Microbial life in boreal soils

On the availability and fate of carbon substrates for microbial activity in boreal soils

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Microbial life in boreal soils - On the availability and fate of carbon substrates for microbial activity in boreal soils

Abstract

The large pool of carbon (C) stored as soil organic matter (SOM) in soils of high-latitude ecosystems contains more organic C than all global vegetation and the atmosphere combined. Global climate change is expected to have especially pronounced effects in these ecosystems, and even small changes in the accumulation and decomposition of their soil C pool driven by heterotrophic microbial activity could profoundly affect atmospheric CO_2 levels and thus the global climate. Because such changes could trigger drastic shifts in the delicate balance of CO_2 between the biosphere and atmosphere, a better understanding of the key regulators of C cycling is urgently needed.

Using advanced molecular and biochemical techniques, I investigated the availability of C substrates and their utilization by microorganisms under controlled but ecologically relevant conditions in soils representative of the boreal landscape. This molecular characterization of SOM revealed that tree species significantly influence SOM genesis by changing its rate of accumulation and organo-chemical composition. More importantly, a strong connection between SOM decomposition and microbial decomposers was observed and shown to be governed by the organo-chemical composition of the SOM. The structural arrangement of cellulose, and particularly its degree of crystallinity, emerged as a key factor determining rates of cellulose hydrolysis and subsequent C decomposition in boreal forest soils.

This work provides some of the first empirical evidence that soil microbial communities in frozen boreal forest soils can hydrolyze cellulose and use the released substrate for both catabolic and anabolic metabolism. These findings, together with results from peat soil experiments, show that both persistent microbial degradation of C (biopolymers and monomers) and the synthesis of new microbial biomass during winter are widespread features in soils of the boreal landscape. More importantly, the results indicated that small differences in winter soil temperatures can have very large implications for the winter C fluxes of boreal soils.

Over the studied temperature range, C substrates were readily utilized and microbial activity was never totally impeded. However, thermodynamic constraints caused strong reductions in metabolic rates at sub-zero temperatures. The rates of these processes at low temperatures are low but their importance should not be neglected given the spatial scale over which they can occur and the prolonged winters these ecosystems experience. Taken as a whole, this thesis provides a valuable contribution to our understanding of microbial C cycling in one of the world's major soil C pools.

Keywords: boreal forest, soil organic matter, organo-chemical composition, frozen soils, CO_2, biopolymers, decomposition, hydrolysis, PLFA, ^{13}C-NMR, Py-GC–MS, microbial metabolism.

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Mikrobiellt liv i boreala jordar - Om substrattillgång och substratutnyttjande av kol för mikrobiell aktivitet i boreala jordar

Sammanfattning

Globalt sett finns det mer kol bundet i marken än vad som finns i både atmosfären och i växter i biosfären tillsammans. Det gör att även små förändringar i nedbrytningen av markens organiska material (OM) som drivs av mikrobiell aktivitet kan ha betydande konsekvenser för den globala kolbalansen. En övervägande stor del av markkolet återfinns i nordligt belägna ekosystem. Både nutida observationer och modellprognoser för framtida förhållanden tyder på att klimatförändringarna särskilt påverkar dessa ekosystem. Mot denna bakgrund är det av stor vikt att undersöka de faktorer som kan påverka den känsliga balansen i utbytet av CO_2 mellan mark och atmosfär.

Med hjälp av avancerade molekylära och biokemiska tekniker undersöker denna avhandling både substrattillgång och substratutnyttjande av kol i marken under kontrollerade, men biologiskt relevanta betingelser för jordar som är representativa för det boreala landskapet. Den molekylära karakteriseringen av OM visade att olika trädslag signifikant påverkar OM genes både genom att ändra ackumuleringshastigheterna och dess organo-kemiska sammansättning. En stark koppling mellan nedbrytning av OM och markorganismerna var tydlig, med den organo-kemiska sammansättningen av OM som länkande faktor. Vidare framkom att cellulosastrukturen och dess kristallinitetsgrad är ett centralt inslag som reglerar hastigheten av cellulosahydrolys och efterföljande nedbrytning av kol i boreal skogsmark.

Detta arbete utgör den första empiriska observationen som visar att markorganismerna i frusen skogsmark kan hydrolysera cellulosa och använda det frigjorda substratet för både katabolisk och anabolisk metabolism. Dessa resultat, tillsammans med resultat från myrmarksexperiment, visar att både fortgående mikrobiell nedbrytning av kol (biopolymerer och monomerer) och syntes av ny mikrobiell biomassa under vintern är en utbredd företeelse i det boreala landskapet. Resultaten visar också att små skillnader i marktemperaturer under vintern kan få stora konsekvenser för kolnedbrytning i skogs- och myrmarken.

Den mikrobiella aktiviteten och substratutnyttjandet upphörde aldrig helt i det intervall av temperaturer som undersöktes. Vid lägre temperaturer fanns dock tydliga termodynamiska begränsningar. Även om processerna vid låga temperaturer är långsamma bör deras betydelse inte försummas, särskilt med tanke på den globala skalan och de långvariga vintrarna typiska för boreala ekosystem. Sammanfattningsvis utgör denna avhandling ett värdefullt bidrag till förståelsen av substrattillgång och substratutnyttjande av kol i marken som drivs av mikrobiell aktivitet i en av världens största sänkor av kol i biosfären.

Nyckelord: boreal skog, organiskt material, organo-kemisk sammansättning, frusna jordar, CO_2, biopolymer, nedbrytning, hydrolys, PLFA, ^{13}C-NMR, Py-GC–MS, mikrobiell metabolism.

Vida microbiana en suelos boreales - Acerca de la disponibilidad y el uso de sustratos de carbono para la actividad microbiana en suelos boreales

Resumen

La gran cantidad de carbono (C) almacenada como materia orgánica (MO) en los suelos de los ecosistemas de latitudes altas comprenden más C orgánico que la vegetación global y la atmósfera combinadas. El avance del cambio climático afecta particularmente a estos ecosistemas. Incluso pequeños cambios en la acumulación y descomposición de la reserva de C en el suelo, derivado de la actividad microbiana heterotrófica, pueden afectar profundamente los niveles atmosféricos de CO_2 y el clima global. Con vistas al cambio drástico que tales alteraciones ambientales podrían desencadenar en el delicado equilibrio de CO_2 entre la biosfera y la atmósfera, una mejor comprensión de los reguladores de ciclo del C es ahora más clave que nunca.

Mediante el uso de técnicas moleculares y bioquímicas avanzadas, investigué la disponibilidad de los sustratos C y su utilización por microorganismos en condiciones controladas, pero ecológicamente relevantes, en suelos representativos del ecosistema boreal. La caracterización molecular de la MO reveló que las especies arbóreas influyen significativamente en la génesis de la MO tanto por su influencia en las tasas de acumulación como en su composición organo-química. Más importante aún, observé una relación entre la descomposición de la MO y los microorganismos descomponedores que a su vez está estrechamente ligada a la composición organo-química de la MO. Además, la disposición estructural de la celulosa, que implica diferentes grados de cristalinidad, surgió como una característica clave en la determinación de las tasas de hidrólisis de la celulosa y posterior descomposición de C en el suelo del bosque boreal.

Este trabajo proporciona una de las primeras observaciones empíricas de que las comunidades microbianas en suelos congelados de bosques boreales pueden hidrolizar la celulosa y utilizar el sustrato liberado para su metabolismo catabólico y anabólico. Estos hallazgos, junto con los resultados de los experimentos en suelos de turberas, muestran que tanto la degradación microbiana persistente de C (biopolímeros y monómeros) como la síntesis de nueva biomasa microbiana durante el invierno es una característica generalizada en los suelos del paisaje boreal. Aún más relevante es que, los resultados indicaron que pequeñas diferencias en las temperaturas del suelo durante el invierno pueden tener grandes implicaciones para los flujos de C emitidos de suelos del paisaje boreal.

Los sustratos de C se utilizaron y la actividad microbiana nunca se vio inhibida. Sin embargo, restricciones termodinámicas fueron evidentes en las investigaciones de suelos congelados. Las tasas de estos procesos a bajas temperaturas son lentas, pero no se debe ignorar su importancia considerando la escala global a la que potencialmente se pueden producir durante los prolongados inviernos que experimentan estos ecosistemas. En conjunto, esta tesis proporciona una valiosa contribución para la comprensión de la disponibilidad y utilización de los sustratos de C, dos controles clave sobre la actividad microbiana y el ciclo de C en uno de los principales reservorios de C del mundo.

Palabras clave: bosque boreal, materia orgánica, composición organo-química, suelos congelados, CO_2, biopolímeros, descomposición, hidrólisis, PLFA, ^{13}C-RMN, Py-GC–MS, metabolismo microbiano.

Dedication

To all of you who made this journey possible.

Somewhere, something incredible is waiting to be known.
Carl Sagan

Contents

List of publications

This thesis is based on the work contained in the following papers, referred to by Roman numerals in the text:

I Javier H. Segura*, Mats B. Nilsson, Björn Erhagen, Tobias Sparrman, Henrik Serk, Julie Tolu, Jürgen Schleucher, Mats G. Öquist (2018). Effects of boreal forest tree species on soil organic matter, decomposition rate and its temperature sensitivity (*Manuscript*)

II Javier H. Segura*, Mats B. Nilsson, Mahsa Haei, Tobias Sparrman, Jyri-Pekka Mikkola, John Gräsvik, Jürgen Schleucher, Mats G. Öquist* (2017). Microbial mineralization of cellulose in frozen soils. Nature Communications, 8 (1154).

III Mats G. Öquist*, Mahsa Haei, Mats Nilsson, Javier H. Segura, Tobias Sparrman, Jyri-Pekka Mikkola, John Gräsvik, Jürgen Schleucher (2018). The influence of cellulose crystallinity on its microbial decomposition in boreal forest soils. (*Manuscript*)

IV Javier H. Segura*, Mahsa Haei, Tobias Sparrman, Mats B. Nilsson, Jürgen Schleucher, Mats G. Öquist (2018). Microbial utilization of simple carbon substrates in boreal peat soils at low temperatures (*Manuscript*)

* Corresponding author.

The contribution of Javier Segura to the papers included in this thesis was as follows:

I Idea and hypothesis, 75%, Planning and performance of work, 100%; Analysis and summary of results, 75%; Writing of manuscript, 80%.

II Planning and performance of work, 50%; Analysis and summary of results, 70%; Writing of manuscript, 80%.

III Planning and performance of work, 50%; Analysis and summary of results, 50%; Writing of manuscript, 30%.

IV Idea and hypothesis, 50%; Planning and performance of work, 100%; Analysis and summary of results, 75%; Writing of manuscript, 80%.

Abbreviations

^{13}C	The stable carbon isotope with a mass of 13 Daltons
^{13}C-WSC	^{13}C water-soluble carbohydrate monomers and oligomers
$^{13}C1$ ß-D-glucose	ß-D-glucose labelled with carbon 13 at the C1 position
1 and 2D HSQC	One and two-dimensional heteronuclear single quantum coherence
BR	Basal respiration
C	Carbon
CO_2	Carbon dioxide
CP MAS NMR	Cross polarization magic angle spinning nuclear magnetic resonance spectroscopy
DNA	Deoxyribonucleic acid
dw	Dry weight
D_2O	Deuterium oxide, heavy water
e.g.	*Exempli grattia*
i.e.	*Id est*
IL	Ionic liquid
KH_2PO_4	Monopotassium phosphate
KOH	Potassium hydroxide
LOI	Loss on ignition
N	Nitrogen
N_2	Dinitrogen
NH_4 $2SO_4$	Ammonium sulfate
P	Phosphorous
PCA	Principal component analysis
PCR	Polymerase chain reaction
Pg C	Petagram (10^{15} grams) of carbon
PLFA	Phospholipid fatty acid
PLS	Partial least squares
Py-GC/MS	Pyrolysis gas chromatography/ mass spectrometry
Q_{10}	Factor by which the rate of a biological or chemical process (here, respiration) changes in response to a 10 °C temperature change
Q_R	Ratio of BR to SIR (BR/SIR)
SIR	Substrate-induced respiration

1 Introduction

The earth's biosphere is the reservoir of a large mass of carbon (C). Much of this C is stored in high-latitude ecosystems as soil organic matter (SOM), which contains more organic C than all the world's vegetation and the atmosphere combined (Figure 1). Therefore, even small changes in the accumulation and decomposition of the soil C pool driven by net primary production and heterotrophic microbial activity can profoundly affect atmospheric CO_2 levels and the global climate. However, the factors controlling soil microbial metabolic activity in high latitude ecosystems are not fully understood and the need to fill this knowledge gap is increasingly acute. Recent observations indicate that high-latitude ecosystems are already experiencing severe perturbations induced by the advance of climate change (Ciais *et al.*, 2014).

The complexity of SOM makes it difficult to obtain a coherent understanding of the factors controlling its formation and degradation (Simpson and Simpson, 2012). Three conceptual models intended to explain the nature of SOM have been outlined in the literature. In the classical view, SOM decomposition products are 'humified' and further transformed or synthesized into large, dark-coloured compounds that remain in the soil (Stevenson, 1994). A second model, the "selective preservation model," suggests that SOM constituents rich in aromatic structures (e.g. lignin) are preferentially preserved in the soil while carbohydrates such as cellulose are degraded more rapidly (Sollins, Homann and Caldwell, 1996). In a third conceptual model, SOM is regarded as a complex continuum of plant and microbial biopolymers and their degradation products in a state of progressive decomposition (Sutton and Sposito, 2005; Kelleher and Simpson, 2006; Schmidt *et al.*, 2011).

Most recent studies have attributed the persistence of SOM to complex interactions between biological and physicochemical factors controlling rates of decomposition (Schmidt *et al.*, 2011; Lehmann and Kleber, 2015).

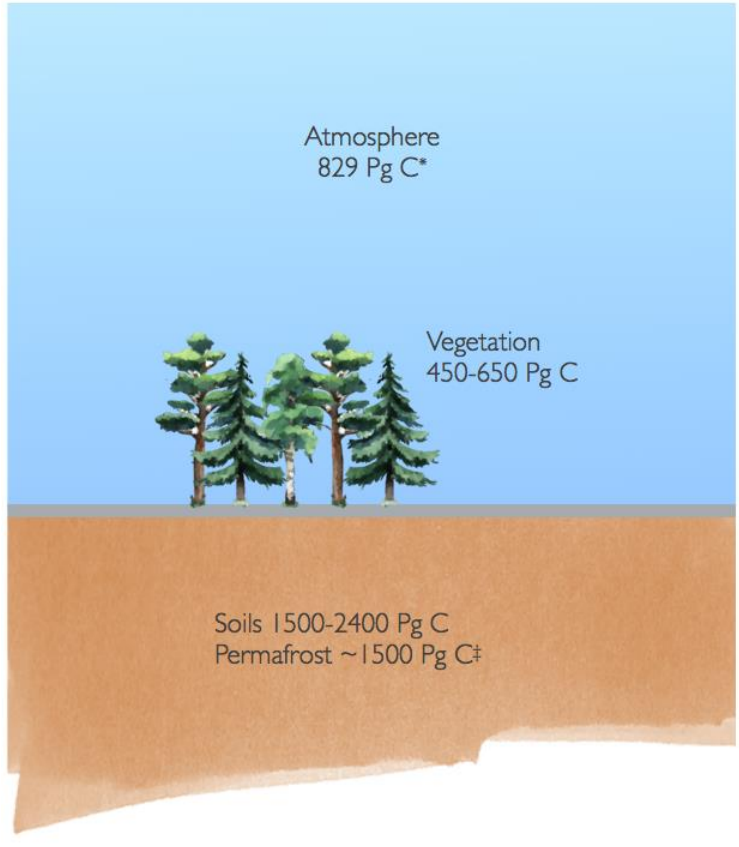


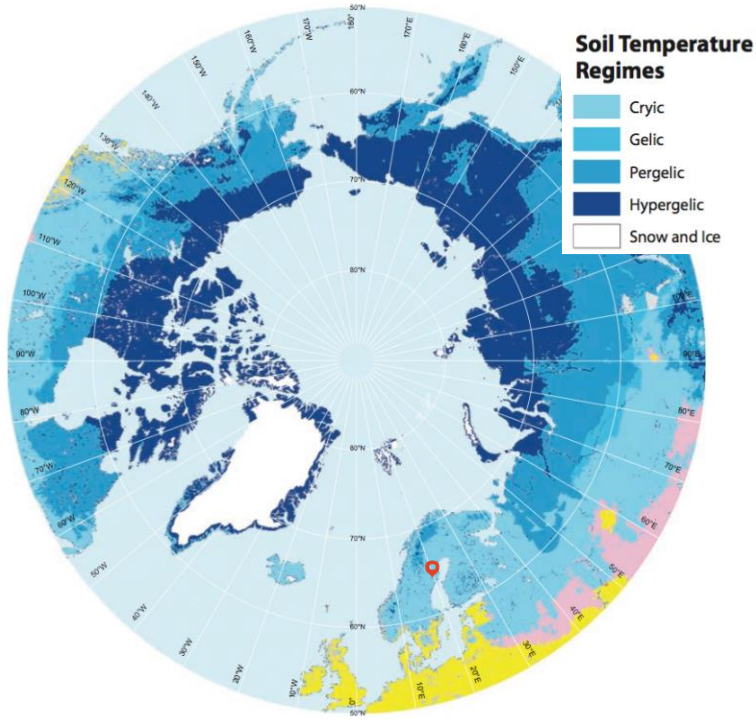
*Figure 1.*Schematic overview of C pools of the terrestrial ecosystem. The numbers indicate the pool mass in Pg C (1 Pg C= 10^{15} g C) based on Ciais *et al*., (2014). * denotes the size of the atmospheric pool of C and is the sum of the preindustrial and cumulative change of anthropogenic C over the industrial period 1750-2011. ‡ denote estimates of the C pool in permafrost adjusted according to Hugelius *et al.*, 2014.

Empirical verification of factors proposed to control SOM formation and decomposition is urgently needed to improve our ability to predict changes in C cycling in high-latitude ecosystems. Boreal ecosystems, are extensively distributed across the northern hemisphere, with boreal forests and peatlands covering 10-15% and 3%, respectively, of the global terrestrial surface area (Lal, 2005; Yu, 2011) (Figure 2). These ecosystems harbour ca. 40% of the total global C pool, much of which is stored in soils (forest soils ~471 Pg C, peatlands ~550 Pg C (Lal, 2005; Yu, 2011). Boreal ecosystems are characterized by strong seasonal variation in air temperature, with short growing seasons, long and cold winters (up to six months), and snow-covered periods.

The typically cold conditions of boreal ecosystems have pronounced effects on their soils, which generally have very high contents of organic matter, low nutrient contents, and low rates of decomposition. The snow cover regulates biogeochemical processes during winter because it insulates the soil surface, reduces heat loss, and controls the formation of soil frost (Groffman *et al.*, 2001; Brooks *et al.*, 2011). Cold and frozen soils are thus common features of boreal landscapes.

Despite low soil temperatures and freezing conditions, soil microbial populations in boreal ecosystems remain metabolically active during winter, as manifested by biogenic CO_2 losses from the soil during the cold months (Coyne and Kelley, 1971; Clein and Schimel, 1995; Fahnestock, Jones and Welker, 1999; Elberling and Brandt, 2003; Öquist *et al.*, 2009). This has been observed in forests and peatlands, two major types of boreal landscape. In boreal forests, winter CO_2 losses from soils can amount to ca. 20% of annual C emissions (Wang, Bond-Lamberty and Gower, 2002; Kim *et al.*, 2007; Sullivan *et al.*, 2008), while CO_2 emissions from boreal peatlands can account for 16–80% of the growing season net CO_2 uptake (Alm *et al.*, 1999; Lafleur *et al.*, 2003; Aurela, Laurila and Tuovinen, 2004; Sagerfors *et al.*, 2008; Peichl *et al.*, 2014; Zhao *et al.*, 2016). Low temperature biogeochemical processes in cold and frozen boreal soils thus contribute significantly to the long-term net ecosystem carbon balance.

The temperature and water content of the soil profoundly affect the rates of heterotrophic microbial activity (Trumbore, 2000; Öquist *et al.*, 2009; Tilston, Sparrman and Öquist, 2010). Winter time soil temperatures in environments with substantial winter snow cover (such as those in the boreal climate zone) are mostly determined by the timing of the snow fall and the insulating properties of the snow cover (e.g. Granberg *et al.*, 1999; Zhao *et al.*, 2016) rather than air temperatures *per se* (Brooks *et al.*, 2011). Even with air temperatures of -20 °C to -30 °C, surface soil temperatures are commonly close to zero or only a few degrees below (e.g. Granberg *et al.*, 1999, 2001; Zhao *et al.*, 2016). The direct effects of temperature on microbial activity (i.e. the kinetics of enzymatic activity) are similar under frozen and unfrozen soil conditions (Öquist *et al.*, 2009; Tilston, Sparrman and Öquist, 2010). However, there are indirect temperature effects that strongly influence the rates of enzyme-catalysed reactions. For instance, when the soil temperature is below the freezing point of the bulk soil solution, the water content and water potential of the soil are dramatically reduced, which affects the microbial community by reducing water availability and substrate diffusion rates (Sparrman *et al.*, 2004; Öquist *et al.*, 2009).


Soil Temperature Regimes
Cryic
Gelic
Pergelic
Hypergelic
Snow and Ice

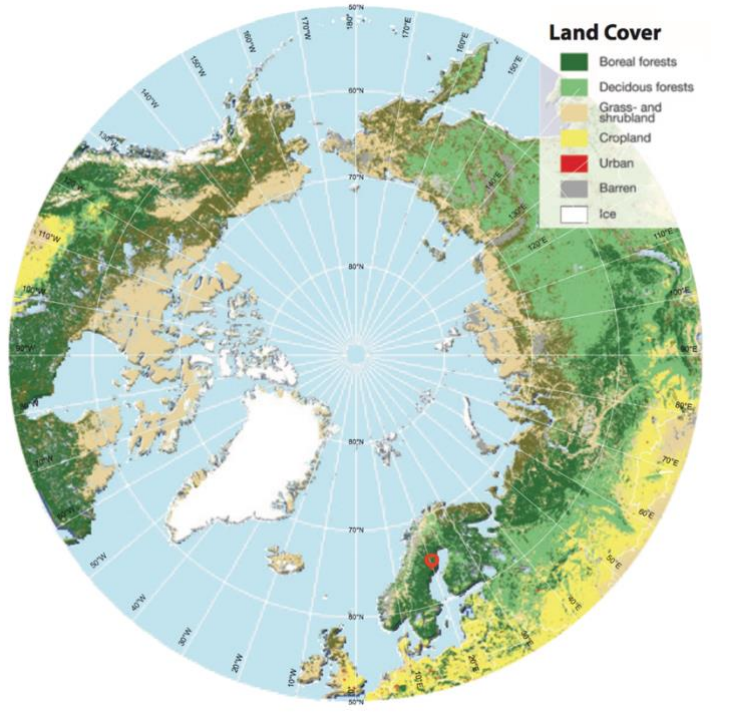

Land Cover
Boreal forests
Decidous forests
Grass- and shrubland
Cropland
Urban
Barren
Ice

Figure 2. Map of the northern circumpolar region showing the field site's location (red marker). The site was chosen to be representative of the central boreal region. Upper map shows the soil temperature regimes. The dark blues of the hypergelic, pergelic and gelic classes indicate very cold soils that broadly coincide with the presence of permafrost. Soils with cryic temperature regimes have a mean annual temperature lower than 8 °C but do not have permafrost. Lower map shows land cover and the extent of the boreal forest (dark green) across Scandinavia, Russia, Canada, and the USA. Modified from the Soil Atlas of the Northern Circumpolar Region (Jones *et al.*, 2010).

Substrate availability and substrate utilization are fundamental concepts in models of low temperature biogeochemistry in high latitude soils. The presence of substrate C and its physical accessibility to microbes have been suggested to be essential for the microbial activities that shape biogeochemical processes (Davidson and Janssens, 2006; Conant *et al.*, 2011; Schimel and Schaeffer, 2012; Erhagen, Ilstedt and Nilsson, 2015; Öquist *et al.*, 2016). This raises two key questions: what substances are good substrates for microorganisms, and what factors affect the availability of these substrates to microorganisms over the boreal temperature range? Heterotrophic microorganisms need energy (i.e. electron donors) and C to sustain the fundamental metabolic processes of respiration and cell growth. The building blocks of SOM, i.e. substances originating from plants and microbes, are the source of this energy and C for soil microorganisms.

The composition of SOM is highly variable, and this variability has a large impact on its decomposition. Studies on the environmental and physicochemical factors that govern SOM formation and decomposition are needed to understand processes critical for soil formation, nutrient cycling and the net ecosystem carbon balance (Swift, Heal and Anderson, 1979; Trumbore, 2000; Kleber, 2010; Schmidt *et al.*, 2011). Unfortunately, the interactions between the composition and decomposition of SOM are incompletely understood because of the challenges associated with characterizing the genesis, turnover, and decomposition of SOM, and the associated biological processes (Lehmann and Kleber, 2015). However, previous studies and recent advances in molecule-level analytical methods have provided new tools for identifying the molecular constituents of SOM and their connection to macroscopic and ecosystem-level responses (Minderman, 1968; Preston *et al.*, 1989; Baldock *et al.*, 1992; Feng *et al.*, 2008; Crow *et al.*, 2009; Clemente *et al.*, 2012; Simpson and Simpson, 2012).

2 Research objectives

The overarching goal of this work was to gain a better understanding of the factors controlling carbon substrate availability and its utilization by microorganisms in boreal forest and peat soils. Specifically, I examined the importance of SOM composition and structure as mechanisms regulating substrate availability to soil microbial populations. I also explored the capacity of these microbial populations for catabolic and anabolic metabolism of these substrates over a temperature range relevant to boreal ecosystems. This included studies on microbial metabolic dynamics below the freezing point (Figure 3). The thesis is based on four studies, which are appended as papers I to IV.

- Paper I focuses on the extent to which different boreal tree species generate SOM with different organo-chemical compositions and how these differences are expressed as differences in SOM accumulation and decomposition, and the temperature response of these processes.
- Paper II explores the capacity of boreal soil microbes to hydrolyze, metabolize, and grow on organic biopolymers (cellulose) under frozen conditions in boreal forest soils.
- Paper III focuses on elucidating the role of cellulose structure, i.e. crystallinity, and its effects on the rates and extent of soil microbial C decomposition.
- Paper IV investigates the capacity of boreal soil microbes to utilize simple C substrates to sustain catabolic and anabolic processes in frozen peat soil.

Taken together, the four chapters of my thesis aim to identify key controlling factors that determine the rate of SOM decomposition in high latitude ecosystems. Each chapter focuses on the influence of one candidate factor – the vegetation type, the metabolic activity of soil microbes under frozen and unfrozen conditions, the organo-chemical composition of the SOM, and the structural organization of the organic substrates in the soil. I investigate each

factor's potential to affect the rate of SOM decomposition under controlled but ecologically relevant conditions. Advanced molecular and biochemical techniques are used to study microbial activity in field-collected soils at several incubation temperatures, both above and below the freezing point, that commonly occur in these ecosystems. This provides valuable information about C cycling during the generally less intensively studied winter conditions. Although microbial activity during the cold months is likely to be slow, the vast geographical extent of these ecosystems and the length of their winters mean that mechanistic understanding of microbial responses to these conditions is extremely important. This is especially true in light of predictions that seasonal dynamics in these regions may be particularly sensitive to the effects of future climate change.

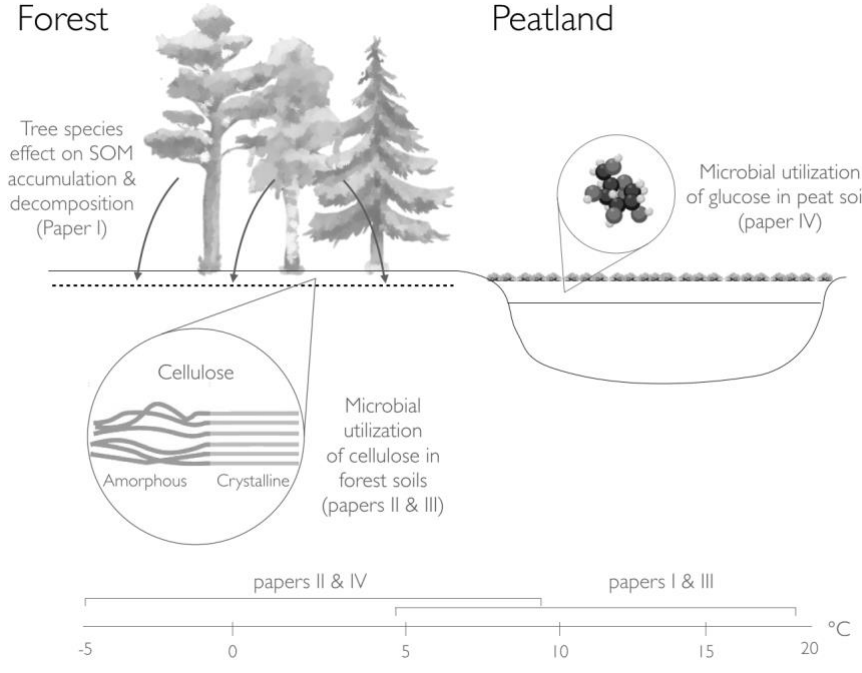


Figure 3. Schematic overview of the studies comprising the thesis. The fundamental questions addressed in this work and formulated as hypotheses in the four papers relate to the factors controlling carbon substrate availability and its utilization by microorganisms in boreal forest and peat soils. The evaluation included the importance of SOM composition and structure (papers I and II) and the potential for changes in metabolic rates (catabolic and anabolic) over an environmentally relevant temperature range for boreal systems, including dynamics below the freezing point (papers III and IV).

2.1 Experimental approach and hypotheses

This thesis describes my use of advanced molecular-level analytical methods and experimental incubations to empirically evaluate the composition of boreal SOM and the extent of C substrate availability and utilization by microbial communities in boreal soils. The hypotheses tested are outlined below.

Paper I: Tree species significantly influence the composition of SOM because they are the sources of much of the diversity of molecular structures found in the litter that subsequently forms the SOM (Preston, Trofymow and Working Group, 2000; Quideau *et al.*, 2001; Erhagen *et al.*, 2013). However, the impact of SOM formation and degradation resulting from changes in tree species composition due forestation and/or global change remains unclear. ***We hypothesized that different tree species generate SOM with different organo-chemical compositions, and that these differences are expressed as differences in the accumulation and decomposition of SOM, and the temperature response of these processes.***

Paper II: Carbohydrate biopolymers such as cellulose typically constitute 40-50% of the SOM mass in the organic horizons of boreal forest soils (Erhagen *et al.*, 2013). This highlights the important effect of cellulose decomposition on soil C balances. It has been suggested that carbohydrate biopolymers may be inaccessible to microorganisms during winter because low temperatures could directly reduce exoenzymatic activity and thus inhibit biopolymer hydrolysis. ***We hypothesized that soil microorganisms in boreal forest soils can hydrolyze, metabolize, and grow on organic biopolymers under frozen soil conditions.***

Paper III: Cellulose in boreal forest soils occurs in two physically distinct forms, crystalline and amorphous. Both forms are abundant in the plant-derived material that constitutes SOM (Newman and Hemmingson, 1990; Andersson *et al.*, 2004). However, little is known about how the amorphous and crystalline structures of cellulose affect its rate of decomposition in boreal forest soils. ***We hypothesized that the structure of cellulose in the soil (i.e. the crystalline or amorphous organisation of its glucose units) has important effects on the rate and extent of soil C mineralization.***

Paper IV: Northern peatlands are important components of the boreal landscape, and even small changes in the rate of SOM decomposition could strongly affect winter C fluxes from these ecosystems. Under non-frozen conditions, boreal peatland soils retain water to a greater degree than upland soils (Öquist *et al.*, 2009) and could therefore potentially sustain higher rates of

microbial activity in a frozen state. However, it is not known how changes in temperature and their effects on the soil water content influence microbial metabolism in boreal peatland soils at temperatures below 0 °C. ***We hypothesized that peat soil microorganisms can use simple C substrates to sustain both catabolic and anabolic processes in frozen peat soils.***

3 Material & methods

3.1 Fieldwork

The results I present in this thesis are based on investigations of organic surface soil samples from forest and peatland sites characteristic of Sweden's central boreal region. In the field, samples were collected from Inceptisols with an incipient organic horizon (paper I) and from well-developed Spodosols (papers II and III) and Histosols (paper IV). These three soil types dominate boreal landscapes (Soil Survey Staff, 2003; Jones *et al.*, 2010)

The soils investigated in paper I were collected from the site of a tree species field experiment located at Sävar, 15 km north of Umeå (63°53′42″N, 20°32′ 34″E) (Figure 4). The annual mean temperature and precipitation at the site over the climate standard reference period 1961-1990 were 2.9 °C and 662 mm, respectively (Alexandersson, Karlström and Larsson-Mccann, 1991). The site was laid out according to a randomized block experimental design with three replicate plots for each of five tree species: Norway spruce (*Picea abies* L. Karst.), Lodgepole pine (*Pinus contorta* Dougl.), Scots pine (*Pinus sylvestris* L.), Siberian larch (*Larix sibirica* Ledeb.) and Silver birch (*Betula pendula* Roth). The site was formerly cultivated land on an Inceptisol, originating from glacifluvium with a silty layer overlaying clay (Soil Survey Staff, 2003). The soils at the site were ploughed and fertilized until the late 1940s. Hay was taken in the 1950s and it was then left unused until the establishment of the tree species experiment in 1971. For further information on the site, see the work of Alriksson and Eriksson (1998).

For papers II and III, I collected samples of typical boreal Spodosols at a site dominated by *Picea abies* L. Karst. and *Pinus sylvestris* L. in the Kulbäcksliden Experimental Area, northern Sweden (64°11′N, 19°33′E), with understory and

field vegetation dominated by *Vaccinium myrtillus* L., *V. vitis idaea* L., and *Pleurozium schreberi* (Brid.) Mitt.

For paper IV, samples were collected from Histosols (acrotelm) at the Degerö Stormyr mire complex, an oligotrophic minerogenic mire covering 6.5 km^2 that is also situated in the Kulbäcksliden Experimental Forest. The ground vegetation at the Degerö Stormyr sampling location is dominated by the mosses *Sphagnum balticum* (Russ.) C. Jens, *S. majus* (Russ.) C. Jens, and *S. lindbergii* Schimp. The site's vascular plant community includes the sedges *Trichoforum cespitosum* L. and *Eriophorum vaginatum* L., as well as the dwarf shrubs *Rubus chamaemorus* L., *Andromeda polifolia* L. and *Vaccinium oxycoccos* L. (Nilsson *et al.*, 2008; Laine *et al.*, 2012).

Over the climate standard reference period 1961-1990, the site's 30-year mean annual precipitation was 523 mm and its mean annual and January temperatures were +1.2 and -12.4 °C, respectively, based on climate data measured 2 km away from the Kulbäcksliden Experimental site (Alexandersson, Karlström and Larsson-Mccann, 1991). The site is characterized as having a cold temperate humid climate with a growing season that usually lasts for approximately 6 months (May–October) and snow cover that normally lasts from early November until late April.

3.2 Laboratory work

3.2.1 Soil processing

Upon collection of the Spodosols and Inceptisols (papers I-III), litter, moss, and underlying mineral soil were removed from the samples, which were then pooled into a single large composite sample per plot to maximize representativeness. For paper I, samples from the surface organic soil layer (0–3 cm) were collected. For papers II and III, soil cores (15 cm diameter) from the organic (O)-horizon (0–5 cm) were collected (Figure 4, d-f and h). The soils were transported directly to the laboratory, where the composite was homogenized by passing it through a sieve (6 × 3.5 mm mesh) in its field-moist state. Needles, coarse and visible fine roots, and other debris were removed manually. The homogenized soil was then stored at −20 °C until the start of the incubations. For paper IV, frozen peat soil from the topmost 20 cm (acrotelm) was collected, transported to the lab, and kept frozen at -20 °C for one week until further treatment. After thawing to 4 °C, the samples were cut with scissors to homogenize the material (Figure 4, g).

a
b
c
d
e
f
g
h

Figure 4. Overview of the experimental sites where soil sampling was carried out. (a) Sävar experimental site; samples collected from 0-3 cm. Subfigures (b), (c) and (d) show soil profiles (ca. 0-20 cm) from *P. abies*, abandoned meadow, and *B. pendula* plots, respectively. (e) The Degerö Stormyr mire complex; subfigure (f) depicts a sample of the collected acrotelm (0-20 cm). (g) Kulbäcksliden experimental site (h) soil cores (15 cm diameter) sampled from the O Horizon (0-5 cm). (*Photos: Javier Segura (e) Meredith Blackburn, (g,h) Björn Erhagen*).

Subsamples of all collected soils were used to determine the soil's dry weight (after drying for 24 h at 105 °C) and SOM content (determined by loss on ignition after heating at 550 °C for 6 h), and the soil pH. The C and N contents of all sampled soils were determined by elemental analysis using a Flash EA 2000 elemental analyser (Thermo Fisher Scientific, Bremen, Germany) to calculate C: N ratios. The total C and N contents reported in paper I were related to the mass of SOM in the soil (measured in g SOM/g dw) and are referred to as soil organic matter C (SOM_C) and soil organic matter N (SOM_N).

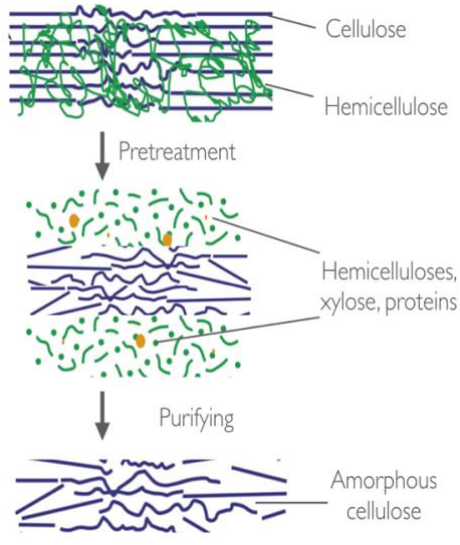
3.2.2 Pretreatment of ^{13}C-cellulose

For the investigations reported in papers II and III, ^{13}C-cellulose (97 at% ^{13}C, from *Zea mays* L., obtained from Iso*Life*, Wageningen, The Netherlands) was treated with an ionic liquid (IL) to disrupt its partly crystalline structure. The IL 1-butyl-3-methylimidazolium chloride (BmimCl, Ab Rani *et al.*, 2011) was used in these studies because it did not break down into toxic compounds or induce the degradation of cellulose to glucose upon mild heating (to ~ 75 °C for 48 h). The ^{13}C-cellulose was pretreated and purified, and the IL-induced changes in its structure were evaluated by cross polarization magic angle spinning nuclear magnetic resonance spectroscopy (CP MAS NMR) and elemental analysis, which revealed complete conversion of the polymer into an amorphous structure (see Box 1 and details of the IL treatment in paper II).

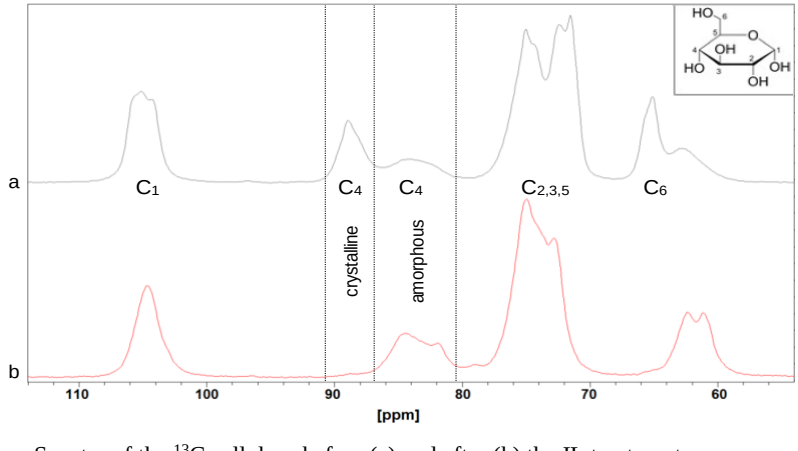
3.2.3 Pyrolysis-GC–MS analysis

The molecular SOM characterization data reported in paper I were acquired by pyrolysis gas chromatography/ mass spectrometry (Py-GC–MS). Briefly, 200 ± 10 µg freeze dried and tube-milled SOM subsamples were weighed and transferred to autosampler containers (Eco-cup SF, Frontier Laboratories, Japan). The samples were pyrolyzed in a Frontier Labs PY-2020iD oven (450 °C) connected to an Agilent 7890A-5975C GC–MS system. 450 °C has been proposed as a suitable pyrolysis temperature for low mass samples because it avoids complete degradation of some organic matter biomarkers (Tolu *et al.*, 2015).

Box 1. Recent advances in "green chemistry" have focused on the study of the structural and chemical obstacles that limit the access to carbohydrates like cellulose. Usually chemical methods, elevated temperature and often also elevated pressure are applied in biomass transformations at industrial level. During the last years, Ionic liquids (IL) have been increasingly utilized for research purposes. Basically, an IL is a salt that exists as a liquid consisting of ions and short-lived ion pairs at room temperature. Many ILs can dissolve plant biomass. In my studies, an IL was used to disrupt the crystalline structure of cellulose, increase its accessibility to microorganisms, and remove impurities such as hemicelluloses, xylose, proteins, and organic acids (Tadesse and Luque, 2011; Brandt *et al.*, 2013). After purification and washing, cross-polarization magic angle spinning nuclear magnetic resonance spectroscopy (CP MAS NMR) confirmed that IL treatment yielded completely amorphous cellulose (see methods in paper II).



Schematic depiction of the disruption of ^{13}C-cellulose using ionic liquids. Figure modified from (Hsu, Ladisch and Tsao, 1980 and Brandt *et al.*, 2013)



Spectra of the ^{13}C cellulose before (a) and after (b) the IL treatment

In our samples, 111 pyrolytic organic compounds were identified, and peak areas were normalized by setting the total identified peak area for each sample to 100% (see Tolu *et al.*, (2015) and paper I for more details).

3.2.4 Respiration measurements & substrate additions

Respirometer experiments

Papers I and III describe soil incubation experiments performed with a respirometer. To optimize moisture conditions for microbial activity during incubation, the water content of the soil samples was adjusted to a water potential of -25 kPa (Ilstedt, Nordgren and Malmer, 2000). Briefly, soil samples containing 1 (paper III) or 11 ± 2.1 (paper I) g of organic material (dry weight, dw) were placed in 250 ml incubation jars (Nalgene, Thermo Fisher Scientific) and incubated at 4, 9, 14, and 19 °C (paper I), and 9 and 19 °C (paper III). During incubation, the CO_2 production from the SOM was measured hourly using the respirometer (Chapman, 1971; Nordgren, 1988, Nordgren Innovations, Djäkneboda, Sweden). The incubation jars were equipped with a small vessel containing 10 ml KOH (0.5M) and two platinum electrodes. The jars were placed in an insulated water bath with a tightly controlled temperature (±0.02 °C). CO_2 produced by SOM respiration was trapped in the KOH solution, reducing its electrical conductivity; this change in conductivity was measured with the platinum electrodes. The changes in conductivity were then used to calculate CO_2 production in mg CO_2 g^{-1} OM dry weight (dw) h^{-1}.

In papers I and III, the basal respiration (BR) was calculated as the average of 100 consecutive hours of CO_2 measurements, starting 34 days after the start of the incubation and after respiration equilibration. The BR rates reflect the inherent metabolic properties of the microorganisms in the incubated sample. After the BR had been determined, a quantity of substrate equivalent to 50 mg C g^{-1} SOM in the form of glucose (paper I) or crystalline and amorphous cellulose (paper III, Box 1) together with solutions of $(NH_4)_2SO_4$ and KH_2PO_4 were added to the soil samples to give a 181:13:1 molar ratio of C:N:P. The amounts of C, N and P were chosen to correspond to optimal conditions for microbial growth in soil samples with a high organic content (Ilstedt, Nordgren and Malmer, 2000). In paper III, a second addition of N & P was done after 40 days of incubation to further evaluate the role of nutrients in cellulose mineralization.

Substrate-induced respiration (SIR) is the immediate response in CO_2 production to the addition of a C substrate at saturation level (Anderson and Domsch, 1978). The SIR was calculated as the average of 5 hourly

measurements following glucose or cellulose additions. According to Anderson & Domsch (1978), SIR is a measure of the soil's microbial potential, i.e. the available soil microbial biomass able to degrade the added carbon substrate. For paper I, the ratio of BR to SIR (Q_R=BR/ SIR) was calculated to obtain an indication of the actual activity of the CO_2-producing community in the soil relative to the maximum potential activity of the microbial population (Blagodatskaya and Kuzyakov, 2013).

To determine how SOM degradation rates changed with the temperature, Q_{10} responses were determined from the BR values reported in papers I and III and SIR values in paper IV. Q_{10} is the factor by which rates of respiration changes in response to a ten degree change in temperature. To describe the respiration rate and its temperature dependence, we used an exponential model as proposed by Fang *et al.*, (2005) (eq. 1)

$$Q_{10} = e^{\beta \times 10} \quad (1.)$$

Here, e is the base of the natural logarithm, *ß* is the exponent of the exponential function that best fits the corresponding respiration and temperature data, and 10 is a factor corresponding to the 10-degree difference in temperature.

3.2.5 Frozen soil incubations

In papers II and IV, incubations were performed to measure the production of ^{13}C-CO_2 and the synthesis of ^{13}C-labeled compounds (corresponding to the rates of catabolic and anabolic processes) under frozen and unfrozen conditions (Drotz *et al.*, 2010, Erhagen *et al*., 2013). To this end, soil samples were placed in autoclaved gas-tight glass bottles. Each soil sample was then treated with a quantity of ^{13}C-labeled cellulose (see box 1, paper II) or ^{13}C-labeled glucose (paper IV) equivalent to 67 (paper II) or 44 (paper IV) mg C per g SOM (dw), respectively. A solution of $(NH_4)_2SO_4$ and KH_2PO_4 was then added to each bottle to give a final C:N:P molar ratio of 182:13:1.

As described previously, the amounts of C, N and P were chosen to correspond to optimal conditions for microbial growth. To quantify the non-biological transformation of the ^{13}C-labelled substrates, I treated some soil samples with an NaN_3 solution to inhibit microbial activity (Wolf *et al.*, 1989) just before incubation and immediately after adding the ^{13}C-labelled substrate and N&P solution to the soil. The bottles were sealed, evacuated, refilled with atmospheric air and then placed in temperature-controlled cabinets. For paper II, the bottles were incubated at -4 and 4 °C for 195 and 28 days respectively. For paper IV, the bottles were incubated at -5, -3, 4, and 9 °C for 115, 77, 11, and 7 days respectively.

On each sampling occasion, NaN_3 solution was added to each of three incubated bottles to inhibit further microbial activity. Headspace gas samples were withdrawn from these bottles and transferred to N_2-flushed GC vials (Perkin Elmer) and N_2-flushed EXETAINER® tubes containing a solution of 500 µl 0.5 M KOH to determine their total CO_2 and ^{13}C-CO_2 contents, respectively. Total CO_2 contents were determined using a gas chromatograph (Perkin-Elmer Auto Systems, Waltham, MA, USA) operating with a methanizer and flame ionization detector (Zhao *et al.*, 2016). Amounts of ^{13}C-CO_2 produced during the incubations were determined as described below.

3.2.6 NMR spectroscopy techniques

Determination of ^{13}C-CO_2 and ^{13}C-labeled water-soluble compounds

For papers II and IV, the amounts of ^{13}C-CO_2 produced in the incubation samples were determined by solution state ^{13}C NMR analysis of the CO_2 absorbed by the KOH solutions in the EXETAINER® tubes. After injecting the sampled headspace gas, the tubes were equilibrated at 4 °C for 1 h. Next, 250 µl of the solution from the tube and 250 µl of 1.0 M KCH_3COO were transferred to a NMR tube (Wilmad-Lab Glass, Vineland, USA) and analyzed using a 600 MHz Avance III HD spectrometer (Bruker Biospin GmbH, Rheinstetten, Germany), equipped with a 5 mm Broad Band Observe Cryo-Probe. The acetate carbonyl signal at 181.4 ppm was used as a natural abundance internal reference to integrate the signal of the ^{13}C carbonate at 168.2 ppm (see details in paper II).
To determine the contents of ^{13}C water-soluble carbohydrate (^{13}C-WSC) originating from the ^{13}C cellulose used as the substrate in paper II, forest soils were homogenized to enable representative subsampling of the labelled material. I achieved this by grinding and homogenizing the samples in a liquid N_2 bath. I then applied established soil water extraction protocols (Giesler and Lundström, 1993; Jones and Willet, 2006) to the homogenized samples so as to monitor changes over time in their ^{13}C-WSC contents (see papers II and IV). The extracted aqueous solutions were passed through a 0.45 µm filter and freeze-dried. The freeze-dried material was then rewetted and dissolved in 1 ml of water containing 10% D_2O. A sample of 500 µl of the resulting mixture was transferred to an NMR tube (Wilmad-Lab Glass) and analyzed using the 600 MHz spectrometer and probe mentioned above. For more detailed information concerning the NMR settings, see papers II and IV.

Analysis of membrane phospholipid fatty acid (PLFA) enrichment

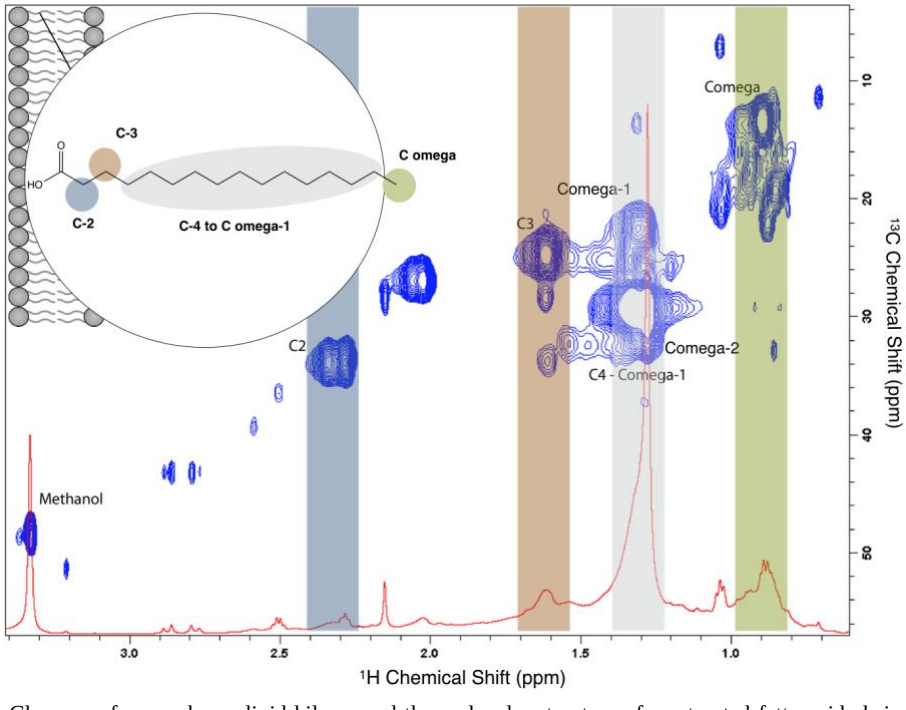
To determine the incorporation of the ^{13}C-label into PLFAs in papers II and IV, lipid extracts were weighed into smaller glass vials, evaporated at 40 °C under a stream of N_2, and re-weighed. 400 µl methanol-d_4 (99.8 at%) and 200 µl of a mixture of 0.1 % methyl ethyl ketone (99.5 %) and Chloroform-d (99.8 at%) were added to the extracts and 480 µl portions of the resulting solutions were transferred to NMR tubes (Wilmad-Lab Glass) and examined by performing a one-dimensional (1D) ^{1}H experiment and a 1D variant of a ^{1}H-^{13}C HSQC experiment (see Box 2) (Szyperski, 1995; Mahrous, Lee and Lee, 2008; Fan *et al.*, 2009) using a 600 MHz Avance III spectrometer (Bruker Biospin GmbH).
The 1D HSQC experiment observes protons connected by a single chemical bond to a ^{13}C carbon. To quantify ^{13}C enrichment in PLFAs, we used the signal of the residual solvent (methanol) as a reference for the natural abundance of ^{13}C. The natural abundance gives rise to a specific intensity ratio of the solvent signals in the 1D HSQC and 1D ^{1}H spectra. This ratio was also evaluated for the PLFA signals; the difference between the ratios for the PLFA and methanol signals reflects the ^{13}C enrichment of the fatty acids (see box 2 and the Methods section of paper II for validation and further details of this approach).

3.2.7 Phospholipid fatty acid (PLFA) analysis

For papers II and IV, 0.5 g portions of soil were extracted and fractionated using the method of Bligh & Dyer (modified according to Frostegård, Tunlid and Bååth, 1991) to determine their phospholipid fatty acid (PLFA) types and concentrations. The abundance of PLFAs was analyzed using a Perkin-Elmer Clarus 500 gas chromatograph (Waltham, MA, USA). Different types of PLFA can be associated with different microbial groups. The PLFAs i15:0, a15:0, a17:0, i16:0, 16:1ω9, 16:1ω7t, 16:1ω7c, i17:0, cy17:0, cy19:0, and 18:1ω7 were considered to be bacterial markers (Bååth, Frostegård and Fritze, 1992; Frostegård, Bååth and Tunlid, 1993); 18:2ω6,9 was used as a general fungal marker (Federle, 1986; Frostegård, Bååth and Tunlid, 1993); 16:1ω5 was used as a marker for arbuscular mycorrhizal fungi (Frostegård, Bååth and Tunlid, 1993; Zogg *et al.*, 1997); and 10me16, 10me17, and 10me18 were treated as actinobacterial markers PLFA quantification produces results comparable to other biomass-related methods and has been identified as a suitable method for detecting changes in biomass after the addition of substrates (Frostegård, Tunlid and Bååth, 2011).

Box 2. Nuclear magnetic resonance spectroscopy (NMR)

NMR is widely used in organic chemistry, medicine, and biophysics. It also has many applications in environmental research, notably in the study of complex, heterogeneous samples for ecology, soil science, and biogeochemistry. When a molecule is placed in a magnetic field, the nuclei of specific isotopes such as ^{1}H or ^{13}C (^{12}C is NMR-inactive) resonate at specific frequencies characteristic of their chemical environment. These frequencies are known as "chemical shifts," and can be used to identify chemical groups and their bonding within a molecule. NMR enables non-destructive analysis of heterogeneous samples in various phases. In this work, solid- and liquid state NMR experiments were used to determine the fate of C substrates and gather information on the metabolic processes of soil microbes. Specific NMR techniques that were used include CP-MAS to characterize soil samples, liquid state ^{13}C-CO_2 NMR to monitor the respiration of ^{13}C-labelled substrates, and 1D ^{1}H analysis together with a 1D variant of a ^{1}H-^{13}C HSQC experiment to monitor ^{13}C incorporation into fatty acids (PLFA). Together, these experiments allowed to follow the fate of C as it was metabolized by soil microorganisms.



Close-up of a membrane lipid bilayer and the molecular structure of a saturated fatty acid chain overlaid on the 2D HSQC spectrum of a representative soil lipid extract and the corresponding 1D ^{1}H spectrum. Peaks in the 2D spectrum correspond to C-H groups; the peak volumes reflect the abundance of the C-H moiety. These NMR experiments allowed to determine where ^{13}C was incorporated into fatty acids during the incubations and the level of ^{13}C enrichment that was achieved. For more detail on the approach, see the Methods section of paper II.

3.2.8 DNA analysis

In paper IV, the composition of the microbial community in peat samples was explored by means of molecular DNA analysis. Peat soil DNA was extracted in duplicate from 0.25 g of soil from each sampling time and incubation temperature using the MoBio PowerSoil® extraction kit (MoBio Laboratories, Carlsbad, USA) according to the manufacturer's protocol. The 16S rRNA gene was amplified and barcoded for Illumina sequencing using a modified variant of Sinclair's two-step PCR protocol (Sinclair *et al.*, 2015). Briefly, the PCR reactions were performed using Illumina adapter-linked 16S rRNA gene-specific 341F forward (Herlemann *et al.*, 2011) and 805NR reverse primers (based on work by Apprill *et al.*, 2015). Each reaction was performed in duplicate using Q5 High-Fidelity DNA polymerase (New England Biolabs, Ipswich, UK) according to the manufacturer's recommendations. After PCR amplification, the purified barcoded PCR products were pooled to equalize their DNA contents and sequenced with the Illumina MiSeq platform using paired-end 300bp read length sequencing with v3 chemistry at the SciLifeLab SNP&SEQ facility at Uppsala University. For more detailed information on the DNA extraction and sequencing process, see paper IV.

3.3 Statistics and data evaluation

In paper I, differences in SOM descriptors (i.e., the pH, C:N ratio, SOM content and SOM_C and SOM_N) between all treatments, including the control (abandoned meadow) treatment, were evaluated by one-way analysis of variance (ANOVA). To analyze for both main and interaction effects of tree species and temperature on the basal respiration (BR) rates of the incubated samples, a two-way ANOVA was performed with temperature and tree species as factors. Two-way ANOVA was also used to explore main and interaction effects of tree species and incubation temperature on the ratio of actual to potential respiration (Q_R). For all ANOVAs, multiple comparisons between tree treatments were performed when significant differences were detected (alpha=0.05).

In paper I, the peak data obtained from pyrolysis-GC–MS experiments revealing the molecular composition of the SOM were integrated using a data processing pipeline developed for R. Peak identification was performed with the "NIST MS Search 2" program using the "NIST/EPA/NIH 2011" library and additional spectra from published pyrolysis studies (Tolu *et al.*, 2015, 2017).
The effects of tree species on the molecular composition of the SOM were evaluated by performing principal component analysis (PCA) on the identified pyrolytic organic fragments. Since we specifically wanted to explore the effects of tree species on the organic chemistry of the SOM, data for the abandoned meadow plots were excluded from this multivariate analysis A second PCA was performed to explore the correlation structure between the pyrolytic fragments, the SOM descriptors mentioned above, and the measured respiratory responses (BR, SIR and Q_R) at all tested temperatures. To test the hypothesis that OM content, BR, SIR, Q_{10}, and Q_R can be explained by the organo-chemical composition of SOM, we performed a partial least squares (PLS) analysis. All PCA and PLS analyses were performed using version 14.0 of the SIMCA-P software package (Umetrics, Umeå, Sweden).

In paper II, the production of ^{13}C-CO_2 and increases in the concentrations of ^{13}C-WSC and PLFA markers in the soil solution were evaluated by linear regression.

In paper III, the C4 spectral region of the CP MAS NMR spectrum of cellulose (80-92 ppm) was used to assess changes in cellulose crystallinity. The assessment was based on the relative intensities of the CP MAS NMR spectra in the crystalline region (86-92 ppm) and the amorphous region (80-86 ppm) (Larsson *et al.,* 1999). The total amount of the added substrate that was utilized metabolically was estimated using a mass balance approach in which the CO_2 production rates were combined with assumed carbon use efficiencies (CUEs) of 0.4, 0.5, and 0.6 (as reported by Öquist *et al.*, 2016). Statistical differences were evaluated using Student's t-test.

In paper IV, the allocation of ^{13}C to catabolic and anabolic processes was examined using PCA. This analysis included data on the consumption of ^{13}C glucose and the concomitant production of ^{13}C-CO_2, ^{13}C-WSC, microbial PLFA concentrations, and the incorporation of the ^{13}C label into membrane fatty acids. In both papers II and IV, differences in ^{13}C incorporation into PLFAs were examined using the Kruskal Wallis-test, which requires measurements to be placed in rank-order but does not assume normality of data. For all analyses, differences were regarded as significant if $p < 0.05$. All multivariate analyses were performed using version 14.0 of the SIMCA-P software package (Umetrics, Umeå, Sweden). The Prism package (Graph Pad Software 6.0, La Jolla, USA) and Minitab 18 statistical software (Minitab Inc. State College, PA, USA) were used for all univariate analyses.

4 Summary of results and discussion

4.1 Tree species effect on SOM decomposition, its temperature response and accumulation

Paper I examined the effect of tree species on soil properties and processes critical for SOM formation and decomposition. This involved an advanced characterization of the SOM developed under trees planted on a tree experimental site established on an abandoned meadow half a century ago. The goal was to determine the extent to which different tree species generate SOM with distinct organo-chemical compositions and whether these differences are expressed as differences in SOM accumulation, decomposition, and the temperature response of these processes.

The PCA based on the 111 identified pyrolytic fragments revealed three significant principal components and explained 80 % of the variation in the organic chemical composition of the forest soil samples (R^2= 0.80, Q^2=0.5 Fig 5). Overall, the organo-chemical composition data showed that SOM formed under plots with coniferous trees contained higher proportions of intact plant-derived molecules, indicating the accumulation of comparatively non-degraded plant material. Intact plant-derived molecules identified in these plots included levosugars, carbohydrate pyrolytic products of ligno-cellulose, complexes strongly associated with lignin, compounds related to guaiacol (which derives from lignin guiacyl (G) subunits), and preserved plant steroids (Figure 5, Table S1 in paper I).

In contrast, the SOM formed in plots with the deciduous *B. pendula* was rich in plant material-derived degradation products. The most abundant fragments in these cases were aliphatic compounds originating from the breakdown of plant suberin and cuticular waxes into shorter chains and N-containing compounds, i.e. specific pyrolytic products of proteins or amino acids (Fabbri *et al.*, 2012).

Lignin compounds related to syringol (derived from lignin syringyl (S) subunits) were also more abundant in these plots. Both the plant and microbial degradation products found in *B. pendula* plots suggest a high degree of microbial activity and decomposition. Taken together, these results indicate that the variation in SOM composition reflects both the identity of the tree species planted half a century ago and the current state of SOM decomposition (Figure 5).

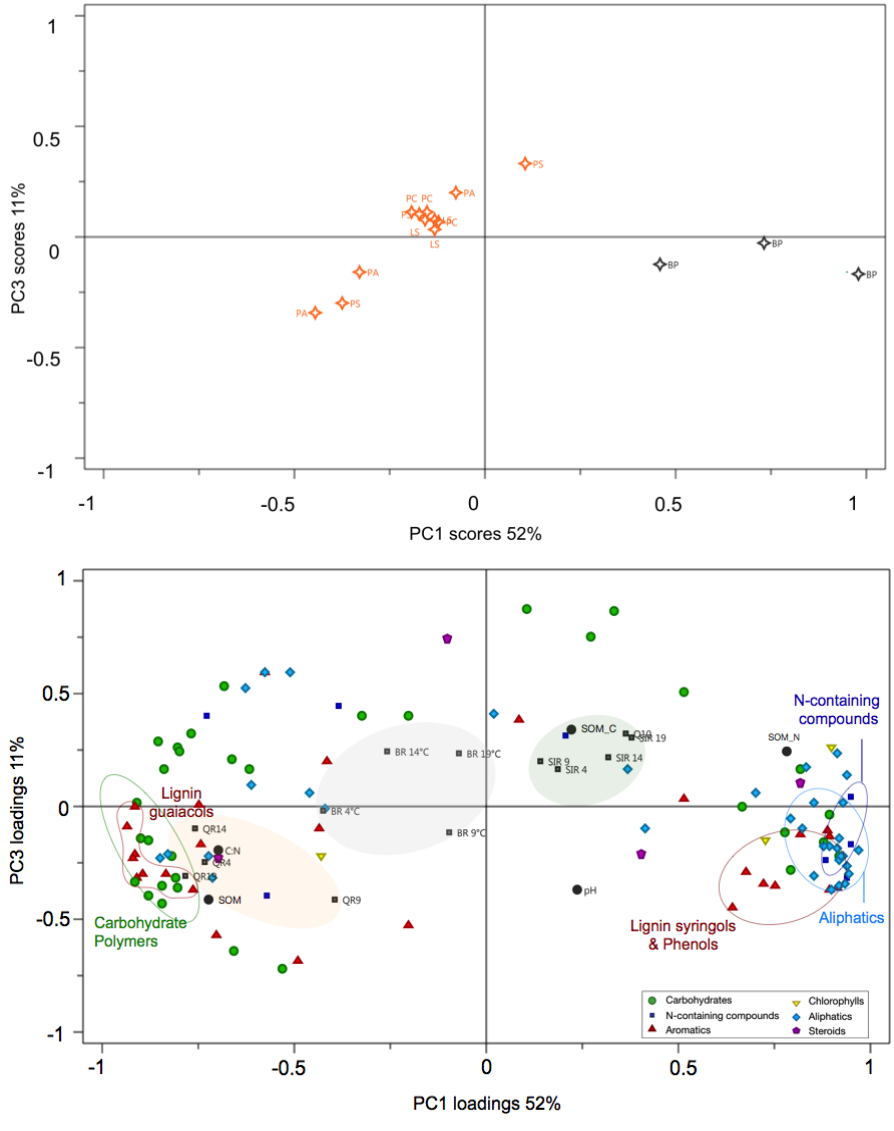


Figure 5. Score and loading plots of the PC model based on 111 pyrolytic fragments of the sampled forest soils (total variation explained: PC1 52%, PC2 11%, PC3 11%, R^2X=0.74 Q^2=0.5, n=15). The variance explained by PC2 and PC3 did not relate to the experimental treatments but to differences between the experimental blocks. For enhanced clarity, the figure shows only PC1 and PC3. PC scores of the forested plots are indicated by orange stars (LS—*L. sibirica*, PA—*P. abies*, PC—*P. contorta*, PS—*P. sylvestris*) and black stars (BP—*B. pendula*). The model also contained the SOM descriptors (pH, C:N ratio, SOM content, SOM_C and SOM_N; black circles) and respiratory measures (Q_{10}, BR, SIR and Q_R; grey squares). Data points corresponding to different compound types are surrounded by enclosures of specific colours: green for carbohydrates, red for lignin and phenolic compounds, light blue for aliphatic compounds, and dark blue for N-containing compounds. The correlation structures between the organo-chemical composition of SOM and Q_R, BR and SIR are indicated by light red, grey, and green shading, respectively.

Paper I also examined the extent to which differences in SOM composition could be related to differences in decomposition rates and the temperature sensitivity of saprotrophic respiration (Q_{10}). Contradicting our hypothesis, the organic chemical composition of the SOM had no significant effect on the Q_{10} of basal soil respiration. The independence of Q_{10} on the organic chemical composition agrees with earlier observations on common European tree species (Vesterdal *et al.*, 2012) and nearby boreal forests (Erhagen *et al.*, 2013).

Two different molecular approaches - ^{13}C-NMR as used by Erhagen *et al.*, (2013) and pyrolysis-GC–MS as reported in this thesis - have indicated that the Q_{10} for soil CO_2 production is independent of the organo-chemical composition of the SOM. This is inconsistent with theoretical predictions of the effects of substrate quality based on the Arrhenius kinetic model (Bosatta and Ågren, 1999; Davidson and Janssens, 2006). Other empirical studies have concluded that the decomposition of complex compounds in SOM is less, equally, or more temperature-sensitive than that of labile compounds (e.g. Melillo *et al.*, 2002; Fang *et al.*, 2005; Fierer *et al.*, 2005; Erhagen *et al.*, 2013). This lack of consensus is presumably partly due to variation in SOM composition and the use of different experimental methods (Conant *et al.*, 2008). However, a recent meta-analysis of empirical studies suggested that most of these studies actually agree with the Arrhenius model and thermodynamic theory, i.e. that temperature sensitivity increases with the complexity of the compounds undergoing degradation (Sierra, 2012),

The CO_2 production data obtained with the respirometer made it possible to acquire three estimates (BR, SIR and Q_R) of microbial metabolic activity. Q_R was found to depend strongly on the organo-chemical composition of the SOM at all tested temperatures. Conversely, the BR and SIR were both only weakly dependent on the organo-chemical composition of the SOM or completely independent of it at all studied temperatures (Figure 5, Table 1). The Q_R values ranged between 0.2 and 0.4, in keeping with previously reported Q_R values for

forest soils (Wardle and Parkinson, 1990; Blagodatskaya, Anan'yeva and Myakshina, 1996; Anan'yeva, Blagodatskaya and Demkina, 2002).

Table 1. Refined model performance statistics for the PLS analysis of basal respiration (BR), substrate-induced respiration (SIR), and the respiratory quotient (Q_R).

PLS model	R^2Y	Q^2	R^2X	Components
BR 4°C	0.37	0.33	0.81	1
BR 9°C	0.36	0.27	0.70	1
BR 14°C	n.s.	n.s.	n.s.	0
BR 19°C	n.s.	n.s.	n.s.	0
SIR 4°C	n.s.	n.s.	n.s.	0
SIR 9°C	n.s.	n.s.	n.s.	0
SIR 14°C	0.32	0.18	0.62	1
SIR 19°C	0.60	0.52	0.39	1
QR 4°C	0.68	0.63	0.85	1
QR 9°C	0.80	0.63	0.75	2
QR 14°C	0.81	0.76	0.88	1
QR 19°C	0.78	0.77	0.76	1

Some emerging ideas and observations suggest that the organo-chemical composition of litter and SOM have less important effects on decomposition than ecosystem-level properties such as environmental and biological factors including mineral protection of substrates and microbial activity (Marín-Spiotta *et al.*, 2014; Lehmann and Kleber, 2015; Bradford *et al.*, 2017; Maaroufi *et al.*, 2017). However, the results presented in paper I do not support the subordination of the molecular composition of the SOM as a factor governing SOM decomposition. On the contrary, this study strongly suggests that the link between SOM decomposition and microbial decomposers is controlled by the organo-chemical composition of the SOM. Thus, although neither the active microbial population nor the potentially active microbial population seem to be governed by the organo-chemical composition, it does seem to control the proportion of the potential microbial population that may become active.

The results presented in paper I agree with the current understanding of SOM as a collection of macromolecular aggregates consisting primarily of small components of < 2000 Da (Simpson *et al.*, 2002; Sutton and Sposito, 2005; Kelleher and Simpson, 2006) that originate mainly from plants and microorganisms (Knops, Bradley and Wedin, 2002; Derrien, Marol and Balesdent, 2007; Hopkins and Dungait, 2010; Miltner *et al.*, 2012). Temperature-driven changes in the physical and chemical environment of the SOM make these aggregates more or less accessible to microbial enzymes, leading to variation in the rate of their decomposition (Figure 3 in paper I). Both

temperature and soil moisture were controlled in the experiments presented in paper I, so the observed responses can be attributed solely to the relationship between the added C, the microbial community's response to the additions and the initial SOM composition.

"Common garden" experiments such as that examined in paper I are seldom performed (Augusto *et al.*, 2002) but can partly eliminate several site-related factors that can confound evaluations of species' effects on soils (Binkley, 1995). There have been few reports on tree species' effects on top-soils based on common garden studies in the boreal region, but those that have been reported consistently show that forest floor C and nutrient levels at sites planted with common boreal tree species decrease in the order *Picea*>*Pinus*>broadleaf (*Populus* or *Betula*) (e.g. Vesterdal et al., 2013 and references therein). Similarly, the mean total SOM and N contents in our samples decreased in the order Picea > Pinus = Larix > *Betula*. However, a study conducted 20 years ago at the experimental site examined in Paper I (Alriksson and Eriksson, 1998) reported no differences between tree species with respect to the accumulation of SOM or C in the forest floor. It may thus require more than 30 years from tree establishment for tree species-dependent differences in the soil-associated processes of SOM formation and decomposition to become apparent in boreal forest soils.

4.2 Microbial mineralization of cellulose in frozen soils

Paper II addressed the potential of the microbial community in boreal forest soils to hydrolyze, metabolize, and grow on organic biopolymers under frozen conditions. The organic layer horizon (O horizon) of boreal forest soils was used as a model system to test this hypothesis and cellulose was chosen as a model substrate because it is the most common C biopolymer, typically comprising 20–30% of the plant litter mass (Kögel-Knabner, 2002). We followed the fate of the added C by determining the transformation rates of ^{13}C-cellulose into ^{13}C water-soluble carbohydrates (^{13}C-WSC monomers and oligomers), ^{13}C-CO_2, and the incorporation of ^{13}C into membrane phospholipid fatty acids (PLFAs).

We found that the added cellulose was hydrolyzed in the soil (Figure 6a) and that ^{13}C-CO_2 was produced until day 113 of incubation (Figure 6b). The net increase in ^{13}C-WSC released from the added ^{13}C-cellulose correlated positively with the amount of ^{13}C-CO_2 produced over 113 days (Pearson $r = 0.66$, $p = 0.014$). Microbial PLFA concentrations initially declined (between days 0 and 16; see Figure 2b in paper II), probably because of the rather quick freezing to the target temperature. Similar declines have been observed as a result of rapid

freezing or after several freeze-thaw cycles (Feng, Nielsen and Simpson, 2007; Schmitt *et al.*, 2008).

However, a large proportion of the microbial cells remained viable and able to grow at -4 °C, as proven by the measured PLFA concentrations, which increased from day 16 onwards. The net ^{13}C-WSC concentration correlated positively with the increases in bacterial and actinobacterial PLFA concentrations between days 16 and 113 (Pearson r = 0.57, p = 0.05, and Pearson r = 0.66, p = 0.019, respectively) (Figure 2b in Paper II). Both bacteria and actinobacteria are important decomposers in forest soils (Štursová *et al.*, 2012; Lladó *et al.*, 2016), and our findings suggest this may also be true in frozen soils.

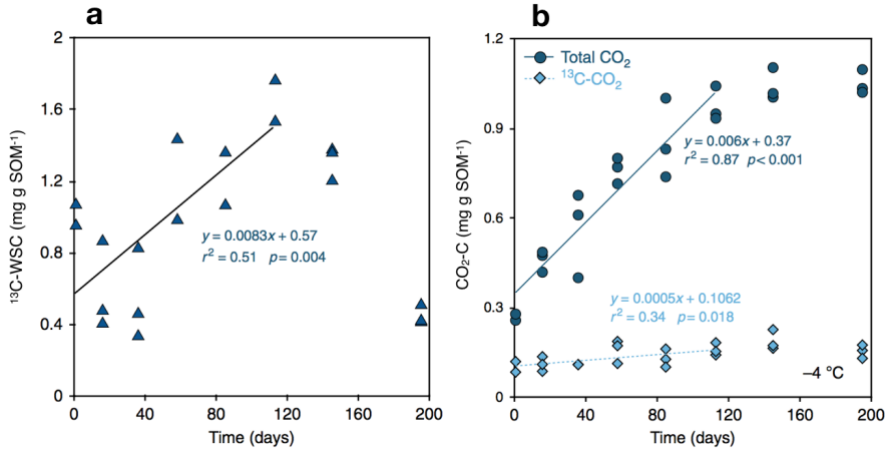


Figure 6. Panel a shows changes over time (and fitted linear functions) in the concentrations of ^{13}C-labeled water soluble carbohydrates (^{13}C-WSC) formed by hydrolysis of the added ^{13}C-cellulose (blue triangles) during incubations at -4 °C. Panel b shows CO_2 (blue circles) and ^{13}C-CO_2 (blue diamonds) produced from the added ^{13}C cellulose in the frozen samples at -4 °C; the lines are linear fits over the first 113 days of incubation.

Substrate availability to bacteria and actinobacteria in the incubated soil may have become limited over time, probably as a result of diffusion constraints in the frozen soil. This is a plausible explanation for the decline in activity observed after ca. 3 months. The stoichiometric relationships between the CO_2 concentrations in the bottles' headspaces and the assumed O_2 consumption suggest that the decrease in activity may also be related to suboptimal oxygen levels that developed as the incubation progressed. However, the increase in microbial PLFA concentrations correlated with total CO_2 production over time, suggesting that microbial carbon mineralization was closely related to changes in microbial biomass during the incubations (Figure 2 in paper II).

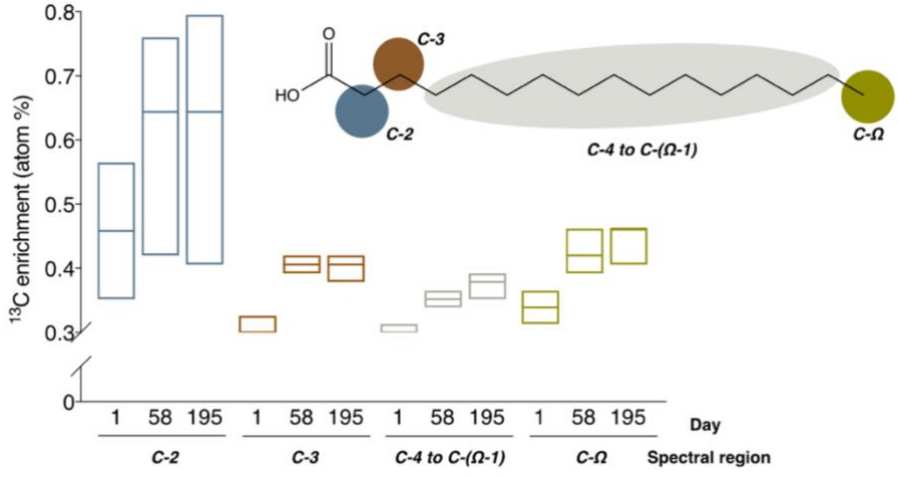


Figure 7. ^{13}C from the added ^{13}C-cellulose was used to synthesize new cell membrane lipids in soil samples incubated at -4 °C. The incorporation of the ^{13}C label was determined by1D ^{1}H analysis and a 1D variant of a ^{1}H-^{13}C HSQC NMR experiment. The bars show the median contents and max and min ranges of ^{13}C-enrichment (relative to natural abundance) in acyl chains at the start (day 1), mid-point (day 58), and end (day 195) of the incubations at -4 °C. We obtained signals for spectral regions assigned to the following hydrogens and the corresponding C atoms (indicated in the model phospholipid fatty acid chain): 2.28 ppm - H-2 (blue circle and bars); 1.62 ppm - H-3 (brown circle and bars); 1.28 ppm - H-4 to H-(Ω-1) (gray ellipse and bars) and 0.9 ppm - H-Ω (green circle and bars) (see Box 2). The median enrichments between the start and end of incubations for the C3 and CΩ signals were 41 and 36%, respectively (Kruskal–Wallis test, $p < 0.05$).

We determined the incorporation of ^{13}C from ^{13}C-cellulose into the PLFA pool and confirmed that the added ^{13}C was a source of C used for growth by the microorganisms (Figure 7).

The detected enrichment occurred along the whole length of the acyl chains in the samples incubated at -4 °C, suggesting new synthesis of fatty acids. Fatty acid chains found in the membranes of microorganisms were modified by elongation and branching (forming additional terminal C-Ω, methyl groups) and exhibited localized enrichment in the C-2 and C4–CΩ-1 groups of the acyl chains (Box 2, Figure 7). This is consistent with previous findings showing that microbial growth at low temperatures affects the degree of unsaturation, chain length, and branching at the methyl ends of fatty acids (Neidleman, 1987; Suutari and Laakso, 1994; Margesin *et al.*, 2003). Our observations thus indicate that the soil microbes' membranes underwent adaptive changes that permitted the maintenance of metabolic processes under frozen conditions.

Cellulose hydrolysis rates were lower in frozen soil than in unfrozen soil, probably largely due to the reduction of the unfrozen water content. The behaviour of unfrozen samples was generally consistent with previous findings

(German *et al.*, 2012; Erhagen *et al.*, 2013; Öquist *et al.*, 2016). The rate restrictions in frozen soils result from the considerable physicochemical changes associated with the dramatic changes in liquid water content and water potential induced by relatively small changes in the temperature of the frozen matrix (Öquist *et al.*, 2009; Harrysson Drotz *et al.*, 2010; Tilston, Sparrman and Öquist, 2010).

It has been suggested that cellulose hydrolysis is hampered in nitrogen-limited soils (Schimel and Weintraub, 2003; Allison and Vitousek, 2005). However, when soils freeze most of the soil pore water transitions into ice and the dissolved compounds become more concentrated in the remaining liquid water pool (Harrysson Drotz *et al.*, 2010). At the same time, metabolic rates and nutrient demands decrease (Tilston, Sparrman and Öquist, 2010). However, one can reasonably expect major differences in unfrozen water content between different soil types and ecosystems (Spaans and Baker, 1996; Romanovsky and Osterkamp, 2000). Additionally, cellulose hydrolysis would have been facilitated by the use of amorphous cellulose as the carbon substrate and by setting the availabilities of N and P to levels expected to be optimal for microbial activity. Naturally occurring cellulose polymers from Scots pine and Norway spruce (the dominant tree species at the studied site) have an amorphous cellulose content of around 50% (Andersson *et al.*, 2004), making it a common constituent of SOM. However, little is known about the influence of the structure of carbohydrate biopolymers (i.e. the arrangements of their glucose subunits) on their rates of decomposition.

Our study demonstrates that microbes in boreal soils can hydrolyse a major constituent of SOM in soils from the boreal landscape. The hydrolysis and mineralization of cellulose observed in our frozen samples contradicts the prevailing view that freezing precludes biopolymer decomposition (Wallenstein *et al.*, 2011). Given the large contribution of C biopolymers to the soil C pool, it is important to account for even small changes in their decomposition rates. Changes in the large biopolymeric SOM pool over decades could lead to important changes in soil C stocks and atmospheric CO_2 concentrations.

4.3 The effect of cellulose structure on microbial C decomposition

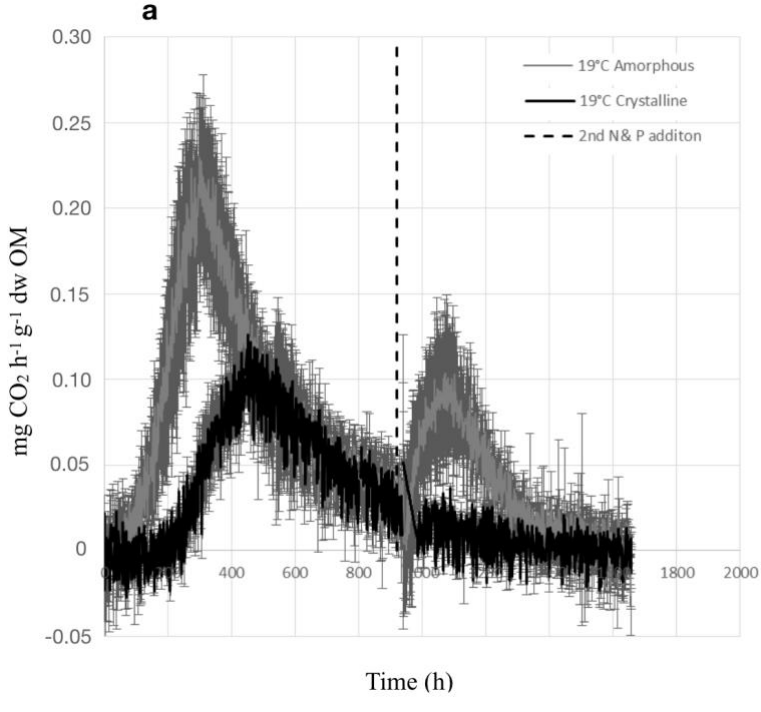
In paper III we investigated how carbohydrate biopolymer structure influences rates of microbial C decomposition in boreal forest soils. Cellulose originating from plant and microorganism cell walls is generally the dominant biopolymer in SOM (Kögel-Knabner, 2002), highlighting the importance of cellulose decomposition for soil C balances. However, little is known about how the decomposition of cellulose is affected by its crystalline or amorphous structure. Cotton linter cellulose (60% crystalline) was used as a model substrate, and completely amorphous cellulose was obtained by treating this material with an ionic liquid (IL, see Box 1). The effect of this structural change on microbial decomposition was evaluated by adding the two types of cellulose to soil incubations and comparing their rates of decomposition.

At both tested temperatures (9 and 19 °C), soils amended with amorphous cellulose exhibited faster initial increases in CO_2 production rates (T-test, $p < 0.05$) and reached maximum respiration rates that were twice as high as those for soils amended with crystalline cellulose (Figure 8a). By the end of the incubations, a mass balance approach showed that the added amorphous cellulose was completely decomposed whereas the crystalline cellulose was mainly undecomposed (Figure 8b). The significantly faster response to the addition of IL-treated amorphous cellulose suggests that the IL treatment may have induced other changes that affected the biopolymer's availability. One likely effect of IL treatment is a reduction of the polymer chain length. This would conceivably increase the number of sites exposed to exoenzymatic attack, leading to a faster substrate utilization response.

Rates of decomposition were calculated based on CO_2 production rates measured with the respirometer and an estimated average carbon use efficiency (CUE) of 0.5; CUE values ranging from 0.4 to 0.6 were previously determined by ^{13}C-cellulose labelling for soil microbial populations in samples taken from the site studied here (Öquist et al., 2016)). A CUE value > 0.5 suggests that some of the utilized substrate originates from the inherent SOM pool, which may reflect a "priming" effect. However, a CUE of 0.6 for utilization of glucose units at 19 °C would correspond to the consumption of ca. 10 mg SOM-C, representing less than 1% of the SOM in the sample. This in turn corresponds to a 0.4% increase in basal respiration rates, which is within the range of uncertainty for the CO_2 production measurements. These results are therefore not conclusive evidence of priming.

Cellulose and stoichiometric amounts of N were added at t=0 and an additional N supplement was added after 920 hours (Figure 8b). No additional effect on crystalline cellulose decomposition was observed following the second nutrient

addition, but the decomposition of the amorphous cellulose was enhanced by extra nutrients (Figure 8b). These results are consistent with the current understanding of hydrolytic enzyme activity and cellulose decomposition in nitrogen-poor soil systems (e.g. Harrington, Fownes and Vitousek, 2001; Schimel and Weintraub, 2003) where the soil microbial population probably lacks access to a readily available N source to rapidly build enzymes (Allison and Vitousek, 2005). More importantly, this result highlights the importance of the interaction between nutrients and the structural arrangement of the cellulose because only the amorphous fraction was decomposed.



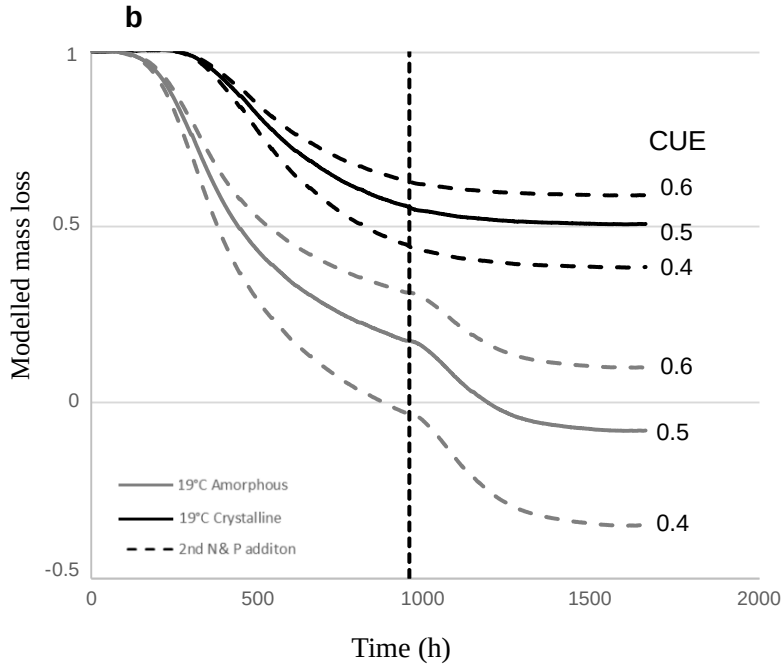


Figure 8. Panel a shows the soil's heterotrophic CO_2 production over time after addition of amorphous (grey line) and crystalline (black line) cellulose. The error bars indicate standard errors (n=3). Panel b shows the cumulative mass loss over time from one unit of cellulose incubated in the moor layer soil matrix (amorphous cellulose (grey line) and a mixture of amorphous (40%) and crystalline (60%) cellulose (black line). Cellulose and stoichiometric amounts of N were added at t=0 and an additional N supplement was made after 920 hours (dashed vertical line). The extent of cellulose decomposition was calculated from the measured CO_2 production and an estimated carbon use efficiency (CUE) of 0.5 for the soil microbial populations derived from a ^{13}C-cellulose labelling study using soils sampled from the same site (Öquist *et al.*, 2016).

Previous studies have attributed the degradation resistance of cellulose to lignification (e.g. McClaugherty and Linkins, 1990; Laskowski and Berg, 2006), i.e. physical protection by lignin that restricts carbohydrate degradation. However, more recent biotechnological studies have shown that the accessibility of cellulose to enzymatic attack is restricted by its crystallinity (Park *et al.*, 2010). Differences in cellulose crystallinity could thus modulate the availability of SOM-C to microorganisms and may explain some of the variation seen in this work. The observed results might help explain why biopolymers such as cellulose typically constitute 40-50% of the contemporary mass in the O horizon of boreal forest soils even though this SOM may have been decomposing for tens, hundreds, or even thousands of years.

Cellulases, which can be either free or cell-associated, hydrolyze β (1→4)-glycosidic bonds between glucosyl residues (Lynd *et al.*, 2002). *Endoglucanases*

catalyze hydrolysis randomly at internal amorphous sites, producing new chain ends and oligosaccharides with different chain lengths, whereas *exoglucanases (cellodextrinases*) catalyze hydrolysis randomly at the ends of cellulose chains, mainly releasing glucose and cellobiose. Thus, IL-induced changes in the hydrogen bonding of the initial cellulose and the resulting increase in amorphicity made the IL-treated cellulose more accessible to endoglucanases at internal amorphous sites and to exoglucanases at the ends of cellulose chains.

The crystallinity of natural plant cellulose varies between species: crystallinities of 52% (Andersson *et al.* 2004) and 62% (Newman and Hemmingson, 1990) have been reported for cellulose from Norway spruce, whereas the reported values for Scots pine range from 51% to 55% (Newman and Hemmingson, 1990; Sivonen *et al.* 2002; Andersson *et al.* 2004). Our results suggest that differences in the crystallinity of the cellulose entering the soil could have important implications for cellulose availability to microorganisms and turnover rates.

SOM dynamics are complex, and SOM decomposition is governed by several factors including substrate quality, enzyme kinetics, the decomposer community, the temperature, and the water content. This study shows conclusively that the structure of cellulose – specifically, its degree of crystallinity – can also control its decomposition rate in boreal forest soils. This factor deserves more attention in studies on the decomposition and recalcitrance of SOM, and should be considered alongside the thermodynamic aspects of SOM decomposition linked to its organic matter content.

4.4 Utilization of simple C substrates in peat soils at low temperatures

Paper IV explored the capacity of the microbial communities to use simple carbon substrates to sustain catabolic and anabolic processes. The results demonstrate that despite being subject to prolonged periods of frozen soil conditions, peat microbial communities are largely viable at typical winter peat soil temperatures. Adding to previous observations of microbial activity in frozen boreal soils (Öquist *et al.*, 2009; Drotz *et al.*, 2010, paper II), this work consolidates the conclusion that both persistent microbial degradation of SOM and synthesis of new microbial biomass during winter are widespread features in boreal soils. In incubations at 9, 4, and -3 °C, ca. 80 percent of the added ^{13}C-glucose was consumed (based on the decline of the $^{13}C_1$ ß-D-glucose NMR signal) after 4, 8, and 63 days of incubation, respectively (Figure 9). At all tested temperatures, the consumption of the added ^{13}C-glucose was strongly negatively correlated to the production of ^{13}C-CO_2 ($r = -1$, $p < 0.05$).

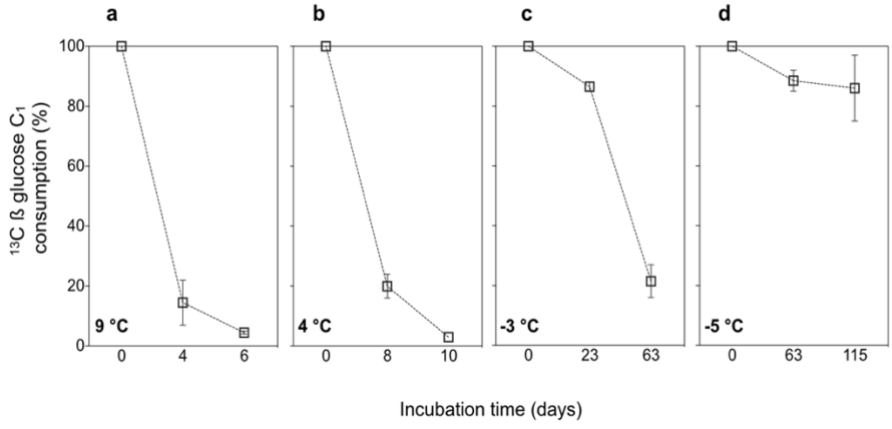


Figure 9. Relative ^{13}C1 ß-D-glucose consumption in incubations at 9, 4, -3 and -5 °C based on the decline of the ^{13}C-NMR signal for C1 ß-D-glucose over time.

The observed rates of ^{13}C-CO_2 production are consistent with previous soil incubation studies over similar temperature ranges (Bergman *et al.*, 2000; Schimel and Mikan, 2005; Drotz *et al.*, 2010). In our frozen samples, ^{13}C-CO_2 production rates accelerated after a "lag phase" lasting for ca. 20 days at -3 °C and ca. 60 days at -5 °C (Figure 2 in paper IV), a period during which the saprotrophs may adapt to utilize the substrate (e.g. Drotz *et al.*, 2010). Evidence of very slow but persistent microbial activity was even found at -5 °C. However, given the timescales involved (>4 months), it is conceivable that this activity would have little impact on the net C balance of peatland.

The delays in the respiratory response could be due to the rapid freezing of the soil to the sub-zero target temperatures, which may have prevented the microbial populations from gradually adapting to the low temperature as they would in a natural winter progression. However, the microorganisms were clearly active during the lag phase because they transformed some of the added ^{13}C to ^{13}C-glycerol and ^{13}C-mannitol. These two compounds are known to enable microbial metabolic function at low temperatures (Weinstein *et al.*, 2000; Robinson, 2001 and refs therein). In other words, they act as physiological antifreeze that limits intracellular damage caused by freezing (Cooke and Whipps, 1993; Weinstein *et al.*, 1997).

By the time the microorganisms had consumed ca. 80% of the added ^{13}C-glucose, an array of newly synthesized ^{13}C compounds were found in the extracted soil solution (Figure 10). Liquid-state NMR experiments (see details in paper IV and Box 2) showed that these compounds were mainly small metabolites found in the alkyl C region. However, broader peaks indicating

protein production were also observed. Most of the NMR peaks exhibited ^{13}C-^{13}C coupling patterns, proving that the carbon source was the added ^{13}C-labeled glucose. Additionally, the increase in ^{13}C-CO_2 respired from the added ^{13}C-glucose was positively correlated to the amount of ^{13}C incorporated into PLFAs ($p < 0.05$) (Figure 10). This suggests that microbial carbon mineralization was closely related to changes in microbial biomass during the incubations.

Moreover, judging by the ^{13}C-CO_2 produced and the ^{13}C incorporated into membrane lipids, the allocation of ^{13}C to catabolic and anabolic pathways in samples incubated at -3 °C may not differ substantially from that at 9 °C, which is consistent with previous observations (Drotz *et al*., 2010). There were however substantial differences in the rates at which the processes occurred. As expected, incubations at 9 and 4 °C yielded turnover times on the order of hours to days for easily assimilated C substrates (Artz, 2009). However, freezing the soils reduced metabolic rates sharply because of the pronounced physicochemical changes resulting from the lower temperatures and changed water content.

The major changes induced by freezing the soil are reflected in the calculated apparent temperature response (Q_{10}) of ^{13}C-CO_2 production. The Q_{10} differed by several orders of magnitude between the unfrozen and frozen soil matrices (Q_{10}= 2.0, 20, and 412 over the intervals 9 to 4 °C, 4 to -3 °C, and -3 to -5 °C). Similar Q_{10} values have been reported for frozen soils by others (e.g. Mikan, Schimel and Doyle, 2002; Panikov *et al.*, 2006). An abrupt increase in the temperature sensitivity of soil respiration upon freezing typically reflects changes in the hierarchy of environmental factors controlling microbial activity (Mikan, Schimel and Doyle, 2002; Elberling and Brandt, 2003; Panikov *et al*., 2006). Öquist *et al*., (2009) have demonstrated that temperature responses at sub-zero temperatures are primarily linked to water deficiency induced by freezing of the soil water.

It has also been proposed that shifts in species composition or substrate use have some influence on the temperature response of frozen soils (Schadt *et al.*, 2003; Schimel and Mikan, 2005). However, these factors also depend on the presence and availability of unfrozen water in the frozen soil matrix. Thus, in the frozen peat matrix, the interactive effect of small temperature changes on water availability and differences in the soil's capacity to retain unfrozen water result in strong apparent temperature sensitivities.

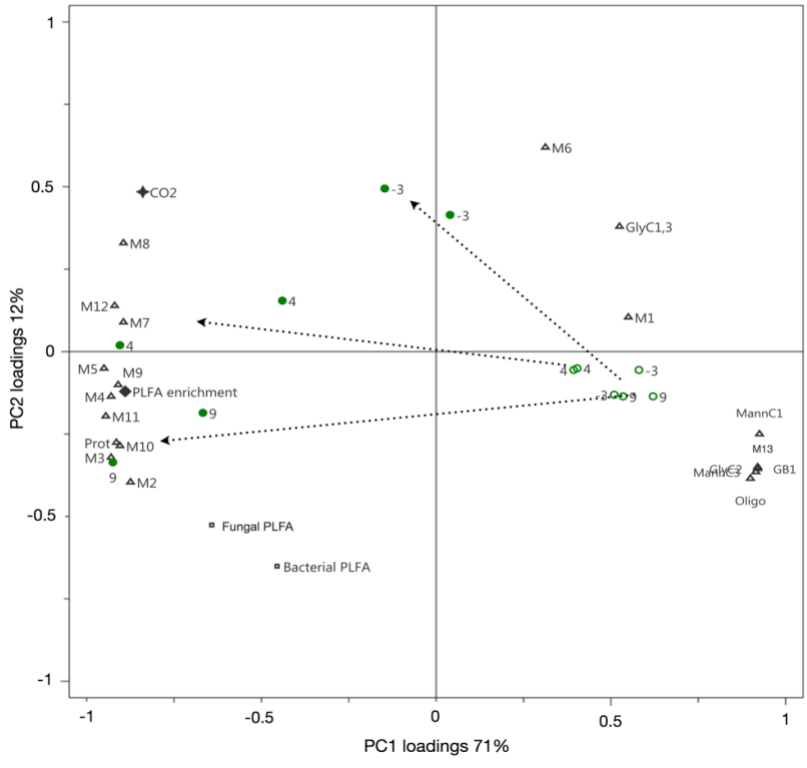


Figure 10. Biplot of the principal component analysis (PCA) of laboratory incubations of peat soils at different temperatures (R^2= 0.83, Q^2= 0.63 n=12). PCA scores show the temperature treatments (-3, 4 and 9 °C) at the start of the incubations and after 80 % of the added glucose had been used by the microorganisms (open and filled circles, respectively). Dashed lines accentuate systematic changes over time at the different temperatures. The PCA loadings show all ^{13}C-labelled water-soluble compounds (open triangles, M1 to M13= metabolite 1 to 13; GB1= C1 ß-D-glucose; Oligo= Oligosugars; Mann= Mannitol; Gly= Glycerol; Prot= Proteins). ^{13}C-CO_2 production (black star), Bacterial and fungal PLFA (open boxes) and level of ^{13}C enrichment in PLFA (black diamond).

Öquist *et al.*, (2009) reported liquid water contents at -4 °C ranging from 0.58 to 0.67 g H_2O g SOM^{-1} for acrotelm peat samples taken from the site sampled in this work. Associated with this water significant substrate diffusion to and from viable microbial cells can be sustained (Tilston *et al.*, 2010). Bacterial and fungal PLFA accounted for 93% and 7%, respectively, of the total PFLA. DNA analysis indicated that the predominant bacterial community was typical for a methanogenic low pH peat environment, and the communities described at the start of the incubation are consistent with those described by Kulichevskaya *et al.*, (2007) (see the discussion of microbial DNA dynamics and taxonomic tree in paper IV).

Among the phyla identified in paper IV, *Acidobacteria*, proved common and widely distributed. Little is known about the function of *Acidobacteria* in terrestrial ecosystems, partly because it is difficult to cultivate these bacteria (Kielak *et al.*, 2016). It is known however that low pH is a regulator of acidobacterial community composition (Jones *et al.*, 2009). Within the phylum *Proteobacteria*, we observed methanotrophic genera with several moderately acidophilic species that have been isolated from *Sphagnum*, peat bogs, forest soils, arctic and subarctic wetlands, and permafrost (Knief, 2015).

All *Planctomycetes* sequences obtained were related to the *Aquisphaera* genus. The genus *Aquisphaera* closely resembles the genus *Singuilisphaera* (genome divergence 7.7%, Rosenberg, 2014), a planctomycete isolated from the acidic peatlands of European north Russia (Kulichevskaya *et al.*, 2008, 2009, 2011). Although few organisms from this phylum have been isolated, *Planctomycetes* seems to be diverse and ubiquitous, and has been described as an important part of the bacterial populations decomposing *Sphagnum* in northern peatlands (representing up to 14% of all bacterial cells (Kulichevskaya, Pankratov and Dedysh, 2006; Kulichevskaya *et al.*, 2007; Ivanova and Dedysh, 2012). Further research into the role of *Planctomycetes* in C mineralization and other biogeochemical cycles in northern peatland ecosystems is thus warranted.

Changes in the physical nature of water (e.g. water potential) at sub-zero temperatures induce physiological adjustments in microorganisms (Russell & Fukunaga, 1990; Margesin *et al.*, 2003; Schimel & Mikan, 2005; Drotz *et al.*, 2010). Schimel and Mikan (2005) suggested that such changes are more likely to occur than changes in the microbial community composition. The ^{13}C-enrichment of fatty acid chains in peat samples at -3 °C was strongest at the C2 position, with a lower but evident enrichment across the C3, C4–CΩ-1 and CΩ groups of the acyl chains. This pattern of enrichment is consistent with the results presented in paper II and suggests that microorganisms readily use the C that becomes available when other microorganisms die.

This is in line with the current understanding that intense and efficient internal recycling of simple C biopolymer building blocks is a widespread metabolic feature of soil microorganisms (Gunina and Kuzyakov, 2015). Changing the composition of the membrane fatty acids is a known response that increases the fluidity of microbial membranes and permits growth at low temperatures (Chintalapati, Kiran and Shivaji, 2004 and references therein). Taken together, our observations support the theory of Schimel and Mikan (2005), whose studies on tundra soils prompted them to suggest that the ability of microorganisms to sustain physiological processes under freezing conditions results from changes in the their physiology rather than changes in community composition.

5 Concluding remarks

This work investigated issues relating to the availability of C substrates and their utilization by microorganisms in boreal soils. The chapters were conceptually linked to provide a better understanding of how C availability controls microbial activity, which in turn shapes biogeochemical processes with profound implications for the C balance of boreal ecosystems. It should be pointed out that experimental laboratory studies, *by definition*, are subject to artefacts to some degree. For instance, the act of collecting soils and bringing them to the laboratory may disrupt the physical structures of microbial associations in the soil. I believe, however, that the behaviour of the model soil system and the studied saprotrophic communities resembles the characteristics of soils *in situ* closely enough to meaningfully address the questions considered in this thesis. That is to say, the studied soil systems are capable of providing answers that will help to disentangle the more complex processes occurring in nature. The following section discusses the goals of the individual chapters of this thesis, their main conclusions, and the implications of the results when taken as a whole.

Paper I

The goal of this paper was to determine whether different tree species generate SOM with different organo-chemical compositions and whether these differences are expressed as differences in the accumulation and decomposition of SOM, and the temperature response of these processes.

- A sophisticated molecular characterization of the SOM revealed that tree species significantly influence SOM genesis by changing both its rate of accumulation and its organo-chemical composition. Accordingly, tree-type specific molecular fingerprints were detected in SOM formed under conifers and *B. pendula*. It was shown that the observed variation in SOM content could be explained by the organo-chemical composition of the SOM. More importantly, a strong connection between SOM decomposition and microbial

decomposers was observed and this connection was linked to the organo-chemical composition of SOM. No effect of tree species on the temperature response of CO_2 production was detected, nor was any relationship between the organo-chemical composition of the SOM and the Q_{10} value. The results presented here suggest that tree growth has a considerable impact on the molecular composition of the SOM and the associated processes of SOM formation and decomposition in boreal forest soils.

Paper II

The goal of this paper was to explore the capacity of soil microorganisms in boreal forest soils to hydrolyze, metabolize, and grow on an organic biopolymer under frozen soil conditions.

- Soil microbial communities in frozen soils were found to hydrolyze cellulose and use the released substrates for both catabolic and anabolic metabolism. This work was the first to demonstrate microbial capacity to hydrolyze biopolymeric SOM constituents in frozen boreal soils, resulting in slow but sustained SOM degradation. The decomposition of C biopolymers is what regulates soil C balances, and even small changes in the decomposition rates of the large biopolymeric SOM pool could cause important changes in soil C stocks, and atmospheric CO_2 concentrations over decades. Given the long periods during which high latitude soils are frozen, the findings presented in this paper provide vital insights into the contribution of winter processes to the global carbon balance.

Paper III

The goal of this paper was to assess whether cellulose structure, i.e. the crystalline or amorphous organisation of the glucose units, has important effects on the rates and extent of soil C decomposition.

- The degree of cellulose crystallinity was found to be a key factor determining the rate of cellulose hydrolysis and subsequent mineralization in boreal forest soils. This observation advances our understanding of SOM decomposition and should be considered alongside the thermodynamic aspects of SOM decomposition linked to the molecular composition of the soil organic matter.

Paper IV

The goal of this paper was to evaluate the capacity of peat soil microorganisms to utilize simple C substrates to sustain catabolic and anabolic processes in frozen peat soils.

- Microorganisms in the acrotelm of frozen boreal peat soils were found to utilize simple C substrates to sustain both catabolic and anabolic metabolism. Although freezing of the soil reduces rates of substrate utilization, the observed microbial responses are consistent with a significant metabolic capacity. This work also showed that very small differences in winter soil temperatures can have very large implications for winter C fluxes. The finding that catabolic and anabolic processes proceed in frozen peat soil over an environmentally-relevant temperature range advances our understanding of the factors controlling winter CO_2 mineralization in northern peatlands.

5.1 Implications and suggestions for future work

The large pool of C stored as SOM in soils of high-latitude ecosystems comprises more organic C than all the world's vegetation and the atmosphere combined (Figure 1). This work shows that both persistent microbial degradation of C in SOM (both biopolymers and monomers) and synthesis of new microbial biomass during winter are widespread features in boreal soils. More importantly, these observations indicate that small differences in winter soil temperatures can have very large implications for winter C fluxes from boreal soils.

Observations and projections for the northern regions suggest that the effects of climate change will be most pronounced during winter, leading to reduced snow cover and altered frost regimes. These trends may continue throughout the 21th century (Ciais *et al.*, 2014), and even small changes in winter soil dynamics caused by such changes could appreciably increase the amount of C available for microbial decomposition. In view of the drastic shift that such environmental changes could trigger in the delicate balance between atmospheric and biospheric CO_2, a better understanding of the key regulators is urgently needed. To this end, my thesis provides novel data about several aspects of the dynamics of soil microbial decomposition, identifying important controlling factors that determine the rate of SOM degradation in these sensitive ecosystems.

The results presented herein also reveal the molecular composition of the SOM and the structural arrangement of its constituents to be important factors controlling C availability. Over the studied range of temperatures, C substrates were readily utilized and microbial activity was never totally impeded. However, thermodynamic constraints were evident at the lower end of the temperature range. While these processes are slow at such temperatures, it is important to recall the immense scale on which they can occur: approximately 57% of the Earth's terrestrial surface experiences long periods of freezing at least occasionally (Duguay *et al.*, 2005), and wintry conditions persist for several months of the year in the arctic and boreal biomes of Scandinavia, Canada,

Russia, and the USA. Thus, while considerable differences in soil characteristics between different soil types and ecosystems can be expected, these processes could occur over vast areas of the Earth and extended periods of time.

Further research is needed to assess the effects of different tree species on the balance between SOM formation and decomposition. For boreal forests soils specifically, an interesting issue to explore is the high prevalence of carbohydrate polymers (e.g. cellulose), which account for 40-50% of the contemporary SOM mass (Erhagen et al. 2013), even in cases where the SOM has been decomposing for long periods of time. Moreover, the variety of complex superstructures exhibited by cellulose and the different scales on which these structures are defined (nano-, micro-, and macro-) makes it an ideal model substrate for this task. I think such studies would be greatly enhanced by characterization at the molecular level (using techniques such as NMR and/or Py-GC–MS) that can link the molecular composition and structure of SOM constituents to SOM formation and decomposition processes.

Studies of this kind would not be complete without efforts to relate the activity and composition of soil microbial communities in order to enhance the understanding of the availability and utilization of C substrates and their effects on the C pools in soils of high-latitude ecosystems.

Questions remain as to whether the microbial metabolic capacity demonstrated here for cellulose in forest soils is also applicable to the decomposition of other biopolymers and over a wider range of environmental conditions (e.g. those found in the active layer or permafrost). This warrants further research because of the large contribution of biopolymers to soil C stocks. Future studies of peatland winter biogeochemical processes should also address the role of C biopolymers as substrates for mineralization and examine anoxic conditions and the microbial capacity for methane production under frozen conditions. There should be efforts to quantify this winter microbial capacity and define its contribution to C cycling in high-latitude ecosystems. Ultimately, winter processes should be incorporated into Earth-system model projections to enable accurate assessment of their contributions to the global carbon balance. Each of the four chapters in my thesis deals with one important piece of the puzzle of microbial metabolic activity in boreal soils, from the influence of vegetation type on substrate characteristics to the ability of microorganisms to decompose C substrates under the harsh conditions of high-latitude soils. This thesis is thus a valuable contribution to our understanding of microbial C cycling in one of the world's major soil C pools.

6 References

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