Durán et al. 2013). This is unlikely to be the case in this soil as such effects do not appear to last much more than 3 days after thaw and no increase in N was observed due to thaw date (Glanville et al. 2012 b). Also, the colder winter would be anticipated to cause more damage and thus have greater nutrient fluxes, which is not the case. The snow pack itself could also have been a source of N to the soil, potentially containing up to 0.45 kg N ha⁻¹ of nitrate-N (Filippa et al. 2010; Viglietti et al. 2013) although atmospheric N deposition is less at arctic latitudes than in the Alps where such values are measured. In Svalbard, nitrate concentrations in snow up to 3-4 μmol L⁻¹ have been measured. The highest values of nitrate in snow, occurred when air masses came from Europe which occur regularly in warm winters such as 2013-2014 (Vega et al. 2015). The snow could have contributed to the observed large concentrations of nitrate in 2014, but would likely only account for a small fraction. Nearby N labelling (approx. 1m away) could have contaminated the plots and as nitrate is more mobile than the other N forms this would likely be the most expected to migrate through the ground water. However, there was a high recovery of ¹⁵N from all forms in the labelled plots which suggests that this is not the case. Both the Heath and Meadow sites are

in close proximity to the road and to Ny Ålesund so they could be more disturbed than is representative for most arctic tundra soils. This could mean that the sites receive more N deposition than is usual for remote arctic regions, but the roads are not used frequently so this may not overly affect N dynamics (Ackermann et al. 2012). Therefore, it seems likely that the high nitrate concentrations and availability in 2014 is due to an increase in mineralisation over the warmer winter.

The microdialysis results show greater diffusion rates for ammonium and nitrate than found by Inselsbacher and Näsholm (2012) whilst amino acid results were in the same range (8-15 nmol N cm⁻² h⁻¹). This could partly be caused by the lower flow rate used which increases recovery (Inselsbacher et al. 2011). However, this should be corrected for by the relative recovery. This experiment was performed in the dark and without leaching. This could increase inorganic N concentrations due to decreased plant uptake and increase nitrate particularly due to no removal in ground water flow. Similar inorganic N values to those found here were obtained by Inselsbacher and Näsholm (2011) for sieved soil (6-8 and 3-9 nmol N cm⁻² h⁻¹ for ammonium and nitrate, respectively) which would also include no mass transport or plant uptake. A reduction in plant exudates could reduce amino acid diffusion. It is also possible that lower temperatures in Svalbard compared to Sweden could decrease diffusion and decomposition which would particularly affect the availability of amino acids in soil (Inselsbacher and Näsholm 2011). This could account for amino acids not being quite as dominant as reported by Inselsbacher and Näsholm (2012) for completely *in-situ* soils. This suggests the results presented here might not be entirely representative of *in-situ* soils. The relative recovery were near the reported values of Inselsbacher et al. (2011) The decrease in recovery during calibration for ammonium might suggest that the probes were getting blocked. The calibration for amino acids was lower in the first year which could be due to an error making the calibration solution. If relative recovery was actually less than reported in 2014 then the amino acid flux in 2014 would be greater than stated, but the relationship between the years would not change.

The ¹⁵N labelling experiment (Fig. 8.11) showed quite high variability and the results could have been affected by the amount of material collected. The amino acid N appeared to be taken up more quickly by both the vascular plants and soil than the inorganic N. This could be due to plant and microbial preference for LMW organic N, although results suggest the amino acids were not intact (Hill et al. 2011; Näsholm et al. 2009). This method would not separate what was taken up by soil microbes, adsorbed to soil particles or remained in soil

solution so even if the material was not taken up by soil microbes they should be recovered by the soil. Ammonium could be being held in the uncollected moss layer yet this is less likely for nitrate. As plant cover is not homogenous the moss/lichen layer could intercept different amounts and confound the results. No effect of snow fence was observed despite that more uptake would be expected due to the earlier thaw. More N from a nitrate source remained in the plant after 1 year than from an amino acid source. This could indicate that nitrate is the main plant N source, but could also indicate differences in how plants utilise the different N source. An increase in nitrate could lead to different plant N allocation which if it was stored in the stems could lead a change in when N is available as root and stem turnover is different (Cambui et al. 2011). The substrate was added from above which is more representative of atmospheric deposition than soil production. It should be mentioned that this method does not distinguish between ^{15}N taken up by plants and ^{15}N adsorbed to the outside. Some material may adhere to the foliage. Also separation of fine roots and soil is not 100% efficient. The N concentration added was of a similar magnitude to annual N deposition and mineralisation rates in high arctic soils (Robinson et al 1995; Woodin 1997). As this was added in a single dose this means that the N concentrations experienced were higher than normal which may have produced effects which are unrepresentative. For example, high amino acid concentrations can decrease inorganic N uptake, high inorganic N may increase N mineralisation and increases in N may affect the plant and microbial community (Blaško et al. 2013; Näsholm et al. 2009)

Nitrate is susceptible to leaching and transport into rivers. Thus the high nitrification observed in warmer winters could result in a large increase in N being input into rivers and the ocean. This could have further reaching implications, possibly increasing plankton blooms in the oceans which could increase the albedo effect in the Arctic Ocean, increase warming and enhance ice melting (Park et al. 2015). However, runoff can occur prior to soil thaw, reducing soil N contributions. Gaseous N losses from denitrification have been observed to increase due to snow accumulation (Brooks et al. 1997).

8.4.4 Freeze thaw

Increases in amino acid and glucose concentration after freeze-thaw were only observed in the ambient soil from the Meadow site (Fig 8.12-8.13). This is counter to the expectation that a milder winter would cause more FT effect due to damaging previously protected soil. It is possible that the small temperature difference that the soils experienced was insufficient

to cause such an effect if it is an important factor. The earlier thaw in the ambient plots may have increased plant production and biological activity compared to the snow accumulation plots, as is suggested by the respiration results and some previous research (Natali et al. 2012; Sutinen et al. 2015). In some past research longer snow cover has enhanced plant growth, but this was later in the season and may have been due to enhanced soil moisture which was not observed here (Bosio et al. 2014). As established in previous chapters, and by Elliot (2013), Giesler et al. (2007) and Tierney et al. (2001), plants can be an important source of DOC during FT and so may have been responsible for observed increase the glucose and amino acids. No significant difference was observed between microbial biomass carbon or nitrogen due to the snow fence, but the microbial community could be more active which might make them more susceptible to freeze thaw damage.

This result could be important as it suggests that with time soils can recover from thaw and be further damaged by freeze-thaw cycles. Currently in the high arctic, thaw does not tend to occur until 24 hour daylight when temperatures are consistently above 0°C. With a shorter duration of snow cover predicted an early spring provides both the chance for earlier plant growth and more freeze thaw cycles when plants and soil microbes are actively growing. This could cause more DOC production by FT and potentially more greenhouse gases. Whilst past studies have suggested that more CO₂ is produced by unfrozen soils than FT subjected soils the same is not true for N₂O (Matzner and Borken 2008).

8.5 Conclusion

The effects of warmer winter temperatures and later soil thaw on carbon and nitrogen cycling in the early growing season of arctic tundra soils were assessed. The small warming affect caused by snow fence treatment appeared to have little effect on soil processes. The increase of average winter temperature of about 3°C between 2012-2013 and 2013-2014 appears to have enhanced amino acid availability and N mineralisation, particularly nitrification. This seems to occur due to carbon limitation in the microbial biomass, as observed in the significant decrease in MBC in the latter year, which could enhance the degradation of organic material with excess nitrogen being mineralised, but changes in the microbial community could also be responsible. Changes to the fungal community were also observed between years at the Meadow site. The later thaw caused by the snow fence in 2013 appears to have delayed plant growth, decreased CO₂ emissions potentially due to less primary

productivity and made soil less susceptible to FT damage. The later thaw did not have a discernible effect on the fate of added ^{13}C or ^{15}N , but plant allocation of ^{13}C appeared to depend on the species and ^{15}N within plant allocation may have been substrate dependent. This work emphasises the potential for warmer winters to increase nitrate production which could affect plant nutrition, species composition and N loss from the terrestrial ecosystem into or by N_2O emissions. The emission of N_2O could be further enhanced with a greater potential for FT damage in an earlier thaw. This is an area that requires further study.

Chapter 9

General Discussion and Conclusion

9. General Discussion and Conclusion

9.1. Introduction

The aim of this thesis was to investigate how freeze-thaw cycles (FTC) affect the composition, concentration, dynamics and fate of low molecular weight dissolved organic carbon (LMW-DOC) in arctic tundra soils. This carbon pool has been shown to be highly diverse and vital for microbial processes, with certain compounds being produced and used rapidly, potentially supporting large amounts of greenhouse gas emissions (Boddy et al. 2007; Fujii et al. 2010). The quality as well as the quantity of the carbon in this pool is important, inspiring study of its composition as well as its concentration (Kiikkilä et al. 2014). Further, it could be a pivotal pool of carbon in many processes occurring in frozen soils (Michaelson and Ping 2003). The arctic tundra was focussed on in this thesis as it represents both an important carbon store and it is an ecosystem predicted to experience a dramatic change in climate, particularly over the winter period (Anisimov et al. 2007; Tarnicai et al. 2009). This could lead to changes in soil temperature, freezing duration and, potentially, an increase in temperature variability around 0°C which could lead to FTC of shorter duration than the entirety of winter (Cooper 2014; Henry 2008).

The last 6 chapters of this thesis have explored the microbial use of different LMW-DOC substrates in soil both before and after thawing; discussed LMW-DOC inputs to soil during FTC and how they affect the DOC composition and concentrations in thawed soils; investigated whether FTC liberated LMW-DOC could stimulate the degradation of soil organic matter; and whether warmer freezing temperatures and shorter freezing durations over the arctic winter impact DOC and associated C and N cycling in the growing season. In this final chapter the results of the last 6 chapters will be summarised and discussed. The significance of the work will be assessed alongside the questions it raises for future research. This will start with discussion of unfrozen water content in soils below 0°C and how in combination with freezing temperature it can affect LMW-DOC dynamics in frozen soil. How this freezing affects LMW-DOC composition, concentration and fate and the connected effects on C and N cycling after thaw will then be discussed.

9.2. Soils below 0°C

Liquid water is vital for biological activity and for the mobility of DOC. Soils do not freeze immediately as air temperatures dip below 0°C largely due to water's high heat capacity and latent heat of fusion (Romanovsky and Ostercamp 2000). Ice crystals can form when soil solution reaches below zero, but above -39°C this requires ice crystal nuclei. The propagation of ice crystals requires water to flow to the ice front which depends on the soils hydraulic conductivity. Thus, soil freezing is quite gradual and offset from air temperature. Even when ice has formed, liquid water still exists in thin films between ice crystals and soil particles and in nanometer scale pores, as illustrated in figures 9.1 and 9.2 (Hansen-Goos and Wettlaufer 2010). Chapter 7 investigated how much liquid water existed at different temperatures below 0°C. It found that using a model that included mineral precipitation (FREZCHEM) vastly underestimated unfrozen water contents, whilst alcohol calorimetry and a pre-melting model that utilised an ideal solution with ice/particulate surface interactions were of a similar magnitude. The pre-melting model suggested that for soils with a large surface area, surface charge was an important factor in determining the amount of unfrozen water, but for soils with surface areas typical of large clay to sand range, solute content was more important. This suggests that salt content is important for the liquid water content in mineral soils, whilst in organic soils the unfrozen water content might be more dependent on surface forces, which agrees with the work of Harrysson-Drotz et al. (2009). Other work has shown that the soil drying curve models the water content of soil freezing, which does not include the effect of solutes (Kurylyk and Watanabe 2013). This in part could be due to the hysteresis effect of freezing as water flow to the freezing front can be retarded by hydraulic properties, while soil frozen for a long time may not have this problem. Past work also suggests that liquid water content between grains which would require ice crystals of high curvature is also important in soils with sand sized grains (Hansen-Goos and Wettlaufer 2010). Unfrozen water can also exist between ice crystals, which could also be an important source of water, particularly when the ice crystals are small. This would not be included in soil drying curves. Little is known about the size of ice crystals in soils and recrystallisation can occur at mild freezing temperatures causing larger crystals to form reducing surface area and liquid water content. This makes calculation of this portion of unfrozen water problematic. Microbes can produce compounds which keep ice crystals small, possibly helping retain water in liquid form in soil below 0°C. However, the thickness of the water films is greater around larger grains/crystals and substrate diffusion is greater in thick films

(Brown et al. 2012). Thus, this strategy could further limit microbial access to external substrate.

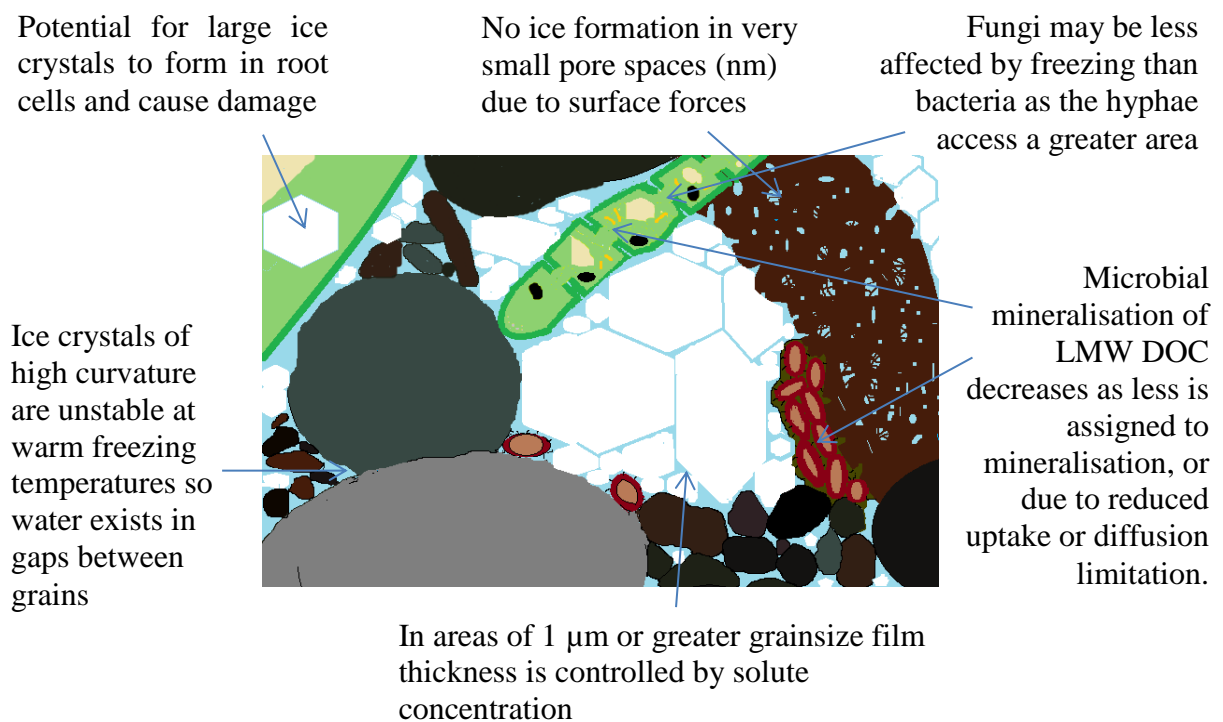


Fig. 9.1. Schematic diagram illustrating processes in a mildly frozen (warmer than -3°C) pore space

Mineralisation of LMW DOC occurs in arctic tundra soils below 0°C . In chapter 7, mineralisation was detected down to -6°C , but neither mineralisation nor uptake was detected at -12°C . CO_2 emissions have previously been measured at temperatures below -6°C with Drotz et al. (2010) and Schimel and Mikan (2005) measuring emissions at -9°C , and Panikov et al. (2006) measured emissions at -39°C . The latter studies added labelled substrate prior to freezing and so did not show if uptake occurred. Chapter 7 suggests that microbes do not take up substrate at -12°C which is within the winter temperature range for some arctic tundra soil (Semenchuk et al. 2013; Schimel et al. 2005). Although only three substrates were tested, glucose is a substrate almost ubiquitously taken up and used by the soil heterotrophic microbial community in catabolic and anabolic processes (Roberts and Jones 2012; Stülke and Hillen 2000). It is possible, but highly unlikely, therefore that the substrates used here do not reflect general microbial activity in soil. Over the long arctic winter period it is possible that microbes could adapt to the temperature and begin to take up substrate, but this appears to take longer than 1 month (Drotz et al. 2010).

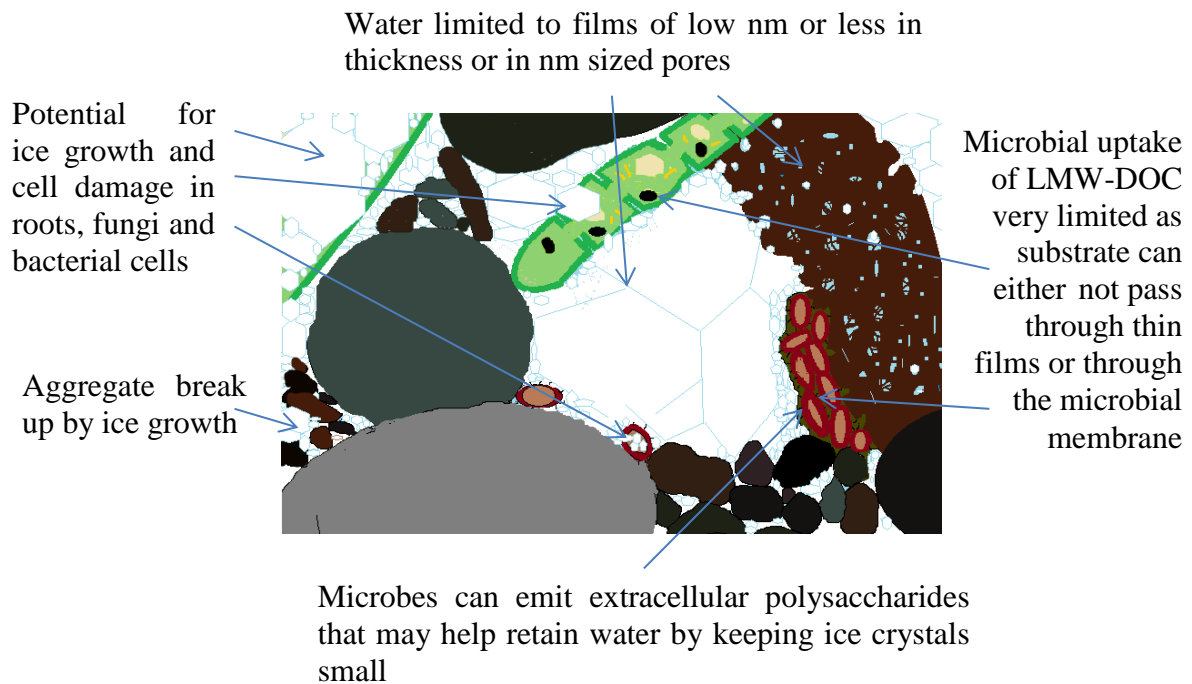


Fig. 9.2. Schematic diagram illustrating processes in an intensively frozen (around -6°C) pore space

The results in chapter 7 showed less carbon substrate was immediately available for mineralisation below zero. Whether this is due to a change in microbial substrate use, to diffusion limitation or changes to microbial membranes affecting uptake is unclear. However, less microbial uptake was measured at -3°C , which implies that at least some of the reduction in available substrate is due to diffusion limitation or changes in microbial uptake. Schimel and Mikan (2005) show that microbial substrate use changes just above 0°C , with a greater proportion of microbial carbon being used for respiration. Chapter 7 did not show any difference until below zero so if this occurred it did not apparently affect uptake or mineralisation of LMW DOC. The effect of freezing temperature on carbon mineralisation was substrate specific with glucose being less affected than alanine which was less affected than trialanine. This could be a sign that microbes prioritise the uptake of glucose over alanine and trialanine, or show that trialanine and alanine are more diffusion limited than glucose when traveling through a concentrated liquid water film that is only a few nanometers thick (Liu and Bruening 2004). If the latter is the case this could suggest that, in freezing conditions, microbes are more reliant on small compounds that are highly soluble. This slower diffusion could also reflect the charged properties of amino acids and peptides and greater reaction with charged surfaces in thin water films. Soil type also seemed to have an effect on substrate diffusion and uptake, with the Heath soil showing less uptake of alanine

than the organic meadow soil. This effect could be due to a higher unfrozen water content in the organic soil or its greater microbial biomass meaning the substrate had to diffuse less far to be used (Harrysson-Drotz et al. 2010; Nunan et al. 2003).

Modelling soil mineralisation below 0°C empirically has been hindered by a lack of accurate measurements, although ongoing work, over the last couple of decades, has been remedying this deficiency (Schimel et al. 2005). In chapter 7 the Dual Arrhenius Michaelis Menten model, modified for enzymatic degradation in frozen soil by Tucker (2014), which incorporated water content to control both substrate and oxygen diffusion limitation was modified to model glucose mineralisation. It produced mineralisation rates of the correct order of magnitude, but requires further testing to see if it works for different substrates and temperatures. This work corroborates with previous studies which suggest that microbes are carbon limited due to slow rates of substrate diffusion in frozen soils (Bukeridge and Grogan 2008; Michaelson and Ping 2003).

Small differences in average freezing temperature (ca 0.5°C) over the arctic winter, did not appear to have a major effect on carbon and nitrogen cycling in the growing season (chapter 8). A longer, and approximately 3°C warmer, winter appears to cause more nitrification and organic N degradation in the growing season than the colder, shorter winter. A decrease in both microbial biomass C (MBC) and the ratio of MBC to MBN was also observed after the warmer winter compared to the colder one. This would be consistent with the results of chapter 7 with a reduction in substrate diffusion or uptake in mildly frozen soils, but sustained microbial activity and depletion of soluble C, whilst colder soils mostly caused diffusion and uptake to cease, significantly reducing microbial activity. A greater survival of bacteria and archaea over the warmer winter and a greater production of hydrolytic enzymes could also potentially explain the observations in chapter 8 (Sistla and Schimel 2013; Strickland and Rousk 2010).

9.3. Soils after thaw

Soils tend to thaw rapidly when temperatures reach 0°C which in the field can be assisted by the infiltration of snow melt water (Sparman et al. 2004). This rapid change in osmotic conditions could be damaging to cells as can internal ice formation during the freezing period (Jefferies et al. 2010; Prickett et al. 2015). In chapters 3 and 4, increases in LMW DOC compounds after a short freeze thaw cycle indicates cellular damage can occur (see figures 9.1-9.3). As occurred in past studies, such damage appears to be finite, with only the first 2

FTC causing an increase in LMW-DOC and freezing maize roots to -5°C not causing greater LMW-DOC concentrations than freezing to -3°C (Morley et al. 1983; Skogland et al. 1988; Yu et al. 2011). However, the results in chapter 8 suggest that with a couple of weeks recovery time and sufficient daylight and plant growth, further freeze-thaw damage can occur. Thus, not only freezing intensity and duration, but also timing is important in assessing FTC greenhouse gas emissions. Chapters 4 and 8 also suggest that FT damage may not cause an increase in LMW-DOC suggesting that a FT induced increase in LMW DOC occurs more in vegetated and relatively organic soils. The results of chapters 3 and 4 indicate that the vegetation could be a major contributor to LMW DOC after FT. The proportion of the contribution was not quantified, but excitation emission spectra and MALDI TOF of soil solution show components that appear to come from the vegetation. Also comparing chapter 3 with previous studies on FT temperature on DOC suggests that plant roots could be more susceptible to FT damage at a warmer freezing temperature than microbes, but this needs to be confirmed with hardier plants than *Zea mays* (Hentschel et al. 2008). *Salix polaris* also seemed to be a source of DOC when frozen to -7.5°C . Whether this is true at milder freezing temperatures could be investigated. Studies that do not include the vegetation could severely underestimate carbon fluxes due to FTC.

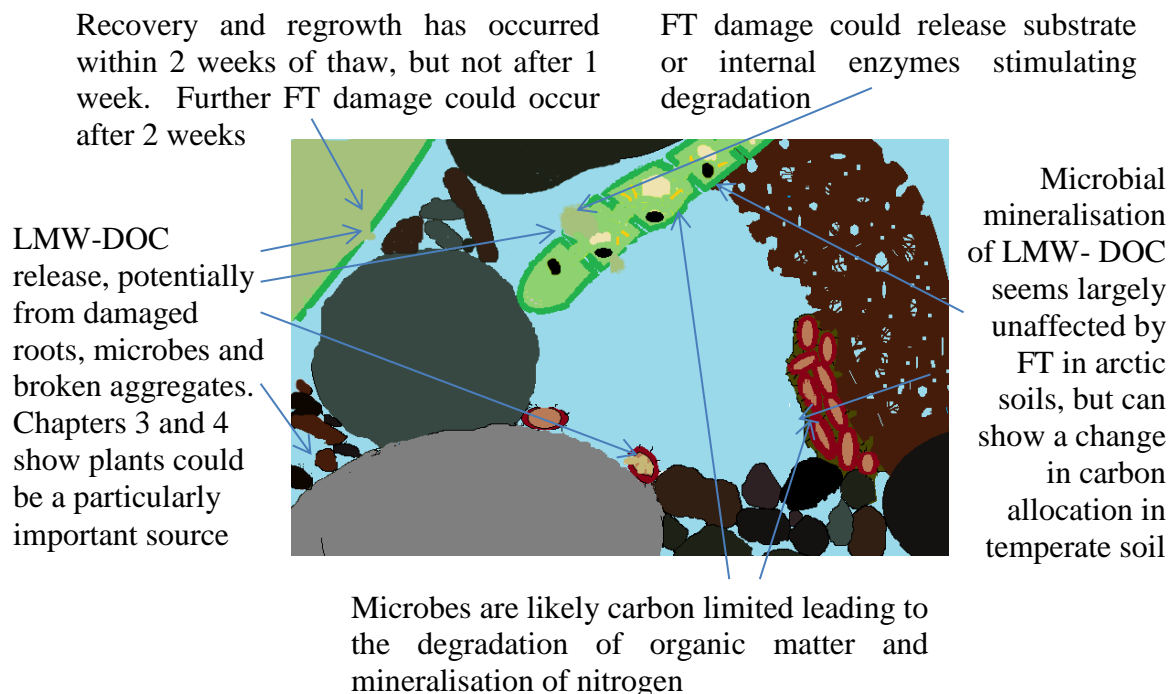


Fig. 9.3. Schematic diagram illustrating processes after thaw in a soil pore space

Microbes appear to recover quite rapidly from short FTC as suggested by the work of Feng et al. (2007) and Morley et al. (1983). Chapter 6 shows that, 12 hours after the air temperature returned to 5°C, the microbial use of certain LMW-DOC substrates, in arctic soils that had been frozen at -7.5°C, was not significantly different from the unfrozen controls. However, temperate soils did show small changes in microbial use of LMW-DOC. This could indicate a lag period with microbes repairing damage sustained during the FTC. The high concentrations of glucose and amino acids measured after the first FTC in chapter 4 could indicate that the FTC delayed microbial uptake of LMW DOC 6 hours after the air temperature returned to control levels. The 2 g of soil used in chapter 6 would also thaw more rapidly than the intact cores used in chapter 4 so the latter is possibly still closer to 0°C. Other explanations for the high LMW-DOC concentrations, such as physical barriers to diffusion or increased enzyme activity could also apply. Whilst Wang et al. (2014) found a decrease in enzyme activity after FT, Yergeau and Kowalchuk (2008) found a potential increase in cellulase activity due to FT. Clearly this is an area requiring further research. Whether the DOC produced on FT causes a priming effect was not answered satisfactorily in chapter 5. The higher than predicted ¹⁴C emissions could be from the microbial pool rather than old soil organic carbon.

The timing of thaw in arctic tundra ecosystems can have a dramatic effect on carbon and nitrogen cycling. An earlier thaw allows earlier plant and microbial growth causing greater CO₂ emissions (see chapter 8), but also greater primary productivity (Natali et al. 2012; Sutinen et al. 2015). As shown in chapter 8, an earlier thaw could also increase the effect of FTC. Chapter 4 shows these can increase LMW-DOC, LMW-DON and potentially NO₃⁻ concentrations. With a long enough interval between FTC, repeated FTC could potentially produce more LMW-DOC and DON than shown in chapter 4 where only the initial 2 cycles had an effect. Changes to thaw date and nutrient availability have been shown to affect vegetation type (Robinson et al. 1998; Sturm et al. 2005; Wahren et al. 2005). The results of labelling studies in chapter 8 suggest that changes in the abundance of evergreen and deciduous shrubs and in the availability of different N forms might affect how carbon and nitrogen is stored in the plant. This could have repercussions for how and when the nutrients can be returned to the soil. With more deciduous shrubs and more carbon stored in the roots in soluble form, this could be released during FT events. If NO₃⁻ becomes more available and is stored in the foliage, then this may only be returned to the soil after senescence. Alternatively, foliage could potentially be more susceptible to FT than roots as it is not as

well insulated from air temperatures. Thus potentially FTC could produce more N and make emissions of N₂O more likely (Buckeridge et al. 2010; Sehy et al. 2004). N released by foliage could be more susceptible to being transported into rivers as it could runoff over the soil surface, particularly if the soil is frozen and the water cannot infiltrate the soil well.

9.4. Significance of results and areas for future research

This work has made some novel contributions to knowledge of C and N cycling in arctic soil over the three quarters of the year where it is frozen or prone to freezing. The results have clearly shown changes to LMW-DOC use and uptake at low temperatures and that these changes are substrate dependent. New methods of measuring unfrozen water content in soils below 0°C have been trialled, providing insight into how water remains in liquid form in different soil types. It also shows that thin films of water are unlikely to collapse at temperatures that the active layer tends to experience in Svalbard, although materials other than quartz need to be tested (Morgner et al. 2010; Semenchuk et al. 2013). This work is also the first to use the DAMM equation to attempt to model LMW-DOC mineralisation in frozen soils. This could be important as microbes maybe limited to dissolved substrate in frozen soil. Further work is required on how liquid water content affects substrate diffusion, if there is a lower temperature limit to substance diffusion and if this temperature varies for the type of substance. For example, enzyme diffusion could be more limited than LMW-DOC diffusion. More *in situ* studies would also be useful as sieving the soil radically affects the bulk density which could affect unfrozen water content and mineralisation. Studies in degradation of SOM in frozen soils and after thaw requires more work as this project suggests that little degradation occurs in frozen soil, but did not conclusively show if it was enhanced on thaw.

This study is the first to make a detailed study of the effects of FTC on LMW-DOC. Little is still known about the composition of DOC. Over the course of this thesis it has been shown that the effects of freezing temperature, FT damage and recovery can be specific to the particular DOC compound being investigated. For example, glucose mineralisation was less affected by freezing soil than amino acid and trialanine, as shown in chapter 7; chapter 6 suggested that the fate of LMW-DOC after FT could depend on compound type; and chapter 3 showed a greater increase in amino acids than glucose due to root FT input. These results show that a more in depth understanding of the DOC pool is required, more than by size fractionation as the LMW-DOC pool is highly diverse. A greater understanding of DOC

composition and utilisation is not limited to FT fluxes, but in many areas where soil C dynamics is investigated. This work agrees with past studies that C fluxes appear to be short in duration following FTC (Matzner and Borken 2008; Schimel and Clein 1996; Yu et al. 2011). This suggests C fluxes caused by FTC are not annually important. This work also finds that microbial recovery is rapid. Further work on FTC cycles, with both longer freezing and thawing periods could be beneficial in proving if recovery can cause more FT damage and if FTC can have a longer term effect than suggested from the LMW-DOC studies.

This work suggests that climate change could affect arctic carbon and nitrogen storage. Further work could be directed on to how this might affect C and N fluxes after FTC. More *in situ* FTC studies could perhaps be done using snow removal in spring, combined with watering to keep soil moisture levels similar. More detail on nitrogen fluxes is required, particularly with a changing climate potentially increasing NO_3^- concentrations. Fluxes of N_2O could become a large feedback to climate change in an area in which they are currently considered to be low, with the exception of some unvegetated, cryoturbated areas (Marushchak et al. 2011).

9.5. Conclusion

This work examined C and N dynamics due to and during FTC in arctic tundra soils, with a primary focus on LMW DOC and LMW DON. When frozen, soils showed decreased liquid water content which could be measured by alcohol calorimetry and modelled by a pre-melting model. However, these methods need further work to check and improve their accuracy. LMW-DOC and DON mineralisation decreased below zero with the change being more apparent for trialanine, then alanine and then glucose. Uptake decreased more for alanine than for glucose at -3°C and this effect was greater in the Heath tundra soil than in the Meadow tundra soil. This suggests that DON uptake is not favoured over DOC in frozen soils and that diffusion or microbial uptake is less in the Heath soil, potentially due to the lower organic carbon content causing less liquid water to be available. Modelling the mineralisation rates of glucose at -3°C using the DAMM and measured or calculated water contents was moderately successful. It suggested that modelling unfrozen water in soils using FREZCHEM may not be appropriate. The results suggest that the DAMM model should be tested further for different substrates and freezing temperatures.

The results of these experiments show clearly that plants can be an important source of LMW DOC to soils after FT with *Zea mays* clearly increasing glucose and amino acid

concentrations in soil after relatively mild FTC (-3°C). *Salix polaris* from the arctic tundra also appeared to be a source of DOC to the soil during FTC. The DOC compounds released from *Salix polaris* could not be identified exactly, but may have included glucose, glucose storage compounds and LMW humic-like material. FTC to -7.5°C caused changes to DOC concentration and composition in arctic tundra soil, but these effects were more pronounced for the vegetated, organic rich Meadow tundra soil than for the bare, low organic Heath tundra soil. Changes to concentration were only observed for the first two cycles which is consistent with previous studies. Increased DON concentrations were observed after the initial cycle and it is possible that nitrate concentrations increased due to FT. Rapid recovery of LMW DOC and DON mineralisation in arctic tundra soil was observed with little difference being observed between control and 1 or 3 FTC 12 hours after thaw.

Whilst in the field it can be hard to identify cause and effect, the results in chapter 9 suggest that winter temperatures and thaw date can affect C and N cycles in arctic tundra soil. An earlier thaw appears to enhance soil respiration, plant growth and could lead to increased fluxes of DOC due to subsequent FTC. Warmer winter soil temperatures (c.a. -2°C) appear to increase nitrification and amino acid turnover in spring compared to colder winter temperatures (c.a. -5°C). Also, much lower MBC/MBN values were observed in soils after the warmer winter due to lower MBC levels. These results are consistent with increased C limitation at milder freezing temperatures, followed by use of DON as a carbon source and N mineralisation. A change in microbial community due to the warmer winters could also be responsible for the observed results.

The course of this work has raised many intriguing questions for further research. The use and production of LMW-DOC in, and the modelling of unfrozen water content and emissions from frozen soil is an area that should be focussed on. It would also be intriguing to see if different results were obtained for longer FTC and longer recovery periods than those used here. This thesis has also shown the importance and usefulness of examining DOC composition. DOC concentration and composition after repeated FTC has been examined in more detail than previously, with plants being observed as important sources. Further understanding of the dynamics of particular LMW DOC compounds during and after soil freezing has been gained. This work has also added to knowledge of C and N cycling over winter in the arctic tundra.

10. References

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Appendix A

R/P	Heath control	Meadow control	Heath FT	Meadow FT	Fumigated	Non-fumigated	<i>Salix</i> control	<i>Salix</i> FT	<i>Ptilidium</i> control	<i>Ptilidium</i> FT
Heath control	n/a	0.49	0.079	0.008	0.018	0.048	0.018	0.018	0.018	0.018
Meadow control	-0.028	n/a	0.016	0.008	0.018	0.048	0.018	0.018	0.018	0.018
Heath FT	0.216	0.403	n/a	0.024	0.057	0.067	0.057	0.057	0.057	0.086
Meadow FT	0.89	0.896	0.413	n/a	0.036	0.048	0.018	0.018	0.036	0.036
Fumigated	0.969	1	0.472	0.701	n/a	0.1	0.1	0.1	0.1	0.1
Non-fumigated	1	1	0.464	0.818	1	n/a	0.1	0.1	0.1	0.1
<i>Salix</i> control	1	1	0.611	0.743	1	1	n/a	0.4	0.1	0.1
<i>Salix</i> FT	1	1	0.611	0.754	1	1	0.037	n/a	0.1	0.1
<i>Ptilidium</i> control	0.764	0.916	0.546	0.549	0.593	0.917	0.407	0.333	n/a	0.7
<i>Ptilidium</i> FT	1	1	0.556	0.713	1	1	0.926	0.889	-0.111	n/a

Table A1 R (bottom left) and p (top right) values for the ANOSIM analysis of the MALDI-TOF results. Bold R values indicate significance $p < 0.05$

Appendix B

Soil	a_1	k_1	a_2	k_2	a_3	k_3
Meadow (m)	10.69 ± 0.42	1.24 ± 0.04	8.32 ± 0.30	4.97 ± 0.74	80.94 ± 0.63	1.62 ± 0.18
Meadow (o)	10.68 ± 0.52	1.10 ± 0.02	8.70 ± 0.52	7.55 ± 0.51	80.53 ± 0.78	1.86 ± 0.24
Heath (m)	8.75 ± 0.94	0.50 ± 0.13	8.99 ± 0.39	3.36 ± 0.67	82.10 ± 1.22	1.73 ± 0.26

Table B1 Triple first order decay mineralisation parameters for the decrease in soil ^{14}C originally added as glucose over 18 months. Values represent mean ± SEM (n = 4).

Soil	Snow treatment	a_1	k_1	a_2	k_2	a_3	k_3
Meadow (m)	Ambient Snow	9.51 ± 0.20	1.32 ± 0.08	6.88 ± 0.95	7.48 ± 1.98	83.58 ± 1.08	3.90 ± 0.70
	Increased Snow	9.60 ± 0.10	1.31 ± 0.03	7.61 ± 0.57	5.94 ± 0.25	82.75 ± 0.57	2.90 ± 0.19
Meadow (o)	Ambient Snow	8.07 ± 0.19	1.21 ± 0.07	7.17 ± 0.87	7.25 ± 1.90	84.71 ± 0.99	2.86 ± 0.66
	Increased Snow	8.17 ± 0.15	1.25 ± 0.03	5.54 ± 0.24	13.61 ± 3.21	86.26 ± 0.28	5.43 ± 0.44
Heath (m)	Ambient Snow	7.47 ± 4.81	0.41 ± 0.14	25.14 ± 4.78	1.04 ± 0.36	67.05 ± 9.51	0.36 ± 0.36
	Increased Snow	4.87 ± 0.88	0.33 ± 0.05	19.20 ± 2.71	3.83 ± 1.40	75.70 ± 2.17	1.88 ± 0.56

Table B2 Triple first order decay mineralisation parameters for the decrease in soil ^{14}C originally added as glucose over 6 months in soil from snow treatment plots. Values represent mean ± SEM (n = 4).