Temperature Sensitivity of Soil Carbon Decomposition

Molecular Controls and Environmental Feedbacks

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Doctoral Thesis
Swedish University of Agricultural Sciences
Umeå 2013

Acta Universitatis agriculturae Sueciae
2013:61

Cover: A soil core sampled from the organic layer of a spodosol
(photo: Lucy Rist)

ISSN 1652-6880
ISBN (print version) 978-91-576-7860-7
ISBN (electronic version) 978-91-576-7861-4

Print: Arkitektkopia AB, Umeå 2013

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- Molecular Controls and Environmental Feedbacks

Abstract

The world's soils contain three times as much carbon as the atmosphere. Thus, any changes in this carbon pool may affect atmospheric CO_2 levels with implications for climate change. Anthropogenic contributions to global carbon and nitrogen cycles have increased in the last century. Both temperature and nitrogen influence decomposition processes and are therefore critical in determining CO_2 return to the atmosphere.

Kinetic theory predicts that the chemical composition of soil organic matter represents a dominant influence on the temperature response of decomposition. However, empirical observations and modeling indicate that this relationship is constrained by other factors. We address a number of research questions related to these factors, which are central to a thorough understanding of temperature sensitivity in decomposition. Specifically it offers one of the first empirical observations consistent with modeling in demonstrating increased temperature sensitivity for the uptake of carbon monomers over microbial cell membranes. Using NMR spectroscopy we were able to demonstrate how temperature response is directly related to the chemical composition of the organic material present. The thesis shows how increased soil nitrogen reduces temperature response. The key mechanism behind this observation, we suggest, is the influence of nitrogen on the chemical composition of organic matter, mediating a direct effect on temperature response. Given that nitrogen availability in terrestrial ecosystems has doubled relative to preindustrial levels, this observation may be vital in understanding the net effect of temperature increase on CO_2 return to the atmosphere. The proportion of carbon in plant litter transformed by microorganisms into biomass (carbon use efficiency; CUE) is a central factor determining global land-atmosphere CO_2 exchange. CUE was highly sensitive to whether carbon monomers or polymers were degraded; yet temperature had no clear effect on CUE. The majority of soil organic matter is comprised of polymers, highlighting the importance of using these as model substrates in studies of CUE.

This thesis represents a major contribution to our understanding of the intrinsic and external controls acting on temperature sensitivity of decomposition, and thus to regulation of CO_2 return to the atmosphere under a changing climate.

Keywords: decomposition, soil organic matter, litter, boreal forest, organic chemical composition, temperature sensitivity, Q_{10}, CUE, CP- MAS NMR, HSQC

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Hade vi inte dig Björn…JA då hade vi någon annan!
Stefan Jungholm

Contents

List of Publications

This thesis is based on the work described in the following papers, which are referred to by the corresponding Roman numerals in the text:

I **Erhagen, B**., Ilstedt, U. and Nilsson, M.B. Temperature sensitivity of saprotrophic CO2 production increases with increasing carbon substrate uptake rate (under *review, Soil Biology & Biochemistry).*

II **Erhagen, B**., Öquist, M., Sparrman, T., Haei, M., Ilstedt, U., Hedenström, M., Schleucher, J. and M.B. Nilsson. (2013). Temperature response of litter and soil organic matter decomposition is determined by chemical composition of organic material. *Global Change Biology 19(11), 12342.*

III Nilsson, M.B., **Erhagen, B**. Ilstedt, U., Sparrman, T., Öquist, M, and J. Schleucher. Increased nitrogen availability counteracts the climatic changes feedback from increased temperature on boreal forest soil organic matter degradation. (*Manuscript*).

IV **Erhagen, B**., Haei, M., Öquist, M., Schleucher, J. Sparrman, T. and Nilsson, M. B. The effect of temperature and substrate quality on carbon use efficiency of saprotrophic decomposition. (*Manuscript*).

Paper II is reproduced with the kind permission of the publisher.

The contributions of Björn Erhagen to the papers included in this thesis were as follows:

I Study design, 40%; Experimental, 50%; Data compilation & evaluation, 80; Manuscript drafting, 60%.

II Study design, 50%; Experimental, 100%; Data compilation & evaluation, 100%; Manuscript drafting, 80%.

III Study design, 50%; Experimental, 100%; Data compilation & evaluation, 90%; Manuscript drafting, 30%.

IV Study design, 80%; Experimental, 80%; Data compilation & evaluation, 100%; Manuscript drafting, 80%.

Abbreviations

A-Index	Availability index
BR	Basal Respiration
CP-MAS NMR	Cross polarization magic angle spinning
CUE	Carbon use efficiency
D	Substrate diffusion rate
EM	Electromagnetic radiation
FID	Free induced decay
HSQC	Heteronuclear single quantum coherence
K	Half-saturation-constant
LOI	Loss on ignition
NMR	Nuclear Magnetic Resonance
OM	Organic matter
PLS	Partial least squares
Q_{10}	Factor by which the rate of a biological or chemical process (here, respiration) changes in response to a 10°C temperature change.
S	Substrate releases rate
SGR	Specific growth rate
SIR	Substrate-induced respiration
SOM	Soil organic matter
μ	Rate of substrate uptake

1 Introduction

1.1 Background

Since the industrial revolution the average global temperature of the atmosphere has increased by 1.5°C and it is predicted to further increase by 4-7°C in the next century (IPCC, 2007). The world's soils are estimated to currently contain 3000 Gt carbon, which is three times the estimated amount of atmospheric CO_2-C (Tarnocai *et al.*, 2009; Batjes, 1996). About 40% of that carbon is stored in boreal forests, which cover a large part of the northern hemisphere (Denman, 2007). Thus, even small changes in the soil carbon pool may severely affect the atmospheric CO_2-concentration, thereby further affecting the global air temperature. The decomposition of organic material has been shown to be more sensitive to temperature than net primary production (Kirschbaum, 2000; Lloyd & Taylor, 1994; Schimel *et al.*, 1994), further supporting the hypothesis that changes in the global air temperature could affect the rate of net C-exchange between the atmosphere and biosphere (Cox *et al.*, 2000; Schimel, 1995).

Therefore, reliable predictions of ecosystem responses to climate change require thorough understanding of the factors and processes controlling the temperature sensitivity of soil organic matter's decomposition (Conant *et al.*, 2011; von Lutzow & Kögel-Knabner, 2009; Davidson & Janssens, 2006). Some of these factors and processes are not yet well understood or under debate and, hence, need further research.

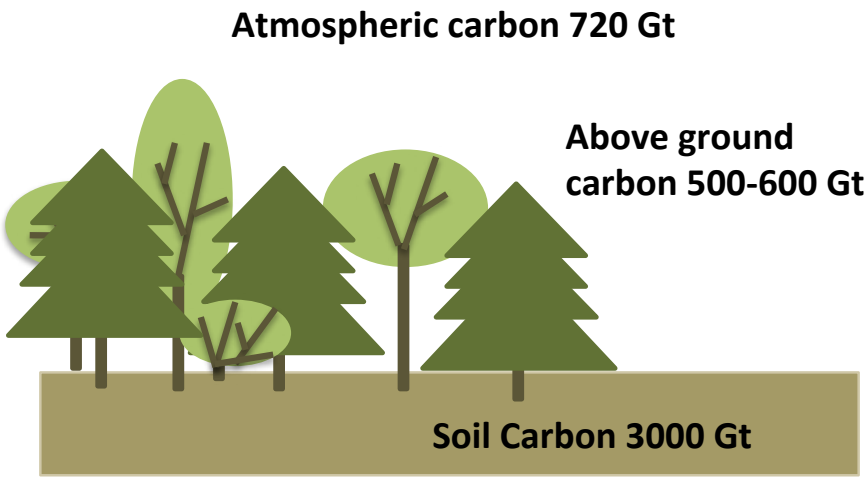

Figure 1. The carbon pools of the terrestrial ecosystem (Tarnocai *et al.*, 2009).

An important factor influencing the temperature sensitivity of soil organic matter (SOM) decomposition is the quality of the organic material (OM). According to kinetic theory, the temperature sensitivity of OM should be inversely related to its "quality", i.e. temperature sensitivity should increase with reductions in the degradability of the carbon forms (Davidson & Janssens, 2006; Knorr *et al.*, 2005; Bosatta & Ågren, 1999; Arrhenius, 1889). However, results of empirical studies do not necessarily follow this pattern. Some have found that the decomposition of stable compounds (low quality carbon) is not temperature sensitive (Giardina & Ryan, 2000; Liski, 1999), others that both labile and stable organic compounds respond similarly to temperature increases (Conen *et al.*, 2006; Fang *et al.*, 2005), and some of the more recent studies have found that the temperature sensitivity increases with decreased quality of the OM (Wetterstedt *et al.*, 2010; Conant *et al.*, 2008a; Conant *et al.*, 2008b; Hartley *et al.*, 2008; Fierer *et al.*, 2005; Leifeld & Fuhrer, 2005). Thus, despite these research efforts no consensus has yet been reached on likely effects of increases in atmospheric temperature on the decomposition of SOM (Conant *et al.*, 2011; Kirschbaum, 2006).

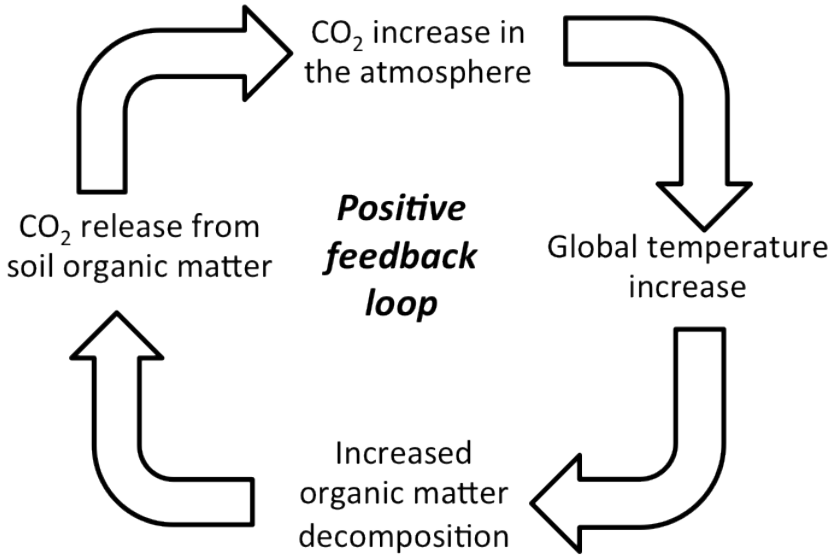

Figure 2. A plausible scenario of the effect of increased soil organic matter decomposition with increased temperature and the positive feedback effect of increased soil organic decomposition with increased temperature.

Since the late 20th century anthropogenic inputs of nitrogen to the terrestrial ecosystem have exceeded natural inputs from nitrogen fixation (Galloway *et al.*, 2008). This has major implications as nitrogen is the most strongly limiting nutrient for carbon fixation in the terrestrial ecosystem (Vitousek *et al.*, 1997), and thus a key determinant of the net ecosystem balance (De Vries *et al.*, 2006). Several studies have also shown that nitrogen strongly affects decomposition rates (Janssens *et al.*, 2010; Berg, 2000; Berg & Matzner, 1997), but there is little knowledge of the effects of nitrogen deposition on the temperature sensitivity of decomposition.

Another important aspect that requires better understanding is the partitioning of the resources acquired via the decomposition of organic carbon between respiration and biomass growth in microorganisms. This partitioning is often expressed in terms of the carbon use efficiency (CUE), a measure of the proportion of a utilized carbon source that is converted into microbial biomass (del Giorgio & Cole, 1998; Clifton, 1946; Winzler & Baumberger, 1938). CUE is highly sensitive to changes in environmental conditions such as temperature increases (Wetterstedt & Ågren, 2011; Allison *et al.*, 2010).

1.2 Objectives

The overall objective of the project this thesis is based upon was to elucidate controls of the temperature sensitivity of OM decomposition. This calls for very detailed molecular level examination of the key factors and processes that influence OM decomposition and their temperature responses. More specifically the work focused upon:

- Effects of OM composition (quality) on the temperature sensitivity of its decomposition.
- Separating effects of temperature on OM decomposition into its effects on rates of three key processes: substrate release, diffusion and uptake by microorganisms.
- Effects of nitrogen (N) on the temperature response of OM decomposition.
- Effects of temperature sensitivity on catabolic and anabolic processes, and thus carbon use efficiency.

1.3 Factors controlling temperature sensitivity of soil organic material decomposition

When studying the temperature sensitivity of SOM decomposition a number of influential factors must be considered. These factors can be categorized as environmental constraints and others linked to the intrinsic temperature sensitivity of the organic material (von Lutzow & Kögel-Knabner, 2009; Davidson & Janssens, 2006; Ågren & Bosatta, 2002).

1.3.1 Environmental constraints on temperature sensitivity of organic matter decomposition

Four major environmental constraints are generally recognized. The first is drought, which reduces water films in soil thus inhibiting the diffusion of soluble extracellular enzymes and reducing the substrate availability for microorganisms at the reaction sites. The second is physicochemical protection of the organic material from microorganisms, which involves physical occlusion of organic material in the interior of soil aggregates and/or chemical adsorption of organic material to mineral surfaces through covalent or electrostatic bonds (Lutzow *et al.*, 2006; Sollins *et al.*, 1996). The third is freezing, which slows enzymatic reactions and the diffusion of substrates. The fourth is flooding, which reduces oxygen diffusion and thus often leads to anaerobic decomposition, which is much slower than aerobic decomposition

under otherwise similar conditions (Davidson & Janssens, 2006). In some cases extreme pH or high concentrations of toxins may also severely limit OM decomposition, although such conditions could be regarded as further types of chemical protection in this context.

1.3.2 Intrinsic temperature sensitivity of organic matter decomposition

According to "carbon quality theory", the intrinsic temperature responses of OM are governed by the carbon quality of the organic material, i.e. how easily it can be degraded, which depends largely on the number of enzymatic steps required for microorganisms to decompose it (Bosatta & Ågren, 1999). The theory is based on the Arrhenius function, which describes the dependence of the rate of a given chemical reaction on temperature and the activation energy required for it to occur (Arrhenius, 1889). The carbon quality theory predicts that the decomposition of low-quality carbon substrates (which require high activation energy for degradation) should have higher temperature sensitivity than the decomposition of higher quality carbon substrates (Davidson & Janssens, 2006; Bosatta & Ågren, 1999).

Much of the variation in the results from studies of the temperature sensitivity of OM decomposition stems from variations in the carbon quality of the material used (Conant *et al.*, 2011; Davidson & Janssens, 2006), but contrasting responses of several key processes involved in decomposition may also act antagonistically or synergistically (Ågren & Wetterstedt, 2007). Figure 3 presents a conceptual illustration of the key processes and parameters involved: 1(S), the substrate release rate; 2(D), the substrate diffusion rate; 3(μ), the substrate uptake rate; and 4(K), the half-saturation constant.

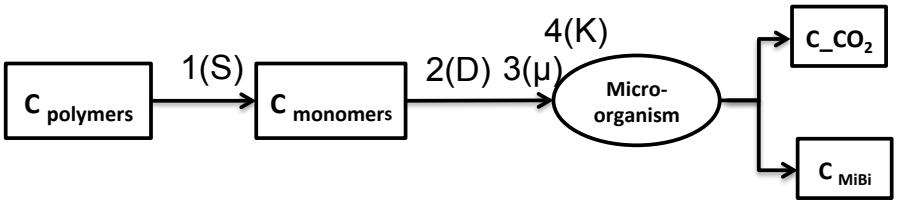

Figure 3. Conceptual illustration of the key processes and parameters affecting the temperature sensitivity of OM decomposition. Substrate release rate (1, S) is directly related to carbon quality while uptake rate (3, D) and half saturation constant (4, K) reflect microorganism characteristics and diffusion rate (2, D) reflects prevailing environmental conditions.

The conceptual model in Figure 3 describes the decomposition of a substrate from a large organic polymer some distance from a microorganism. It begins with substrate release (1) by exoenzymes and diffusion of the substrate

(2) to the microorganism at rates S and D, respectively. At the surface of the microorganism the substrate's uptake (3) is described by the Michaelis-Menten equation, with a maximal rate μ and (4) half-saturation constant K. All of these processes are temperature-dependent, and the overall temperature response is governed by the integrative changes in activation energy for all of them (Ågren & Wetterstedt, 2007).

Organic chemical composition of the organic material and its relation to decomposition

The organic chemical composition of litter and soil organic matter and its influence on degradation and recalcitrance have been a key focus in terrestrial biogeochemistry and soil ecology (Berg, 2000; Bosatta & Ågren, 1999; MacFee & Kelly, 1995; Melillo *et al.*, 1982; Swift *et al.*, 1979). Much of the general understanding of variation in organic degradability stems from a combination of various wet chemical digestion protocols and analytical techniques (Berg & McClaugherty, 2003; Minderman.G, 1968). Based on such an approach the general understanding is that cellulose and hemicellulose are easily decomposed. These are followed by polymers of aromatic or alkyl carbon compounds; or polymers made up of aromatic and alkyl carbons such as lignins, cutins, suberins (Berg & McClaugherty, 2003; Swift *et al.*, 1979; Minderman.G, 1968).

However, based on investigations utilizing a range of other analytical techniques, e.g. NMR, Fourier transform infrared spectroscopy (FT-IR); Pyrolysis gas chromatography mass spectroscopy (PyGCMS) this view appears too simplistic. For example, based on several NMR spectroscopy approaches it is evident that soil organic matter composition is generally dominated by O-alkyl C and di-O-alkyl C accompanied by varying proportions of alkyl C methoxy/N-alkyl C, aromatic, O-aromatic C and carbonyl C (Preston *et al.*, 2000; Kögel-Knabner, 1997; Preston *et al.*, 1997). Alkyl carbon originates from fatty acid chains, and therefore in litter mainly comes from surface waxes and cutin (Lorenz *et al.*, 2000). Methoxy carbon originates from lignin, and N-alkyl carbon largely from protein compounds (Nelson & Baldock, 2005). O-alkyl carbon originates from carbohydrates such as cellulose and hemicellulose (Baldock *et al.*, 1990b). Aromatic and O-aromatic molecules originate from lignin and tannins (Kögel-Knabner, 2000; Preston *et al.*, 2000). Carbonyl carbon is mainly a constituent of lipids or amino acids (Kögel-Knabner, 2002; Baldock *et al.*, 1990b).

O-alkyl and di-O-alkyl carbons are major constituents of cellulose and hemicellulose, and are commonly considered to be readily degradable, yet

surprisingly they represent up to 20-50% of SOM (Erhagen *et al.*, 2013; Lorenz *et al.*, 2000; Preston *et al.*, 2000; Preston *et al.*, 1994; Kögel-knabner *et al.*, 1992). In contrast, polymers of aromatic carbons are considered to be recalcitrant to microbial degradation. Thus, the relative contribution of aromatic polymers in SOM should be substantially higher in comparison to the plant and microbial biomass constituting the carbon source. Published data clearly reveal that the relative proportions of aromatic carbons are approximately the same in litter and SOM (Erhagen *et al.*, 2013; Preston *et al.*, 2006; Kögel-Knabner, 2002; Lorenz *et al.*, 2000; Preston *et al.*, 2000). Both examples above clearly indicate that simply assigning carbohydrate polymers as easily degradable and aromatic polymers as recalcitrant is far too simplistic.

The role of nitrogen in decomposition

Increases in nitrogen deposition have been shown to affect soil carbon storage positively, because they reduce saprotrophic respiration via mechanisms that shift the composition of the OM towards more chemically stable compounds (Janssens *et al.*, 2010; Liu & Greaver, 2010; Berg & McClaugherty, 2003). High N content also appears to suppress activities of ligninolytic fungi and their enzymes (Berg & Matzner, 1997). However, N has complex effects in decomposition processes as high N concentrations appear to enhance degradation in early stages of fresh litter decomposition but suppress it in late stages (Berg, 2000; Berg & Matzner, 1997). Thus, it plays a key role in the sequestration of soil carbon. Because of the importance of carbon sequestration for the climate, the interactive effects of soil N content and temperature on the temperature sensitivity of CO_2 production from OM decomposition is a very important issue.

Microbial metabolism

Microorganisms obtain both energy and substrates for cell growth from the decomposition of OM, via processes often referred to as catabolic (breaking down) and anabolic (building up), respectively. Ratios of soil microorganisms' catabolic and anabolic reaction rates depend (*inter alia*) on the stoichiometry of the available carbon and nutrient sources. The ratio between anabolic and catabolic reaction rates is often referred to as carbon use efficiency (CUE), and provides a measure of the proportion of acquired carbon that is used to synthesize new microbial biomass (Manzoni *et al.*, 2012; Clifton, 1946; Winzler & Baumberger, 1938). How this relationship is affected by temperature is relatively poorly understood (Manzoni *et al.*, 2012; Allison *et*

al., 2010; Drotz *et al.*, 2010a). However, it is generally believed that the CUE should decrease with increases in temperature (Steinweg *et al.*, 2008; Hall & Cotner, 2007; Farmer & Jones, 1976; Mainzer & Hempfling, 1976), because of relatively high increases in maintenance respiration rates driven by rises in energy costs of maintaining ion gradients across the cell membranes and increases in protein turnover rates (Hall & Cotner, 2007; Farmer & Jones, 1976; Mainzer & Hempfling, 1976). Some more recent studies have supported this view (Frey *et al.*, 2013; Steinweg *et al.*, 2008), but others have found that the CUE is not temperature-sensitive (Dijkstra *et al.*, 2011b; Drotz *et al.*, 2010a).

To estimate the CUE, a ^{13}C-labeled substrate can be added to monitor amounts of newly synthesized microbial biomass and ^{13}C-CO_2 respired by the microorganisms, then CUE can be calculated by dividing the amount of ^{13}C incorporated into biomass by the total amount incorporated and respired as ^{13}C-CO_2. The fate of a carbon substrate follows a well-established pattern (especially for highly labelled ^{13}C-glucose), which can be divided into several distinct metabolic phases (Ilstedt *et al.*, 2003; Nordgren *et al.*, 1988): an initial response reflecting the microbial population's capacity to use it (Substrate-induced respiration, SIR; (Anderson & Domsch, 1978) followed by a lag phase until the microorganisms start to grow and a specific growth rate (SGR) reflected in an exponential increase in CO_2 production.

Microbial communities in boreal forest soils

Bacteria and fungi are the main decomposers in boreal forest soils and are responsible for more than 95% of the decomposition of OM (Persson, 1980) and the microbial biomass in the boreal forest is dominated by fungi (Högberg *et al.*, 2007; Frostegard & Baath, 1996). The fungal community has a dual function in being either saprotrophs, i.e. decomposing organic material or biotrophs through mycorrhizal relationship with living plants (Clemmensen *et al.*, 2013; Johnson *et al.*, 1997; Smith & Read, 1997). Much of the high relative contribution from fungi to the total microbial biomass in forest soils most likely originate from the mycorrhizal fungi (Clemmensen *et al.*, 2013). Besides being crucial in carbon and nutrient cycling the microorganisms also contribute most significantly to the secondary biomass production in the soil, both from saprotrophic and mycorrhizal fungi (Bradford *et al.*, 2013; Clemmensen *et al.*, 2013; Baldock *et al.*, 1990a; Swift, 1973). Thus, both the accumulation of soil carbon and the rate of soil carbon decomposition relay on biomass from both plant primary production and microbial heterotrophic secondary production. The vertical distribution of fungi in a soil profile reflects a distinct successional

gradient with saprotrophic fungi dominating the young and less decomposed material while the older humus material deeper down in the profile is dominated by mycorrhizal fungi (Lindahl *et al.*, 2007).

1.4 Application of NMR spectroscopy in biogeochemistry

Nuclear Magnetic Resonance (NMR) spectroscopy has been successfully used for several decades in soil science to characterize both the structure of OM and its turnover in soil (Lundberg *et al.*, 2001; Preston, 2001; Preston *et al.*, 2000; Baldock *et al.*, 1990a; Wilson, 1987). The major advantage of NMR in soil science applications is that it enables highly detailed investigations without any destructive extraction steps.

The concept of NMR is based on that nuclei of an isotope have a quantum-mechanical property called spin. This spin gives them a magnetic momentum and in a magnetic fields such a nuclei acquires two or more energy levels and depending on the spin quantum number. Nucleic like ^{1}H and ^{13}C have spin quantum number I=1/2, which gives them two energy levels. For ^{12}C the spin quantum number equals 0 which means that they are NMR-inactive. When placing a sample in a magnetic field the magnetic momentum of the nucleus will originate itself according to the applied magnetic field. The energy difference that occurs between the energy fields is proportional the applied magnetic field and the transition of the energy levels is associated with either emission or adsorption of electromagnetic (EM) radiation. The EM frequency corresponding to the energy difference is called the Larmor frequency and applying a radio frequency at the Larmor frequency induces transitions between the energy levels and coherences between the spins. After applying the radio frequency the excited spin system relaxes back to equilibrium and during the relaxations emits decaying radiation. To create a spectrum this free induced decay (FID) is measured as a function of time and Fourier transformed into a spectrum. Depending on what frequency applied different nuclei can be excited separately which allows for individual manipulation for setting up experiments. The most common analyzed isotopes in NMR are ^{13}C, ^{1}H, ^{31}P and ^{15}N.

The chemical shift is a consequence of the electrons around the nuclei that shields the local magnetic field at the nuclei and thus shifts the exact resonance frequency at the ppm level. Depending on the structure of the molecule this electronic environment changes and also the separates the atom a different position along the frequency axis (Wilson, 1987).

1.5 Scientific approach

Essentially, the scientific approach applied in the work was to incubate soil and litter samples collected from boreal forests in a high-resolution respirometer (Respicond), add pure carbon substrates (both unlabelled and $^{12}C/^{13}C$ labelled), then monitor CO_2 production rates hourly. To relate the temperature sensitivity of decomposition to the chemical composition of the OM (quality), and investigate the microbial allocation of carbon we used NMR spectroscopy, as outlined above.

An important aspect to realise with most laboratory soil incubations is that the biotrophically association of mycorrhizal with living trees is terminated. The organic material provided by the mycorrhizal fungi will act as source of recent necromass for the opportunist saprotrophic microorganisms. Decomposition of this newly dead fungal biomass results in high initial respiration but levels off when the high labile C substrate from necromass is depleted. This takes normally 2-4 days (Erhagen *et al.*, 2013; Ilstedt *et al.*, 2003).

In Paper I we empirically investigated a theoretical model describing the key processes influencing the temperature sensitivity of OM decomposition: substrate release, diffusion and uptake by the microorganisms.

In Paper II we thoroughly investigated the chemical composition of OM from a large range of boreal forest ecosystems using CP-MAS NMR and HSQC NMR. We also incubated samples of the same soil and litter at various temperatures to investigate the temperature responses of their decomposition and relate the responses to the OM chemical composition.

In Paper III the aim was to examine the effects of the OM's nitrogen content on the temperature response of saprotrophic CO_2 production emanating from decomposition of OM. We collected soil and litter samples from plots used in a long-term fertilization experiment (which started in 1971), then analysed the chemical composition of their OM by CP-MAS NMR.

In paper IV we investigated effects of temperature, the decomposition of carbon monomer and polymer, and metabolic phases, on the CUE.

2 Material and Methods

2.1 Site description

All the soil and litter that were used in the work underlying this thesis came from sites in boreal forests of northern Sweden, where spodosols and histosols are the main soil types The soil samples were confined to the organic (O)-horizon, mainly because this is where the microbial activity is highest. The soil used in Paper I was collected in the outskirts of Umeå from a boreal forest with *Pinus sylvestris* (L.) and *Picea abies* (L.) being the dominated three species.
In Paper II we wanted to investigate temperature responses of a large range of boreal forest types. Therefore, we collected soil and litter from eight sites located around Kulbäcksliden Experimental Park, representing the major types of forests in the boreal landscape. The first four (1-4) sites were sampled along a 500 m gradient. The vegetation at site 1, located at the top of the discharge area, is typical boreal forest dominated by pine and spruce. Further down the gradient the vegetation changes due to the increasing availability of water and nutrients. Spruce becomes increasingly abundant, and at sites 3 and 4 it is completely dominant. The ground vegetation also changes, with more high herbs at the bottom of the gradient (See Table 1, Paper II, for a more detailed description of vegetation at each of the sites). Sites 5, 6 and 7 were located on a dry sandy, poor pine heath dominated by pine, in a *Betula pendula* (L.) grove and a clear-cut area dominated by small *B. pendula,* recently planted spruce and *Deschampsia flexuosa* (wavy hair grass) plants (L.) The last site (8) was also a *B. pendula* grove where the dominating species apart from *B. pendula* was *Vaccinium myrtillus* (L.).

Figure 4. Two sampling locations: left, a mixed spruce and pine forest (Site 2 in Papers II and IV); right, a nutrient-poor pine heath (Site 5 in Paper II).

Norrliden long-term fertilization experiment (Paper III)

The soil and litter used Paper III were sampled at Norrliden, Vindeln, from plots used in a long-term experiment, established in 1971, testing effects of N fertilization on a pine-dominated forest stand. Ammonium nitrate (NH_4NO_3) is being annually added to 30×30 m plots at three levels (30, 60 and 90 kg N ha^{-1} yr^{-1}; designated N1, N2 and N3, respectively) and withheld in a control treatment (N0). The N3 treatment was terminated in 1991, but the other treatments are still being applied. The doses used correspond to standard forest fertilization regimes, although the lowest dose also corresponds to the highest levels of atmospheric deposition in Europe and North America.

Figure 5. A plot of the Norrliden fertilization experiment, showing the litter traps used in Paper III to collect pine needles.

2.2 Soil and litter processing

In all the experiments soil cores with a 15 cm diameter were sampled from the organic (O-) horizon. Litter and mosses were removed then the soil samples were bulked in plastic bags and placed in a refrigerator at 6ºC in the laboratory before processing. The soil samples were then passed through a sieve with 5 mm mesh to remove coarser roots and plant residues while gently homogenizing the soil, and placed in a freezer (-22°C) until the incubations and other analyses. Their water and organic contents were determined by measuring changes in the weight of sub-samples after drying at 105ºC for 24 h and losses on ignition (LOI) after drying at 550ºC for 6 h. To optimize the water content for microbial decomposition the water potential was adjusted to -25 kPa before each incubation (Ilstedt *et al.*, 2000).

Figure 6. A soil core (Left figure) sampled from the organic layer (O-horizon) of a spodosol (Right figure).

Paper I included two incubation experiments (designated 1 and 2), using soils sampled from Liljansberget (a site dominated by spruce within Umeå municipal boundaries) and Kulbäcksliden Research Park (70 km west of Umeå, dominated by spruce and pine), respectively. The soils at both locations are classified as spodosol, one of the major forest soil types in the boreal region (Soil Survey Staff, 2003).

In Paper II and III newly shed litter was collected by litter traps (five within a 100 m^2 area at each site, Paper II, three traps in Paper III within each treatment plot), each consisting of a net bag with a 50×50 cm opening held in place a few dm above the ground by a wooden frame. Collected litter was sorted to exclude inputs from non-dominant vegetation (and thus minimise potentially confounding data). The remaining litter was chopped into ca. 1 cm pieces, its water content and LOI were determined as for the soil samples (see above) and placed in a freezer until the incubations. To optimise water contents for microbial decomposition, the litter samples were mixed with 20 g of Perlite with a water potential adjusted to -25 kPa. Ilstedt (2007) showed that microbial respiration is proportional to the amount of soil in such mixtures, and not affected by the perlite.

The soil used in paper IV were sampled in October 2012 within Kulbäcksliden Experimental area, Vindeln Experiment Forests which are located in the northern parts of Sweden (64°11`N,19°33`E). Sub samples of the soils and litter were taken out for the CP-MAS NMR analyses. Before the NMR-analyses the soil and litter were air-dried (60°C), and then milled with

steel ball to achieve the same size distribution. Drotz, H et al (2010b) compared the use of steel ball to agate balls and saw no difference in NMR results.

2.3 Respiration measurements

For all the soil incubations, soil samples containing 1 g organic of material (dry weight, dw) were transferred to 250 ml incubation jars. The saprotrophic respiration was measured using a high-resolution respirometer (Respicond VI, Nordgren Innovation, Djäkneboda, Sweden) that monitored the rate of CO_2 production hourly. The respirometer technique is based on a simple procedure presented by Chapman (1971) and further developed by Nordgren (1988). The Respicond instrument consists of a series of incubation vessels, each equipped with a small measurement jar containing a KOH solution that traps respired CO_2. The resulting reductions in electrical conductivity are measured by pairs of platinum electrodes, and are used to calculate the amounts of CO_2 released per unit time.

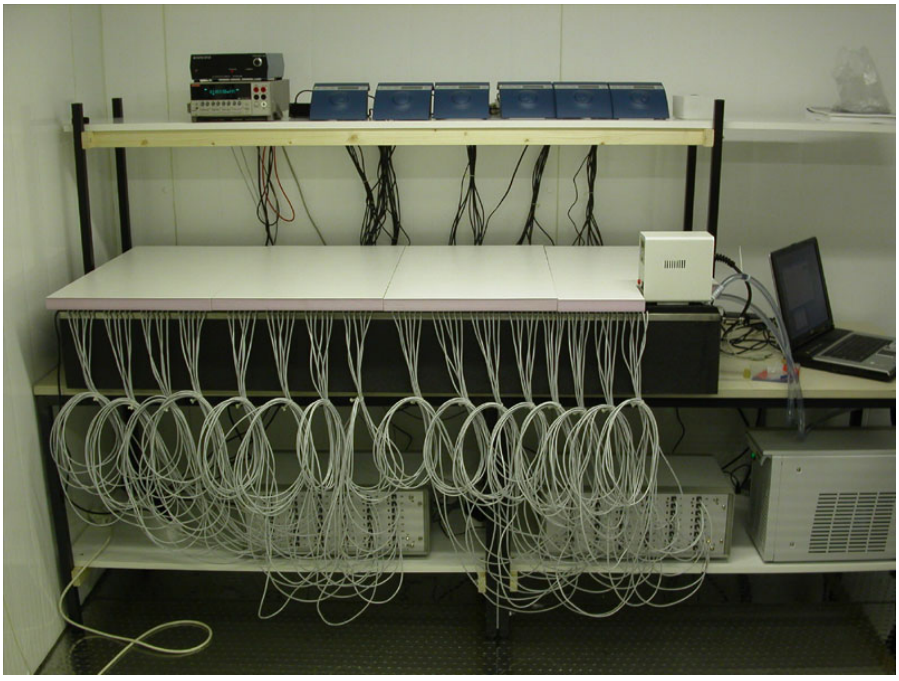

Figure 7. A Respicond IV respirometer, containing 96 incubation vessels each equipped with a small jar of KOH and two platinum electrodes that measure the decrease in conductivity as the CO_2 produced by microorganisms in the incubation vessels is captured by the KOH.

2.3.1 Carbon substrate addition (Paper I and IV)

In Paper I we separately added two sets of pure carbon substrates representing major constituents of plant and microbial biomass (Berg & McClaugherty, 2003) purchased from Sigma-Aldrich (Stockholm, Sweden). In Experiment 1 we added four 6-C carbohydrates (glucose, fructose, galactose and rhamnose), two 5-C carbohydrates (xylose and arabinose), one aromatic compound (vanillic acid), one fatty acid monomer (palmitic acid), and three dimers (disaccharides) of 6-C carbohydrates (maltose, lactose and sucrose). In Experiment 2 we added six polymers (crystalline cellulose, amylose, amylopectin, xylan, glycogen and chitin) and two monomers (mannitol and glucosamine). The carbon substrates were added to soil sub-samples for the incubations, together with $(NH_4)_2SO_4$ and KH_2PO_4 as nitrogen and phosphorus sources, respectively, to C:N:P mass ratios of 1:13:182. In Experiments 1 and 2, the quantities of added C-monomer corresponded to 0.46 and 0.27 M g^{-1} OM (dw), respectively (sufficient for substrate saturation in both cases).

In Study IV we added ^{13}C-labelled glucose and ^{13}C-labelled cellulose (>97 atom %) supplied by Isolife B.V., Wageningen, The Netherlands. We added 50 mg of C-glucose and cellulose to each incubation vessel together with a nutrient solution of $(NH_4)_2SO_4$ and phosphorus KH_2PO_4 to obtain a C:N:P mass ratio of 1:13:182.

2.3.2 Description of metabolic phases after substrate addition

Three metabolic growth phases were characterized in the incubations (Nordgren *et al.*, 1988): the basal respiration, substrate-induced respiration (SIR), and specific growth rate (SGR) phases. The periods for these phases are indicated in Figure 4 (A - basal respiration, B - SIR and C - SGR). The basal respiration reflects the decomposition of the incubated sample in the absence of added substrate, this was calculated from the average of 100 hourly measurements after respiration had stabilized, which typically occurred 2-4 days after the start of the incubation. SIR is the immediate response of CO_2 production following addition of a carbon source, and was calculated as the average of 5 hourly measurements after the addition of the substrate. SIR is commonly described as the soil microbes' potential capacity to degrade an added C-source (Anderson & Domsch, 1978). The SGR was calculated by linear regression after logarithmic transformation of measured CO_2 production rates, and is the slope of the log-linear curve (r^2 >0.95).

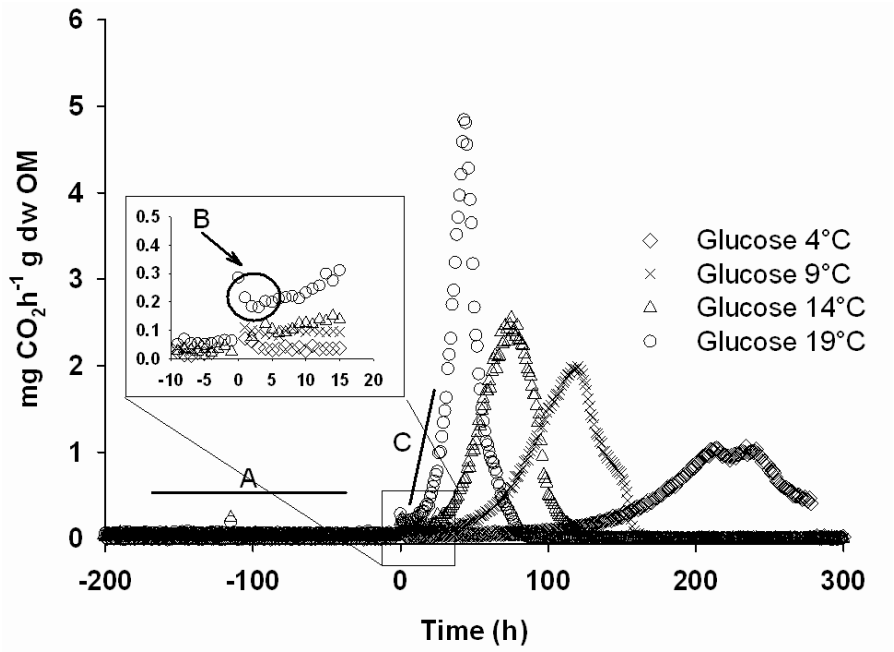

Figure 8. Microbial respiration rates at four temperatures (4, 9, 14 and 19˚C) before and after addition of glucose (plus nitrogen and phosphorus) to soil samples from the O-horizon of a coniferous forest close to Umeå, Sweden. The substrate was added at time 0. The time intervals used for estimating the basal respiration, SIR and SGR parameters (A, B and C, respectively) are also indicated in the graph. The SGR was estimated after logarithmic transformation of the respiration data recorded in period C.

In Paper IV for each of the labeled substrates (i.e. ^{13}C glucose and ^{13}C cellulose), 6 replicate soil samples were incubated at each temperature. This made it possible to analyze both the disappearance of the added ^{13}C labeled substrate and the synthesis of new ^{13}C labeled microbial biomass at six different points in time, during different metabolic conditions. The exact timing of sample collection for the analysis of the consumption and synthesis of ^{13}C labeled compounds was determined based on the real-time data on total CO_2 production generated by the Respicond system. Samples were collected (Figure 1, Paper IV) during the exponential growth phase (SGR), and during the period when the rate of CO_2 production started to decrease as the metabolic condition changes and substrate availability become a limiting factor (After peak, AP). The first two samples for the ^{13}C-glucose experiments were collected during the SGR phase. The third sample was collected when the CO_2 production peaked, (Peak) (For 19°C the peak sample was missed, resulting in one additional sample after the peak) and the last three were collected at various points while the rate of CO_2 production declined (AP1, AP2 and AP3).

For the ^{13}C-cellulose experiments, at 19°C the following samples were analyzed SGR1, AP1, AP2, AP3. At 14°C following samples were analyzed: SGR1, AP1, AP2, AP3, AP4. At 9°C three samples were analyzed: SGR1, AP1 and AP4. At 4°C the following samples were analyzed: SGR1, Peak, AP1, AP2, AP3, AP4 and AP5. Immediately after being removed from the Respicond, the samples were sterilized by the addition of 0.5% NaN_3 (Wolf *et al.*, 1989) and stored in a freezer at -20 °C until required for NMR analysis.

2.4 Applied NMR techniques

2.4.1 Solid-state CP-MAS NMR (Papers II and III) and HSQC

To characterize soil and litter in Paper II and III a Varian/Chemagnetics CMX400 spectrometer was used, with a ^{13}C operating frequency of 100.72 MHz. For more detailed description of the NMR settings used see Paper II.

Figure 9. A Varian/Chemagnetics CMX400 400 MHz NMR magnet.

To characterize the OM in paper II and III solid state CP-MAS NMR and also in paper II 2D Heteronuclear Single Quantum Coherence (HSQC) spectroscopy were used. Solid state CP-MAS NMR stands for cross polarization magic angle spinning and is a technique used to increase sensitivity by transferring sensitive ^{1}H magnetization to less sensitive ^{13}C nuclei nearby using a double spin lock during the CP step. Furthermore, the 1H nuclei normally have much shorter relaxation times (T1) than ^{13}C in a solid

state, making it possible to repeat experiments much more frequently to increase signal to noise ratios. In our studies this dual enhancement allowed ^{13}C experiments to be completed in 1-2 hours instead of days. Unfortunately, the CP step introduces signal integration problems since the ^{1}H-^{13}C proximity and dynamics influence the efficiency of the magnetization transfer. To counter this problem we used spin counting (Smernik & Oades, 2000). The transfer of magnetization between ^{1}H and ^{13}C requires the carbon atoms to be sufficiently close to a proton for the transformation to occur. Therefore "black carbon" like graphite is invisible to CP. To reduce the line widths in the spectra the CP is accompanied by MAS (Magic Angle Spinning), which averages out orientation-dependent interactions in the solid state by rapidly mechanically spinning the sample around an axis 54.7° relative to the magnetic field. In our experiments we used a spinning rate of 8 kHz.

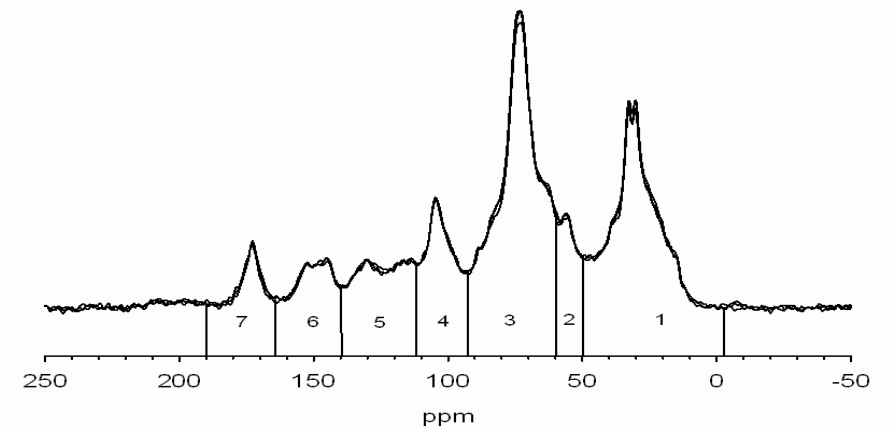

Figure 10. Replicate (n=3) ^{13}C CP-MAS NMR spectra. The vertical lines indicate the partitioning of the spectra into regions of signals assigned to the following groups of carbon compounds: 1 (0-50 ppm), alkyl carbon; 2 (50-60 ppm), methoxy/N-alkyl carbon; region 3 (60- 93 ppm) O-alkyl carbon; region 4 (93-112 ppm), di-O-alkyl carbon; region 5 (112-140) aromatic carbon; region 6 (140-165), O-aromatic carbon and region 7 (165-190 ppm), carbonyl carbon (Preston, 2001; Preston *et al.*, 2000; Kögel-Knabner, 1997; Preston *et al.*, 1997).

The characteristic ^{13}C chemical shifts of organic material lie between 0 and 190 ppm (Smernik, 2005). The NMR spectra were divided into the following chemical shift regions to define the composition of SOM: 0-50 ppm (alkyl C), 50-60 ppm (methoxy/N-alkyl C), 60-93 ppm (O-alkyl C), 93-112 ppm (di-O-alkyl C), 112-140 ppm (aromatic) 140-165 ppm (O-aromatic C) and 165-190 ppm (carbonyl C) (Preston, 2001; Preston *et al.*, 2000; Kögel-Knabner, 1997; Preston *et al.*, 1997).

To further enhance the resolution and characterization of the OM we used in Paper II a technique called 2D Heteronuclear Single Quantum Coherence (HSQC) spectroscopy (Kim *et al.*, 2008). ^{13}C-1H HSQC provides more detailed information on short-range C-H bonds, and thus on the structural compounds, than ^{13}C CP MAS alone. In HSQC samples are transferred to NMR tubes and suspended in deuterated dimethyl sulfoxide (DMSO-d6) (Simpson *et al.*, 2007).

To investigate microorganisms partitioning of ^{13}C-labeled substrates in Paper IV, direct excitation of ^{13}C, by ^{13}C MAS NMR was used. This provides information particularly on soluble and semi-solid compounds (and solid compounds, but with lower intensity and broader linewidths).

In Paper IV the production of ^{13}C-CO_2 during the incubation experiments was determined by performing solution ^{13}C NMR analyses of the KOH solutions from the Respicond apparatus. A subsample (250 µl) of the 10 mL of KOH solution in the Respicond was taken and mixed with 250µl of a stock solution containing 1.00M potassium acetate (CH_3CO_2K) and 20% D_2O in a 5mm NMR tube (giving a final KAc concentration of 0.500M KAc in the NMR tube). For more detailed description of the NMR settings used see Paper IV.

2.5 Statistical analysis and data evaluation

In all studies, observed effects of treatments were considered statistically significant if $p<0.05$. To evaluate the acquired data linear and partial least squares (PLS) regression were used. The statistical analyses were performed using several software packages, including SPSS version 11 (SPSS Inc., Chicago, IL USA), Simca-P, version 10.5 (Umetrics, Umeå, Sweden), Minitab 16, JMP version 9.0.0 and Excel (Microsoft Office Excel 2011).

2.5.1 Q_{10} calculations

In all studies temperature responses were expressed as Q_{10} values, defined as the factor by which the respiration rate changes in response to a temperature change of 10°C. To calculate these values an exponential model (eq. 1) was used to describe the respiration rate as a function of temperature.

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

Here, e is the base of the natural logarithm, β is the exponent from the best fitting exponential function for the respiration and temperature data, and the factor of 10 corresponds to the 10-degree temperature difference. The variance and standard error (SE) of sets of Q_{10} values were obtained from the slope of the Taylor expansion. A curve-fitting model was used to calculate the standard deviation of the exponent β; the SE of the Q_{10} was calculated using equation 2:

$$SE(Q_{10})=10\times e^{\beta\times 10}\times SE(\beta) \quad (2)$$

2.5.2 Statistical calculations

In paper I and II the following regression model was used to evaluate effects of different soils and litters on the observed Q_{10} values:

$$y_{ik} = \alpha_k + \beta \times T_i + \varepsilon_{ik} \quad (3)$$

Here, y_{ik} is the natural logarithm of the respiration rate for soil k (k=1,…,K) and temperature i. T_i is the temperature, α_K and β_K are regression coefficients, and ε_{ik} are normal, independent random variables $N(0, \sigma^2)$. The null hypothesis that changes in temperature affect all soils and litter equally (i.e. $\beta_1 = \beta_2 = \ldots = \beta_k$) was tested separately for BR, SIR and μ using the *F*-test. If the null hypothesis was rejected, soil-specific β values were compared pairwise using Tukey's test, see (Zar, 1996).

In paper II we tested whether the temperature responses of BR and SIR, or SIR and μ, differed according to soil and litter type. The hypothesis that β (BR) =β (SIR) was tested using equation 4, with y_{diff} being equal to the difference between the natural logarithms for BR and SIR rates. The regression model from equation 3 was applied on this occasion using the difference between BR and SIR according to equation 5.

$$y_{diff} = y_{BR} - y_{SIR} = \alpha_{BR} - \alpha_{SIR} + (\beta_{BR} - \beta_{SIR})\times T + (\varepsilon_{BR} - \varepsilon_{SIR}) \quad (4)$$

$$y_{diff} = \alpha_{diff} + \beta_{diff} \times T + \varepsilon_{diff} \quad (5)$$

BR data were obtained from the same vessels as those used when acquiring the SIR and μ data, thus β_{BR}, β_{SIR} and βμ may potentially have been sample-

dependent and (hence) correlated. Sample dependence was therefore tested using correlations between the residuals for the vessels involved. The correlation coefficients were close to zero, indicating that any correlation between β_{BR} and β_{SIR} could be considered negligible.

To evaluate differences in organic chemical composition between the litter and soil humus in Paper II, a one-way ANOVA was used, with the carbon forms derived from the CP-MAS NMR specified as the dependent variables.

In Paper II and III partial least square (PLS) regression was used to evaluate the relationship between the organic chemical composition of the soil and litter, and the temperature response of basal respiration (Software, Simca-P, version 10.5, Umetrics, Umeå, Sweden). PLS uses two data matrices: X (explanatory variables), and Y (response variable) and relates these to each other using a linear multivariate model (Eriksson et al., 2001). The performance, and model fit is explained by R^2 with the prediction power estimated by internal cross validation and described by Q^2. In our analysis, the Q_{10} of the BR constituted the response variable (Y), while the X-matrix consisted of the relative integrals of signals from regions of the CP-MAS NMR spectra corresponding to specified C forms (alkyl C methoxy/N-alkyl C, O-alkyl C, di-O-alkyl C, aromatic C, O-aromatic C and carbonyl C, see above). Both individual X-variables and the two-way interaction terms for the X-variables were included in the PLS analysis. The X-variables were scaled and mean-centred prior to the analysis. The PLS models were refined, i.e. the non-significant X-variables (with 95% confidence intervals of the coefficients $\neq 0$) were removed. The results from the PLS-analysis are presented as histograms displaying the coefficients. The coefficient plots are based on scaled and centred variables, i.e. the influence of the coefficients on the response variable in the model is directly reflected by the size and sign of each coefficient.

For Paper III the following complete mixed model was used to describe the respiration rate associated with decomposition of the soil and litter samples collected from plots under k=4 nitrogen treatments (N0, N1, N2, and N3) incubated at j=4 temperatures:

$$y_{kij} = \mu + \alpha_k + \beta + (\alpha\beta)_{kj} + ci_{(k)} + e_{kij} \qquad (6)$$

Here y_{kij} is the natural logarithm (ln) of the respiration rate for nitrogen treatment k (k=0,...,3), field replicate within treatment i (i =1,...,3) and temperature j (j=1,…, 4), μ is the grand mean, α is main treatment effects and β is temperature effects, $\alpha\beta$ their interactions, c is the replicate within treatment

random effects and e is the individual random error. The model was reduced according to model 2 to test for linearity of the temperature effects:

$$y_{kij} = \mu + \alpha_k + \beta_k * Temp_{kij} - c_{i(k)} + e_{kij} \quad (7)$$

The null hypothesis implied by equation 2 was tested by the general F-test

$$F=(SS_{res,H0}-SS_{res})/q/(SS_{res}/(n-p)) \quad (8)$$

Here SS_{res} and $SS_{res,Ho}$ are the sums of the squares of the residuals in the complete and reduced models, respectively, q = 8, n = 48 and p = 28. The p-values were not low enough to reject the hypothesis of linearity for either of the two soil layers (0-3 and 4-7 cm) or bilberry leaf litter ($p_{soil\ 0-3}$ =0.271; $p_{soil\ 4-7}$ =0.394; $p_{Bilberry\ leaves}$ =0.520). Thus, the effect of temperature on respiration of the OM in these samples can be described by a linear regression function. For pine needles we obtained a p value, $p_{Pine\ needles}$ =0.000, thus for pine needles the temperature effect cannot be described by a linear regression. To test if the slopes (regression lines of ln respiration rates vs. temperature) of samples representing the four nitrogen treatments (N0, N1, N2 and N3) significantly differed, equation 2 was used as a basic model. The null hypothesis, formulated as H0:βk=β (samples representing all nitrogen treatments yield equal, β, slopes) was tested with an F-test. If the hypothesis was rejected an intermediate hypothesis was formulated: $H_0=\beta_1=\beta_2=\beta_3\neq\beta_0$ to test if the slope for samples representing the control treatment (N0) differed from the slopes for samples representing the other three nitrogen treatments, all of which are equal. For this purpose, the following equation was used.

$$y_{kij} = \mu + \alpha_k + \beta * Ind_{N1N2N3} * Temp_{kij} + \beta_0 * Ind_{N0} * Temp + c_{i(k)} + e_{kij} \quad (9)$$

Here μ is the grand mean, α are the main treatment effects, β is the slope common to N1, N2 and N3, and β_0 the slope for N0. Ind_{123} and Ind_0 are indicator variables for N1, N2, N3 (as a group) and N0, respectively. If H0 was rejected a Tukey-Kramer test was performed according to equation 5, since sizes of samples of the lower soil layer were slightly unequal. For the other layer the test statistic coincides with the standard Tukey test statistic. Critical values were determined according to the Studentized range distribution.

$$T_{ij} = \frac{(\hat{\beta}_i - \hat{\beta}_j)}{\sqrt{SE^2(\hat{\beta}_i) + SE^2(\hat{\beta}_j)}} * \sqrt{2} \quad (10)$$

Effects of the nitrogen fertilization treatments on saprotrophic CO_2 production (Figure 20) were evaluated by linear regression using the model $y_{ji} = a + bx_i + e_{ij}$, where y is the CO_2 production rate (mg CO_2 h^{-1} g^{-1} dry weight), x is the nitrogen concentration in the litter or soil samples, a is a constant, b is the regression coefficient and e is the random error.

Effects of the field nitrogen fertilization treatments on the chemical composition of OM as derived from CP-MAS NMR spectroscopy were evaluated by one-way ANOVA for each carbon type and soil or litter type (cf. Fig. 4, Paper 4). The quantitative relationship between nitrogen content and CP-MAS NMR chemistry was also further evaluated by partial least square (PLS) regression (Wold *et al*., 2001; User guide to SIMCA, Umeå, Sweden) with nitrogen concentration (%) as the dependent variable and organic chemical constituents as independent variables. The quantitative relationships between temperature sensitivity (Q_{10}) and the organic chemical constituents were also evaluated using PLS regression with Q_{10} and the organic chemical constituents as dependent and independent variables, respectively.

The CUE was determined in Paper IV according to Drotz et al (2010a), were the new synthesized ^{13}C-compounds derived from the ^{13}C MAS NMR spectra's and the ^{13}C-CO_2 from the respired CO_2 were used to calculate the CUE:

$$CUE = \frac{Synthesized\ 13C\ compounds}{(synthesized\ 13C\ compunds + 13C - CO2)} \quad (11)$$

To analyses the difference in CUE between the monomeric and the polymeric substrates, and to investigate the CUE between the metabolic phases, the CUE values before the peak (SGR1, SGR2 and Peak) were compared with the CUE values after the peak (AP1, AP2 and AP3). According to a Kolmogorov-Smirnov test data was normal distributed and the CUE-values of the two groups were compared with a T-test (SPSS Inc., Chicago, IL, USA).

3 Results and Discussion

3.1 Temperature responses of additions of pure carbon monomers and polymers (Paper I)

Confounding effects related to substrate quality and substrate availability

In Paper I we addressed confounding effects on the temperature response of decomposition related to substrate quality and substrate uptake by the microorganisms. As the conceptual model in Figure 3 shows, the decomposition processes are regulated by several key parameters: 1) the substrate release rate (S), 2) substrate diffusion rate (D), 3) substrate uptake (μ) and 4) the half-saturation constant (K).

Some of the conflicting results in previous publications may have arisen from confounding effects related to both substrate quality and availability. The simultaneous importance of both substrate quality and availability, as well as substrate diffusion and uptake rates, has been theoretically demonstrated (Ågren & Wetterstedt, 2007).

Therefore, the aim of Paper I was to empirically investigate how Q_{10} values of both SIR and SGR phases are affected by substrate quality, substrate uptake and the metabolic status of the saprotrophic microorganisms in a boreal mixed coniferous forest soil. We hypothesized that: 1) the addition of readily available carbon substrates to a carbon-limited system will result in higher temperature sensitivity, compared to that of basal respiration; 2) the temperature sensitivity, after adding a readily available substrate, will increase in proportion to its rate of uptake (μ) by the organisms; and 3) after adding carbon polymers the temperature sensitivity will depend on the activation energy of the substrate release rate (S) rather than the uptake rate, as is the case for the carbon monomers (see conceptual Figure 3).

To test these hypotheses, we created a model system using the organic layer (O-horizon) of a boreal forest soil (Soil Survey Staff, 2003) to specifically test

effects of varying carbon monomers and carbon polymers**.** The added carbon sources were various monomers and polymers with different degrees of degradability (Figure 11), selected to represent common constituents of plant and microbial biomass. We followed the saprotrophic CO_2 production of the soil microorganisms after the additions of the different substrates.

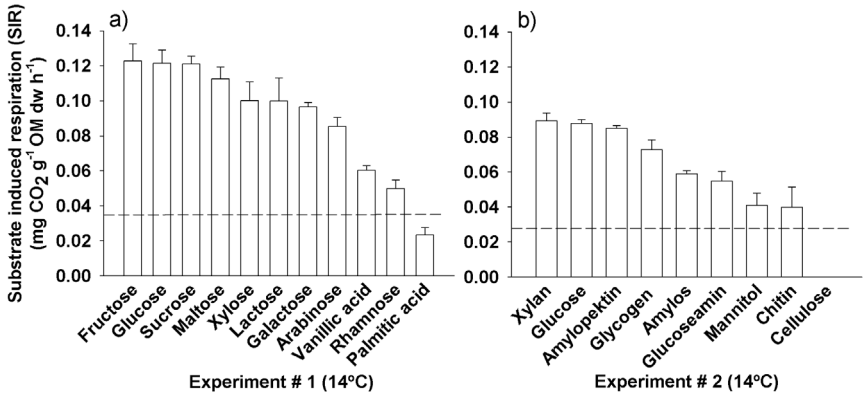

Figure 11. Substrate-induced respiration rates (mg CO_2 h^{-1} g^{-1} OM dw) measured at 14°C and arranged in decreasing order for carbon substrates used in Experiments 1 (a) and 2 (b).

The experimental conditions were designed to test specific hypotheses regarding effects of substrate uptake and release rates on the temperature sensitivity of saprotrophic soil CO_2 production rather than necessarily to mimic natural conditions. For this purpose we added various pure carbon sources to soil and litter samples incubating in the laboratory as model systems. Water potential was maintained at optimal conditions (Ilstedt *et al.*, 2000) and the high content of OM excluded any potential impact of mineral fractions on the temperature sensitivity (Sollins *et al.*, 1996). The substrate-induced Q_{10} values obtained averaged 2.8 (SE±0.13), which is typical for saprotrophic CO_2 production in soils and litters, i.e. measurements that exclude plant root respiration (Conant *et al.*, 2011; Conant *et al.*, 2008b; Fierer *et al.*, 2005; Davidson *et al.*, 1998; Kirschbaum, 1995)**.**

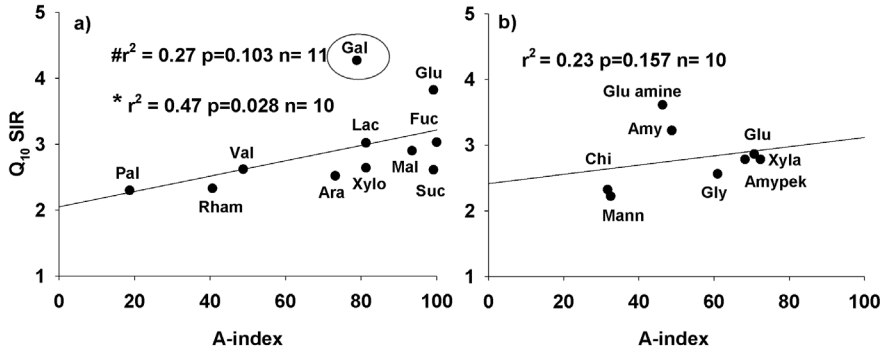

Figure 12. The relationship between the temperature sensitivity (Q_{10}) of substrate-induced respiration (SIR) and substrate availability (A-index). Figure 12a shows data from Experiment 1; the equations marked with # and * were respectively obtained from regression of data sets including and excluding data from galactose incubations (circled). Figure 12b shows data from Experiment 2.

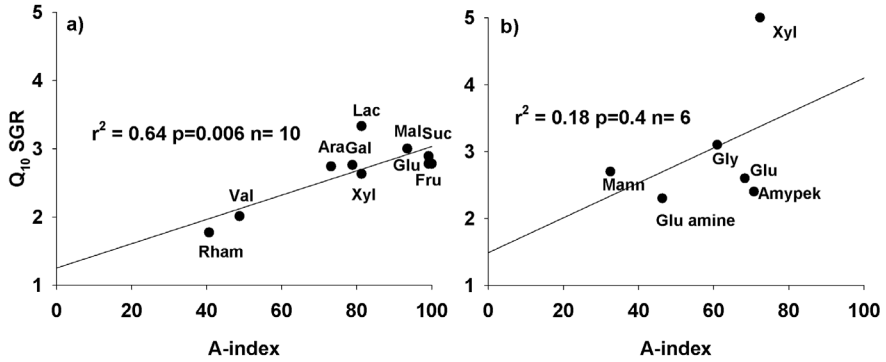

Figure 13. The relationship between the temperature sensitivity (Q_{10}) of the specific growth rate (μ) and substrate availability (A-index). Figure 13a shows data from Experiment 1 and 13b data from Experiment 2.

Previous attempts to explain variation in the intrinsic Q_{10} responses of OM decomposition have focused on differences in substrate quality, i.e. substrate release rates according to the model by Ågren & Wetterstedt (2007). Based on the quality theory, more readily available substrates should yield lower Q_{10} values (Hartley & Ineson, 2008; Davidson & Janssens, 2006; Fierer *et al.*, 2005). In this study addition of both monomers and polymers resulted in increased Q_{10} values (Figures 6 and 7, Paper I) relative to the Q_{10} value of basal respiration, opposite to the response predicted by the quality theory (Conant *et al.*, 2011; Davidson & Janssens, 2006). However, the results

support predictions based on the temperature sensitivity of uptake processes over cell membranes (Ågren and Wetterstedt, (2007), and thus our first hypothesis that adding a readily available carbon substrate to a carbon-limited system will generate a higher Q_{10} value than that of the basal respiration. These results are also consistent with results reported by Gershenson et al. (2009).

The quality of SOM is often assessed in terms of saprotrophic CO_2 production rates, normalised against the amount of OM present in the sample (Mikan, 2002; Wardle *et al.*, 1998; McClaugherty & Berg, 1987). Analogously, we used CO_2 production rates at 14^oC after substrate addition to rank the substrate-specific potential rates of respiration. Although relative levels of anabolic and catabolic metabolism differ when different substrates are available, we assume that CO_2 production (catabolic metabolism) and the uptake rate over the membrane will be strongly correlated. The substrate availability index (A-index) is based on the rankings of initial respiration rates after addition of the substrates (SIR) at 14^oC (Figure 11) in relation to that for glucose at the same temperature. Thus, the A-index for each substrate represents the total potential microbial uptake, i.e. the overall capacity of membrane-bound transport proteins to take it up, and the microbes' potential capacity to metabolise it.

The saprotrophic temperature sensitivity of both SIR and SGR was positively correlated with the A-index for the carbon monomers (Figure 12a and 13a). This positive correlation is contrary to expectations based on the carbon quality theory, which only accounts for carbon quality and predicts that the temperature sensitivity of saprotrophic decomposition of OM should increase as the activation energy of the relevant enzymatic processes rises (Conant *et al.*, 2011; Conant *et al.*, 2008b; Davidson & Janssens, 2006; Bosatta & Ågren, 1999). A possible explanation for the positive correlations between the A-index and Q_{10} values of SIR and SGR is that when a substrate is abundant at the surface of microorganisms, saprotrophic respiration is controlled by the uptake rate, so the temperature response is determined by the activation energy of the uptake into the microorganisms (Ågren & Wetterstedt, 2007). A decrease in activation energy for the uptake process (and thus faster uptake) would increase temperature sensitivity (i.e. result in a higher Q_{10}), as observed in our experiments. This confirms our second hypothesis.

After adding carbon polymers the Q_{10} response was not at all correlated with the A-index (Figures 12b, 13b), further supporting the second hypothesis. Polymeric compounds cannot be taken up directly, unlike the carbon monomers, and need to be decomposed outside the cell by exo-enzymes. Under these conditions, the temperature response is determined by the rates of

substrate release rate (S) and diffusion (D) to the surface of the microorganisms. This confirms our last hypothesis, i.e. that the temperature response of decomposition of carbon polymers will depend mostly on the activation energy of substrate release (S) and diffusion (D).

The Q_{10} values for both SIR and SGR were generally higher than those for basal respiration, but there was no general difference between Q_{10} values for SIR and SGR (Figures 6 and 7, Paper I). The major difference between SIR and SGR is that the uptake rate per unit volume of soil is considerably faster during SGR than SIR, due to a larger microbial population utilizing the added substrate. However, the uptake rate per cell is not very different between SIR and SGR. The temperature sensitivities of SIR and SGR were both significantly positively related to the A-index, but the temperature sensitivity of SGR increased more steeply with increasing A-index, in accordance with the increases in activation energy that follow increases in membrane uptake rates (Ågren & Wetterstedt, 2007).

Paper I provides empirical support for models predicting that both increased substrate availability and increased substrate uptake rate will result in increased temperature sensitivity (Ågren & Wetterstedt, 2007). Our results therefore also resolve the common confusion associated with the increased temperature sensitivity observed in response to the addition of readily available carbon substrates, which is contrary to model predictions based on substrate quality. Increases in substrate quality result in decreased temperature sensitivity (Davidson & Janssens, 2006), while increases in substrate availability and increases in uptake rate result in increased temperature sensitivity (Ågren & Wetterstedt, 2007), as revealed in this study. The study also clearly reveals that the relationship between catabolic and anabolic saprotrophic activity in any sample significantly contributes to the variation in temperature sensitivity.

3.2 Temperature responses of decomposition of soil and litter in relation to their chemical composition (Paper II)

The main aim of Paper II was to test the hypothesis that the chemical composition of OM is a key determinant of the observed temperature sensitivity of the decomposition of both plant litter and SOM. For this purpose we used a range of plant litters and soils from the O-horizon of the boreal forests. We incubated the litters and soil materials at four temperatures (4, 9, 14 and 19°C) in a respirometer, and measured the saprotrophic CO_2 production hourly. To characterize the OM in the litter and soils we used NMR

spectroscopy. This allowed us to link the organic chemical composition and temperature sensitivity of saprotrophic activity.

The findings presented in Paper II (Figure 14) strongly support the kinetic quality theory, and correspond with previous findings that Q_{10} values are higher for soil with relatively well-decomposed OM than for litter containing less decomposed OM (Wetterstedt *et al.*, 2010; Fierer *et al.*, 2005).

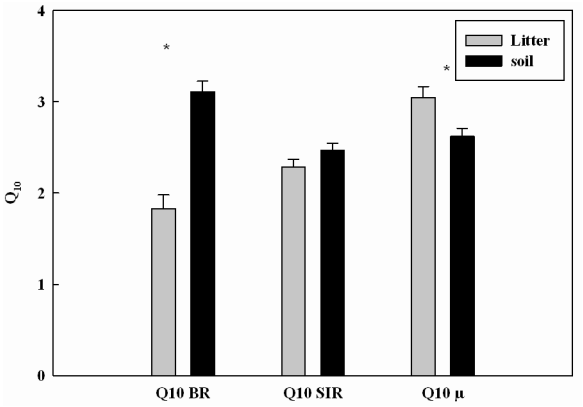

Figure 14. Mean (± SE) temperature responses (Q_{10}) of three metabolic parameters: basal respiration (BR), substrate-induced respiration (SIR) and specific growth rate (SGR). Asterisks (*) indicate significantly different Q_{10} responses between litter and soil.

Paper II also revealed significant relationships between specific organic chemical compounds and the Q_{10} response of saprotrophic CO_2 production. However, the organic chemical compounds correlating with the Q_{10} response of saprotrophic CO_2 production differed between the SOM and litter. Among litter samples the Q_{10} response decreased with increasing amounts of both aromatic and O-aromatic compounds, and increased with increasing amounts of O-alkyl and di-O-alkyl carbon. In contrast, among SOM samples the Q_{10} response did not change following changes in aromatic, O-aromatic, alkyl or O-alkyl carbon contents, but decreased strongly with increasing amounts of carbonyl carbons, and was also influenced by two-way interactions of O-, and di-O-alkyl carbons.

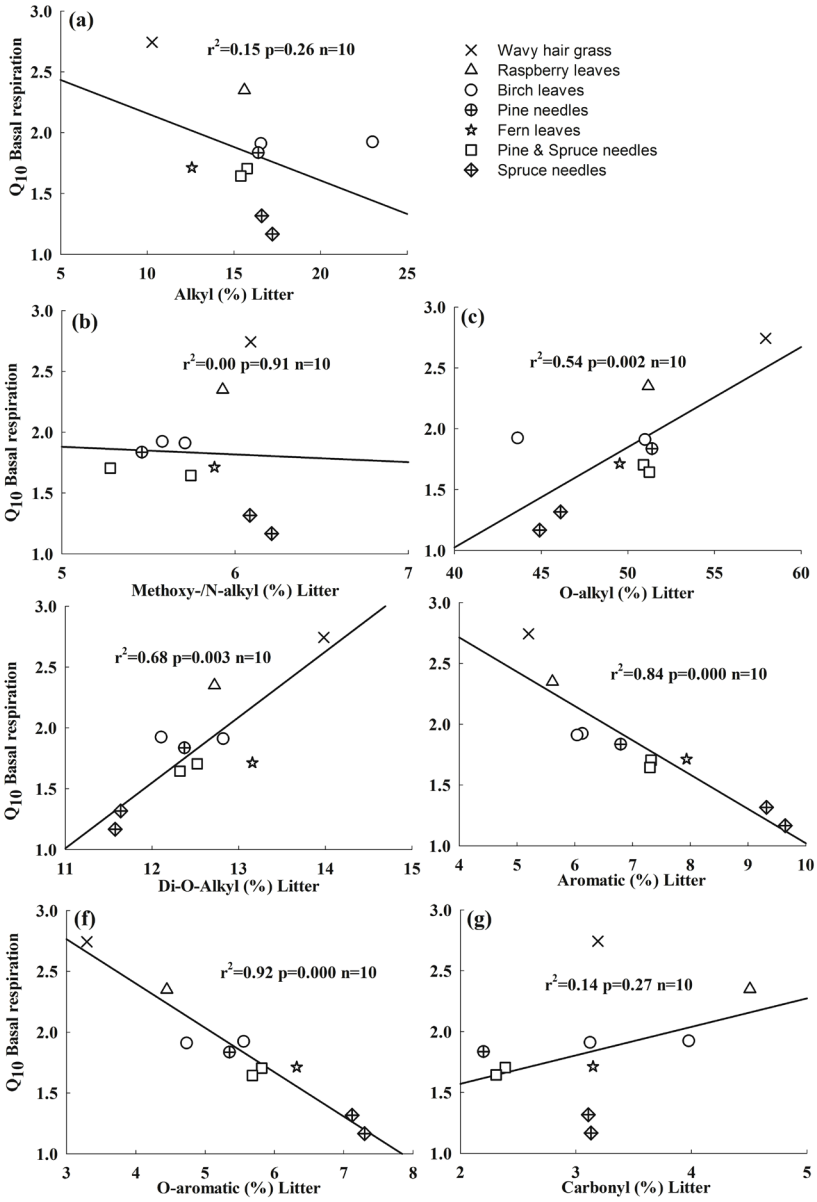

Figure 15. Temperature responses (Q_{10}) of basal respiration as a function of the proportions (%) of the following organic constituents of the litter prior to incubation: a) alkyl-, b) methoxy-/N-alkyl and carbonyl, c) O-alkyl; d) di-O alkyl, e) aromatic, f) O-aromatic and e) carbonyl carbon.

A high level of aromatic constituents in OM is often considered to be strongly linked to recalcitrance and thus restricted microbial availability (Vogt *et al.*, 2004; Weishaar *et al.*, 2003), implying that Q_{10} values should increase with increases in aromatic contents. Our results showed the opposite pattern for litter (Fig. 15 e, f), i.e. higher aromatic contents were associated with lower Q_{10} values. However, several studies (including our HSQC analysis, Figure 17) suggest that the main chemical constituents contributing to aromatic carbons in litter and SOM can, in fact, be quite different (Nierop *et al.*, 2006; Lorenz *et al.*, 2000; Preston *et al.*, 1997). Sources of aromatic carbons include both low-quality structural compounds, such as lignin, condensed tannins and suberin, but also relatively high quality compounds, for example hydrolysable tannins and aromatic amino acids (Lorenz *et al.*, 2007; Preston *et al.*, 2006; Kraus *et al.*, 2004). The very different relationships between the CP MAS ^{13}C-determined aromatic and O-aromatic contents and the Q_{10} responses of saprotrophic CO_2 production from litter and SOM also suggest that the aromatic constituents of litter and SOM strongly differ

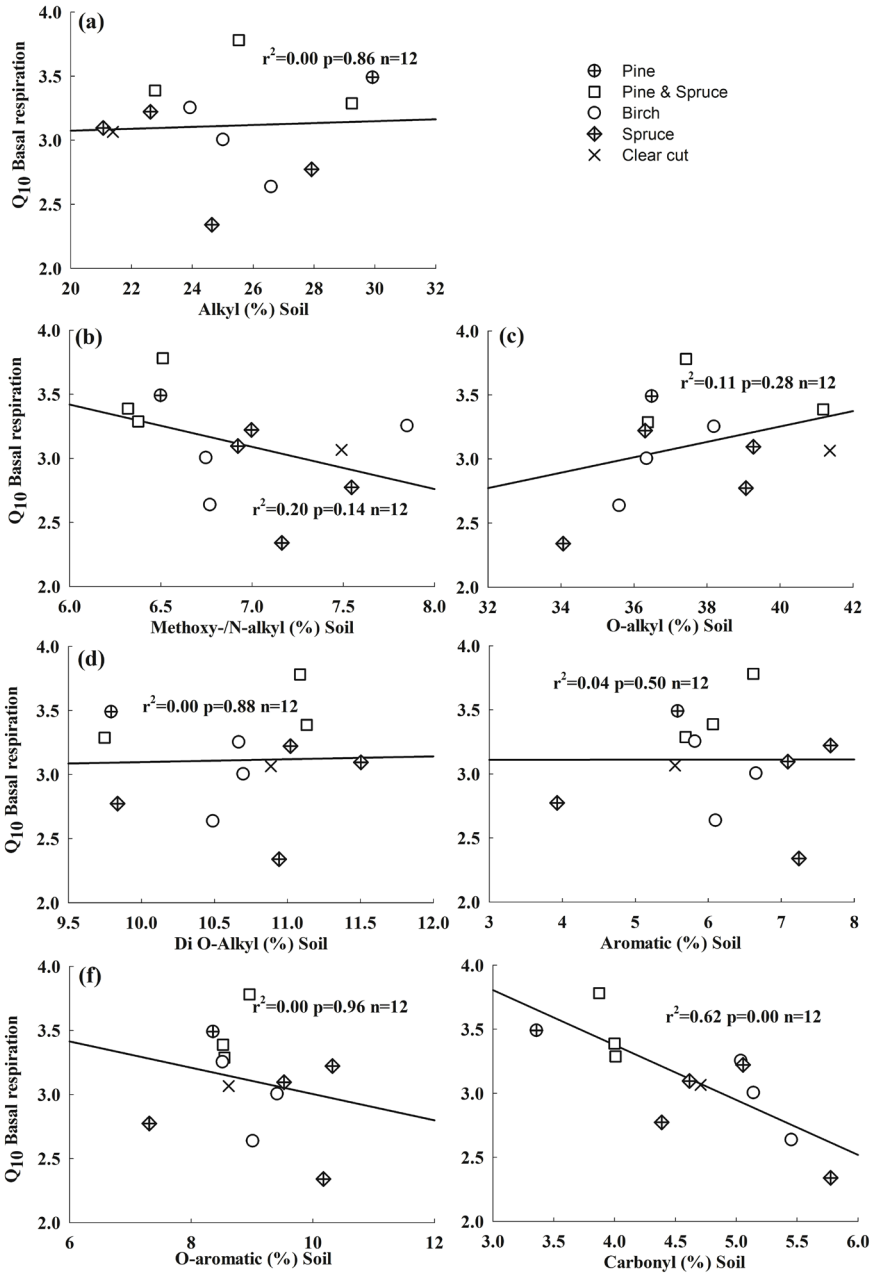

Figure 16. Temperature responses (Q_{10}) of basal respiration as a function of the proportions (%) of the following organic chemical constituents of humus prior to incubation: a) alkyl-, b) methoxy-/N-alkyl, c) O-alkyl, d) di-O alkyl, e) aromatic and O-aromatic, and e) carbonyl carbon.

More detailed analysis of NMR CP MAS signals from the aromatic region in the NMR spectra of the SOM samples also revealed a broader signal at 148 ppm, while litter spectra had sharper peaks at 155 and 145 ppm (Figures S4, S5, Supporting information, Paper II); the former indicating higher contents of guaiacyl lignin and the latter higher contents of tannins and aromatic amino acids (Preston *et al.*, 2000; Preston *et al.*, 1997). Thus, differences in tannin content and composition between litter and SOM might contribute to the difference in correlations between aromatic- and O-aromatic contents and the saprotrophic CO_2 production in litter and SOM, e.g. hydrolysable tannins may represent more easily degradable carbon yielding a lower temperature response (Arrhenius, 1889). The 2D HSQC analysis did not provide any additional information on tannins, but it revealed several other striking differences that may explain the differences in effects of the aromatic content on the saprotrophic Q_{10} responses of litter and SOM. Litter samples with a low Q_{10} response (spruce needles) had the most complex spectra in the aromatic- and O-aromatic region (Figure 17c). In contrast, the Q_{10} responses of the litter samples with the highest Q_{10} responses (*D. flexuosa* and *R. ideaus*) were similar to those of SOM. Their spectra in the aromatic and O-aromatic region were also the least complex of all litter samples (Figure 17d), but very similar to SOM spectra in this region (Figure 17a and b). Thus our data from aromatic- and O-aromatic regions of the 2D HSQC spectra suggest a possible explanation, that the complexity in the guaiacyl region explains most of the variability in the Q_{10} response, while the bulk aromatic C has substantially less impact.

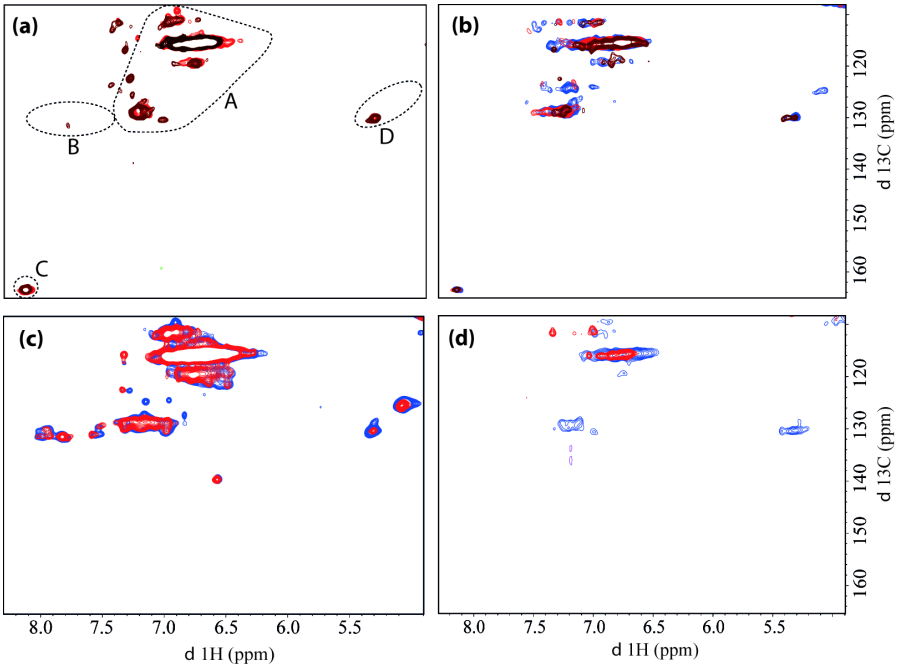

Figure 17. Solution-state 2D NMR (^{1}H-^{13}C HSQC) data for the aromatic region (δ ^{1}H ppm 8.3-4.9; δ ^{13}C ppm 166-108 ppm) illustrating differences in this region between SOM (a and b) and litter (c and d) and between litters giving low (c) and high (d) Q_{10} responses, respectively. a) Soil organic matter (SOM) from a spruce-dominated forest (site 4: red shallow, brown deep). The chemical origins of the spectral signals in specific areas are indicated by capital letters: A – largely from guaiacyl and parahydroxybenzoate units of lignin; B - Benzoic acid or structurally similar aromatic compounds; C - Putatively assigned to formate; D - Unsaturated fatty acids (Mansfield *et al.*, 2012). b) SOM from a pine-dominated forest (blue; site 5, shallow) and from mixed pine and spruce forests (red, site 1, shallow; purple site 2, deep). c) Two litters with the lowest basal respiration Q_{10} (Blue, spruce needles, site 3; red, spruce needles, site 4). d) Two litters with the highest basal respiration Q_{10} (blue, wavy hair grass, *Deschampsia flexuosa* (L.), site 7; red, raspberry, *Rubus ideaus* (L.), site 4).

The positive correlations between Q_{10} values for BR and both O-alkyl and di O-alkyl contents in litter (Fig. 15c,d) were opposite to expectations based on kinetic theory from the general understanding that these C forms represent easily degradable sources. Polymeric carbohydrates, mostly cellulose and hemicellulose, are the main constituents of the O-alkyl region and previous work has shown that the relative contribution of O-alkyl decreases as fresh litter is decomposed (Zimmermann *et al.*, 2012; Kögel-Knabner, 2000; Preston *et al.*, 2000; Preston *et al.*, 1997). The average O-alkyl contents in litter and SOM were ca. 49% and 37%, respectively, thus the O- and di-O-alkyl carbon contents clearly decreased during the litter degradation. Nevertheless, O- and di-O alkyl are clearly still the dominating C fractions in old “decomposed”

SOM, indicating that a significant proportion of the carbohydrate carbons constitute a rather low quality carbon pool. This would also explain an increasing Q_{10} response with increasing O- and di-O-alkyl carbon contents, as found in litter. The significant interaction between O- and di-O-alkyl effects on temperature sensitivity, revealed by the PLS analysis (Figure S7a, Supplementary Information Paper II) also indicates that these carbon forms may be derived from the same compounds. The temperature sensitivity of the BR of SOM material decreased with increasing carbonyl content. Increasing contents of carbonyl and carboxyl groups are commonly interpreted as indicating increasing decomposition (Dijkstra *et al.*, 1998; Preston *et al.*, 2002). If so, the increased temperature sensitivity with increasing carbonyl content is opposite to expectations. However, carbonyl groups can be associated with a vast array of organic compounds that cannot be further identified by CP-MAS NMR methodology (Kögel-Knabner, 2002; Baldock *et al.*, 1990b).

For both SOM and litter the addition of a readily available carbon source removed any correlation between the temperature response and OM composition. The Q_{10} values of both SIR and μ from SOM were also lower than for the BR of the same samples. Thus, as a result of inherent substrate conditions imposed by OM composition, the easily available carbon source completely took over as a substrate-related determinant of temperature sensitivity of degradation. From this we conclude that the relationships observed between the temperature response of BR and the OM composition are related to the inherent substrate signatures of the samples. This is consistent with kinetic theory (Davidson & Janssens, 2006; Bosatta & Ågren, 1999).

Arrhenius-based theory has long been used to predict the temperature sensitivity of OM decomposition from its organic chemical composition. However, despite these theoretical predictions, there have been no empirical studies of the relationship between the temperature sensitivity of decomposition and the chemical constituents of SOM and fresh litter. Here, we used CP-MAS and 2D HSQC NMR spectroscopy of SOM and litter from typical boreal forests to characterize their organic chemical composition. We then used the NMR-derived organic chemical composition to account for the variation in temperature sensitivity of CO_2 production rates measured in laboratory incubations of litter and SOM. Most (>90%) of the variation in temperature sensitivity among the litter samples could be explained by variations in the organic chemical composition, particularly variations in alkyl-, O-alkyl, aromatic- and O-aromatic carbon contents, according to R^2-values derived from the linear regression analysis. The variation in temperature sensitivity among the SOM samples was mainly related to the content of

carbonyl carbon and the two-way interactions of O-, and di-O-alkyl and O-alkyl and carbonyl carbons, which explained 70% of the variation.

To our knowledge, this is the first study to clearly connect variation in specific carbon forms of litter and SOM to variation in the temperature sensitivity of saprotrophic CO_2 production. These findings will form a basis for mechanistic modelling of variation in temperature sensitivity of OM decomposition based on its organic chemical composition.

3.3 Effect of nitrogen on temperature response of decomposition of soil and litter (Paper III)

In paper III we investigated effects of nitrogen on the temperature response of SOM decomposition. Nitrogen strongly affects decomposition in several ways (Janssens *et al.*, 2010; Berg, 2000; Berg & Matzner, 1997; Fog, 1988). It is an essential nutrient for microorganisms, but it also significantly affects the organic chemical composition of plant litter and thus influences of other environmental factors on degradation of the soil C pool. Generally, increases in nitrogen concentration result in faster initial mass losses of organic C, while proportions of more recalcitrant C-compounds increase (Berg, 2000; Melillo *et al.*, 1982). Despite this quite well-developed understanding of the effects of temperature and nitrogen on OM decomposition, very little is known about their interactive effects on the temperature sensitivity of OM degradation.

Therefore, we used a long-term nitrogen fertilization experiment in a pine forest stand in the boreal region to examine the effect of nitrogen availability on the temperature sensitivity of OM degradation. Samples of newly shed litter of both pine and *Vaccinium myrtillus*, and the organic soil layer (O-horizon), were collected from the field and incubated at selected temperatures under laboratory conditions.

Nitrogen concentrations in litter and soil

Results from this study showed that the nitrogen concentration was significantly higher in both litter and soil from plots under all N-treatments compared to controls (N0) (Figure 17). The N contents of pine needles rose extremely significantly (ANOVA, r^2=0.91, p=0.000) from 0.38±0.01% in control (NO) to 0.62±0.03% and 0.44±0.01% in N2 and N3 samples, respectively. The N contents in samples of green bilberry leaves also rose significantly (ANOVA, r^2=0.68, p=0.022) from 1.2±0.08% in control to 1.64±0.07% and 1.37±0.16% in N3 and N2 samples, respectively. Similarly,

the N contents in the 0-3 and 4-7 cm soil layers rose extremely significantly (ANOVA: r^2=0.96, p=0.000 and r^2=0.94, p=0.000, respectively) from 1.33±0.03% and 1.12±0.17% in NO samples to 1.99±0.08% and 1.78%±0.09% in N2 samples and to 1.77±0.08% and 1.78±0.02% in N3 samples, respectively.

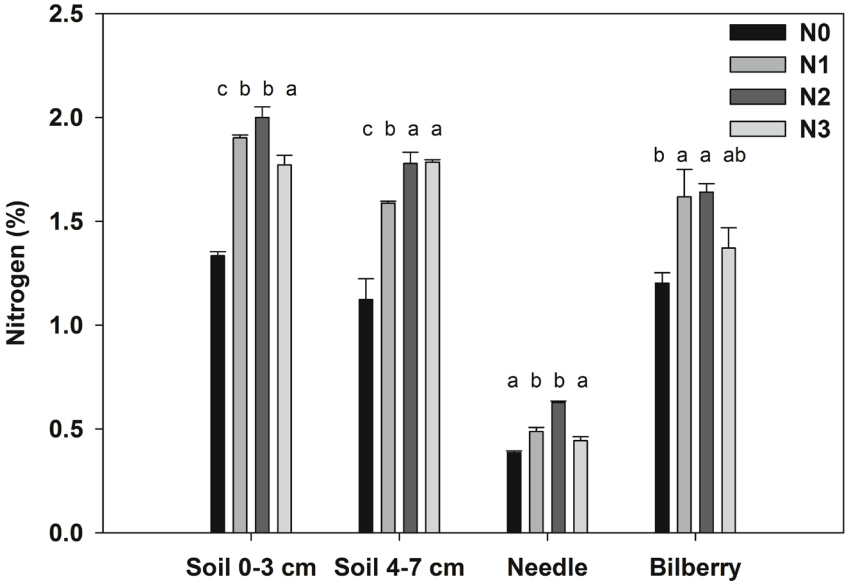

Figure 18. Average nitrogen (%) contents of 0-3 and 4-7 cm soil layers, pine needles and bilberry leaves. The error bars represent standard errors. Different letters indicate significantly different nitrogen contents.

Temperature responses of soil and litter

The temperature sensitivity (Q_{10}) of CO_2 production from litter was not significantly different (Q_{10}±SE) between bilberry leaves (1.9±0.17) and pine needles (2.3±0.40) and was also unaffected by nitrogen additions (Figure 18). In contrast, the temperature sensitivity of SOM decomposition was highly sensitive to nitrogen content, as shown in Figure 18. The Q_{10} of CO_2 production from the superficial layer (0-3 cm) decreased significantly ($p<0.05$) from 2.5±0.35 for N0 samples to 1.9±0.18 for N2 samples. The Q_{10} of CO_2 production from the 4-7 cm layer also significantly decreased from 2.2±0.19 for N0 samples to 1.6±0.15 in response to the highest N addition level. The response of Q_{10} to the lowest N addition (N1) was intermediate, although not significant, between the control and N2-level responses.

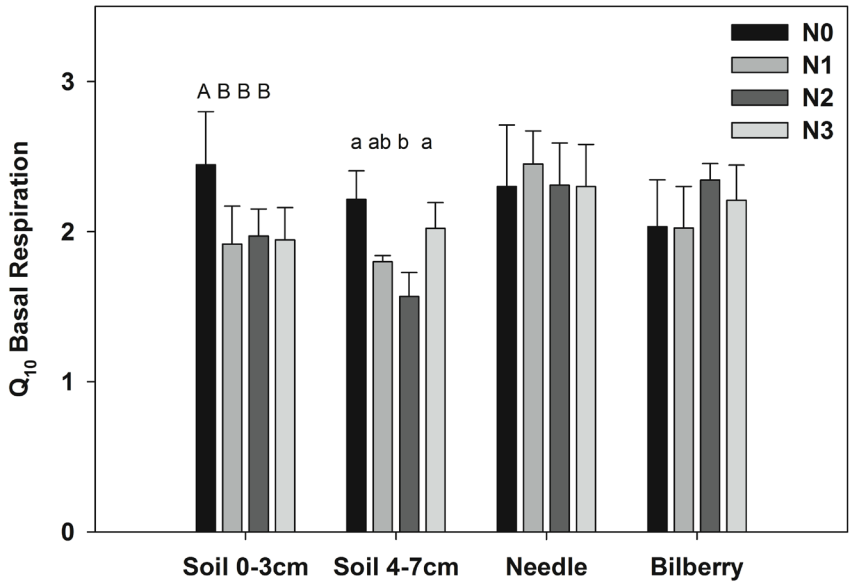

Figure 19. Average (n=3) Q_{10} values of CO_2 production from degradation of soil and litter materials representing three N-treatments, and controls (N0). Different letters indicate significantly different between-N treatment Q_{10} responses. Nitrogen addition significantly reduced the temperature sensitivity of saprotrophic CO_2 production from soil organic matter, but had no significant effect on saprotrophic CO_2 production from litter degradation.

Nitrogen addition had a much stronger effect on the temperature sensitivity of OM degradation in the 0-3 cm layer than in the 4-7 cm layer (Figure 19). However, the pattern of responses was the same for both layers; nitrogen had no effect on decomposition at the lowest temperature (+4^{o}C) but increasing effect with increasing temperature (Figure 20). At 19^{o}C the CO_2 production from the samples representing the treatment with highest N addition was reduced by 42% in the 0-3 cm layer and 50% in the 4-7 cm layer samples.

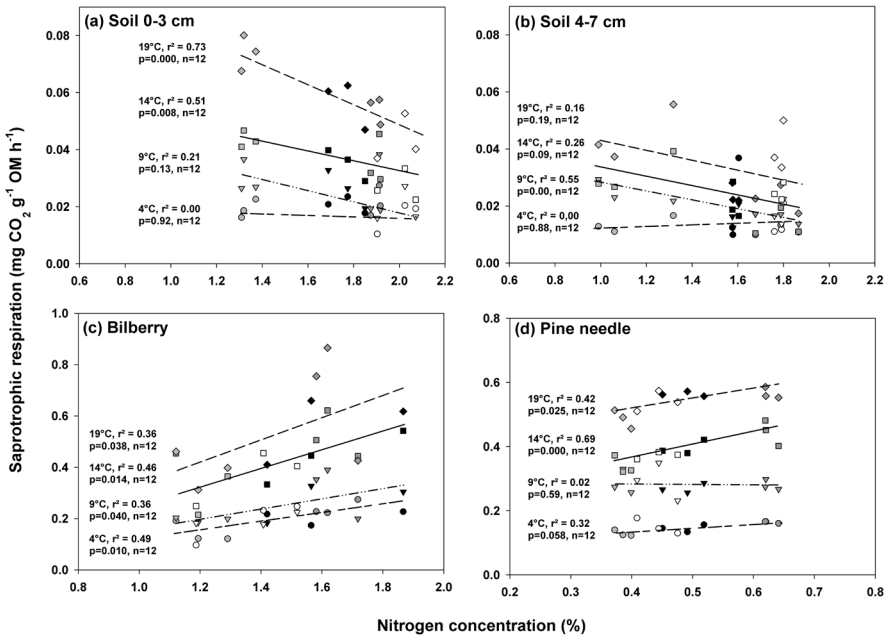

Figure 20. Saprotrophic CO_2 production from SOM and litter samples representing each of the field nitrogen addition treatments: a) soil, 0-3cm layer; b) soil, 4-7cm layer; c) pine needles; d) bilberry (*Vaccinium myrtillus*) leaves. Saprotrophic CO_2 production from the litter incubations increased in response to both temperature and nitrogen additions and temperature had the same effect on litter samples representing all nitrogen treatments. Increased temperature also increased the CO_2 production from the soil layers, but very weakly in incubations of the 0-3 cm soil layer (from a Q_{10} of 2.4±0.35 for N0 samples to 2.0±0.25 for all other N treatment samples). A similar effect was detected in incubations of the 4-7 cm soil layer, temperature sensitivity (Q_{10}) declined from 2.2±0.19 for N0 samples to 1.6±0.16 for N2 samples.

In addition to the effect of N on temperature sensitivity of OM degradation our results also very clearly reveal that increasing N-contents affect saprotrophic CO_2 production from litter and SOM very differently (Figure 19). While CO_2 production from litter samples increased significantly with increasing N content at practically all tested temperatures, CO_2 production from SOM decreased with increasing N content, more weakly as temperature decreased. The highly significant decrease in the negative effect of nitrogen on saprotrophic CO_2 production with decreasing temperature might also importantly help to explain much of the controversy in the scientific literature on the effects of nitrogen on OM decomposition (Janssens *et al.*, 2010). Our results clearly reveal that the negative effect of nitrogen on saprotrophic CO_2 production from SOM decreased as temperature declined, to non-existence at +4°C (Figure 20).

Addition of nitrogen changed the organic chemical composition of both soil layers and bilberry leaves, but not the pine needles (Figure 3a-d, Paper III). According to PLS regression analysis, the changes in organic composition were quantitatively related to the N content in the soil samples, but not in the litter samples (Table 1, Paper III). Generally amounts of alkyl carbons decreased in response to N additions while amounts of aromatic and carbonyl carbons increased. The organic chemical composition and N concentration explained equal amounts of variability in Q_{10} of the 0-3 cm layer, while the organic chemical composition of the 4-7 cm soil layer explained 65% ($p<0.05$) of the variance compared to only 36% ($p<0.05$) when using only the N concentrations. The PLS regression analysis revealed that the Q_{10} value decreased in response to increases in methoxy-, aromatic-, O-aromatic- and carbonyl carbon contents. These results are consistent with results from a survey of a wide range of soil and litter samples, which also showed that increasing concentrations of aromatic-, O-aromatic- and carbonyl carbon contents decreased temperature sensitivity (Erhagen *et al.*, 2013).

The effects of nitrogen on the temperature sensitivity of saprotrophic respiration demonstrated in this study indicate the existence of strong negative feedback mechanisms acting on the global carbon cycle, which may ameliorate increases of CO_2 concentrations in the atmosphere, as follows. Increases in nitrogen inputs to the terrestrial biosphere, either from fertilization or atmospheric deposition, may lead to increased production of biomass and hence higher litter production, removing CO_2 from the atmosphere. The effect of a global temperature increase on saprotrophic CO_2 production from this plant litter will consequently decline in response to the N-induced changes in litter carbon chemistry. Thus, increased nitrogen availability in the biosphere may not only enhance CO_2 removal from the atmosphere, but also substantially reduce the OM decomposition-increasing effect of air temperature increases via the increased nitrogen content in SOM

3.4 The effect of temperature and substrate quality on the carbon use efficiency of saprotrophic decomposition (Paper (IV)

The aim of paper IV was to investigate how CUE of saprotrophic microorganisms is affected by temperature and by the complexity of the organic substrate. It aims to determine whether CUE varies during the different metabolic phases, induced by the addition of a carbon substrate together with required amounts of nitrogen and phosphorus.

Our results show that CUE depends on substrate quality (Frey *et al.*, 2013; Manzoni *et al.*, 2012) and was lower for a carbon polymer than for a carbon monomer (Figure 21). This finding is consistent with the results of previous studies using monomers of different quality (Frey *et al.*, 2013). However, since polymeric substrates have to be degraded using extracellular enzymes before they can be taken up, they present a challenge to saprotrophic microorganisms that is not encountered when using monomers (Manzoni *et al.,* 2012). The average CUE for the monomeric substrate (glucose) used was around 0.70 (SE±0.02), which is relatively high but still consistent with results obtained in previous studies; CUE values ranging from 0.40 to 0.80 have been reported for soil microorganisms utilizing glucose (Frey *et al.*, 2013; Dijkstra *et al.*, 2011a; Thiet *et al.*, 2006; Shields *et al.*, 1973). However, CUE values ranging from 0.50 to 0.90 were observed at different temperatures and different metabolic conditions. It is therefore possible that much of the variation in data in the literature regarding CUE may be due to investigators having conducted measurements at time points when the microbial population is at different metabolic stages, for example due to changing substrate availability.

To our knowledge, this is the first reported CUE value for soil microorganisms growing on a polymeric substrate. The comparatively low CUE (0.50 SE±0.03) for the polymer probably reflects a requirement for the production of extracellular enzymes to enable its decomposition and mineralization (Bradford & Crowther, 2013; Manzoni *et al.*, 2012), and further the greater number of enzymatic steps involved in its degradation compared to glucose (Bosatta & Ågren, 1999). It is likely that CUE values determined by using polymeric substrates in incubation experiments will be more directly relevant to the real-world decomposition of soil OM than those obtained using monomeric substrates specifically because the carbon sources available in situ tend to be polymeric. Indeed, it is possible that models of soil OM decomposition based on studies that use monomers alone may, in fact, overestimate CUE values (Manzoni *et al.,* 2012). A possibility compatible with our findings. Another substrate quality-related factor that affects CUE is the metabolization of different monomers via different metabolic pathways, these therefore yield different respiration rates per unit carbon assimilated (Manzoni *et al.*, 2012; van Hees *et al.*, 2005; Gommers *et al.*, 1988; Gottschalk, 1986). However, in this study, the compounds taken up by microorganisms would have been either monomeric glucose or cellobiose produced by the enzymatic cleavage of cellulose. It is therefore likely that the metabolic pathways used to degrade the monomer would have been very similar to those for the polymer (Gottschalk, 1986).

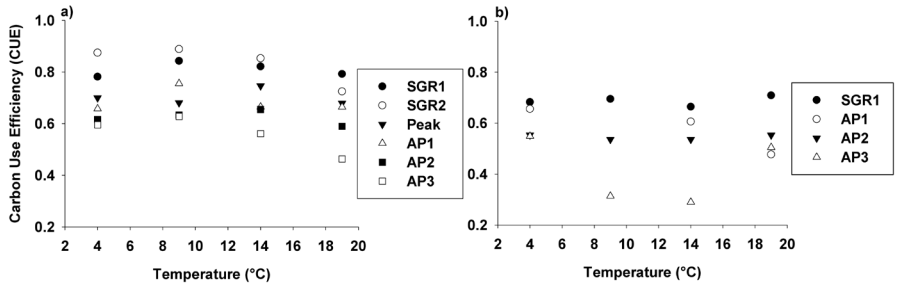

Figure 21. CUE for the different metabolic phases during the decomposition of the labeled substrates ^{13}C-glucose (a) and ^{13}C-cellulose (b) at each of the four incubation temperatures. The different symbols represent samples taken during different metabolic phases during the incubation period.

A second objective in this investigation was to determine how CUE varies between metabolic phase. We found that different metabolic phases were associated with significantly different CUE values for both monomeric and polymeric substrates. In general, CUE values observed before peak CO_2 production were significantly higher than those after the peak (Figure 21). This highlights the importance of substrate availability and stoichiometric conditions and should be considered when estimating CUE values.

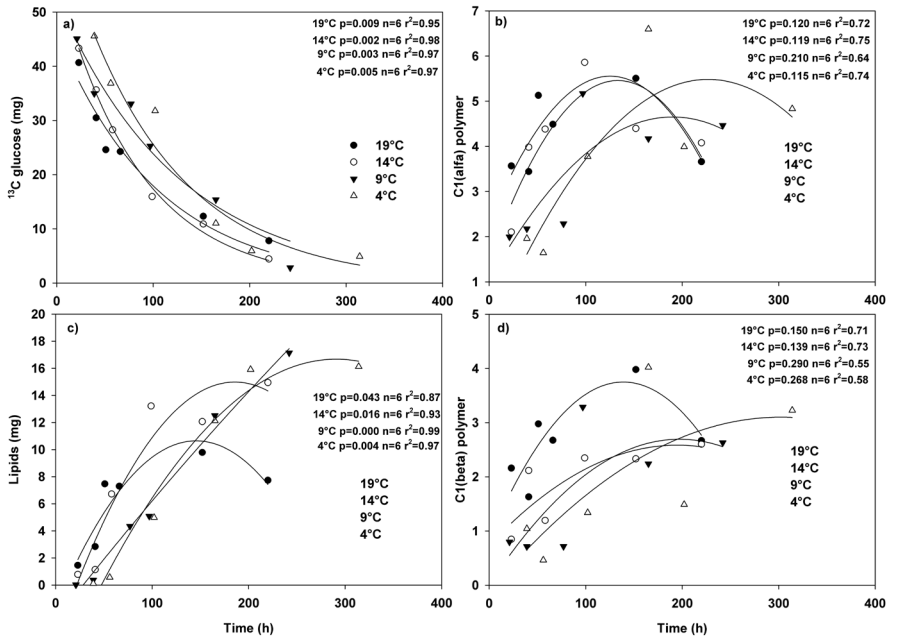

Figure 22. Microbial consumption and production of ^{13}C-labeled carbon compounds over time following the addition of ^{13}C-glucose. The measured concentrations of ^{13}C-glucose (panel a) were fitted to an exponential function while those of ^{13}C-labelled compounds were fitted to a second order polynomial function (panels b, c, and d). The sub-figures show the changes in the concentrations of the following compounds over the course of the incubations and the corresponding fitted curves: ^{13}C-glucose (a), ^{13}C1 (alpha) carbohydrate polymers such as starch and glucogen (b), ^{13}C-labeled lipids (c), and C1 (beta) ^{13}C-carbohydrate polymers such as cellulose and chitin (d).

The utilization of the carbon monomer decayed exponentially towards the end of the experiment, indicating that the rate of respiration had become limited by substrate availability (Figure 22a). Interestingly, substrate availability was limiting under these conditions even though the glucose concentration in the soil solution was still relatively high, at around 7mM. This may reflect a limitation arising from the constraints on substrate transport processes, which might explain why CUE was lower during the later stages of the incubations. In contrast, the rate of utilization for the polymer decreased more linearly over time (Figure 23a), suggesting that substrate limitation was less prominent during the later stages of the incubations, as compared to the samples amended with glucose.

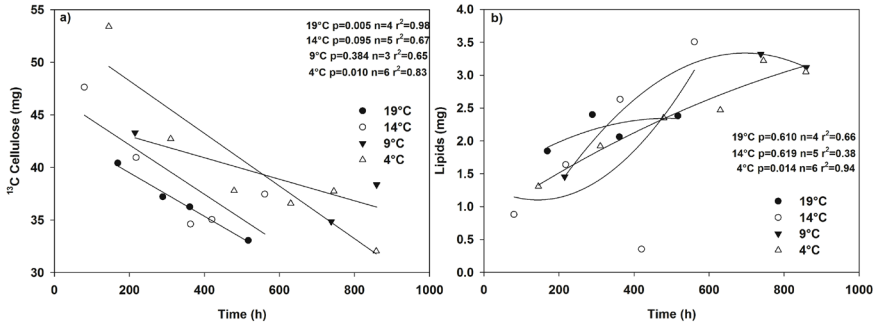

Figure 23. Microbial consumption and production of ^{13}C-labeled carbon compounds following the addition of ^{13}C-cellulose. The measured concentrations of ^{13}C-cellulose (panel a) were fitted to a linear function while the concentrations of ^{13}C-labelled lipids (panel b) were fitted to a second order polynomial function. No regression analysis is presented for the 9 °C results in panel b because of the limitations on the degrees of freedom available to a second order polynomial function.

Changes in temperature had no statistically significant effects on CUE for either substrate (Figure 21). This was not consistent with results obtained in earlier studies (Farmer & Jones, 1976; Mainzer & Hempfling, 1976) nor with the findings of more recent studies (Frey *et al.*, 2013; Steinweg *et al.*, 2008;

Hall & Cotner, 2007). Steinweg *et al.,* (2008) observed that CUE for the decomposition of cellobiose decreased when temperature increased from 15 to 25°C. However, Frey *et al.,* (2013) observed a decrease in CUE for glutamic acid and phenol as temperature increased from 5 to 25°C. Nevertheless, some results consistent with those obtained here have also been presented. For example, Frey *et al.,* (2013) found that CUE values for soil samples amended with glucose or oxalic acid did not change with temperature. In addition, Drotz *et al.,* (2010a) reported that CUE of glucose exhibited no temperature dependence at temperatures of +9, +4 and -4^{o}C.

The use of ^{13}C MAS NMR spectroscopy in this investigation enabled us to quantify the amount of ^{13}C labeled carbon that had been used for anabolic activity and to determine which compounds had been synthesized. The allocation of labeled carbon was relatively temperature independent aside from the longer time constants observed for lower temperatures. The compounds that are most strongly associated with microbial growth are lipids. During the incubation experiments, the concentration of ^{13}C-labeled lipids initially increased rapidly but then leveled off in a way that could be described using a second order polynomial (Figure 22). In addition, quite a lot of the labeled substrate taken up by the microbes was used to produce polymeric carbohydrates. These compounds were not subsequently broken down and converted into CO_2; it is possible that their synthesis was due to the formation of new microbial cells or the production of storage compounds such as glucogen (Lundberg *et al.*, 2001). These storage compounds might subsequently be used for respiration if other nutrients were available, allowing for the continued synthesis of new microbial biomass (Lundberg *et al.,* 2001). This suggestion is consistent with the gradual decline in the abundance of these compounds towards the end of the incubation experiments. The potential for these storage compounds to be used in catabolic reactions at some later stage after they have been synthesized may introduce bias into estimated CUE values, and might be responsible for some of the differences in calculated CUEs for different metabolic phases. The production of C1(β) polymeric carbohydrates (Figure 22d) also increased rapidly during the early stages of the incubations at all temperatures but later leveled off. The production of ^{12}C-CO_2 increased after the addition of substrates due to the regeneration of cytoplasmic carbon compounds in the microorganisms (Ekblad & Hogberg, 2000). The relative contribution of ^{12}C-CO_2 to total CO_2 output of the soil microorganisms was higher when using the polymeric substrate than in the experiments using glucose.

These results allow us to draw three main conclusions regarding the CUE of soil microbes. The first relates to the quality of the carbon substrate: CUE for

a carbon polymer was found to be lower than that for the corresponding monomeric constituent. This finding emphasizes the importance of using polymeric substrates rather than exclusively focusing on monomers when studying CUE. The second is that the time constant applied for sampling significantly affects the measured CUE values and should therefore be recorded when conducting studies of this sort. A recommendation for further studies investigating CUE by substrate addition is to determine CUE after a fixed amount of the added substrate has been consumed. The third conclusion is that CUE was not dependent on temperature when using either the monomeric or the polymeric substrate.

4 Conclusions

The main objectives of the work this thesis is based upon are listed below with the main findings.

1. To separate the effects of temperature on decomposition of organic material into its effects on key processes, i.e. substrate release, substrate diffusion and substrate uptake into microorganisms.
   - *Increases in both substrate availability and substrate uptake rates result in increased temperature sensitivity (Paper I).*

2. To relate OM chemistry (quality) to the temperature sensitivity of OM decomposition.
   - *The temperature response of litter and SOM decomposition can be explained by the chemical composition of the OM (Paper II).*

3. To investigate effects of nitrogen (N) on the temperature response of decomposition of organic material.
   - *Increased nitrogen content in the organic material decreases the temperature sensitivity of SOM decomposition. The effect of increased nitrogen is most likely through changed organic matter composition. However, the nitrogen content does not affect the temperature sensitivity of litter decomposition.*

4. To determine temperature sensitivity on catabolic and anabolic processes, and thus carbon use efficiency.
   - *The CUE was not dependent on the temperature when using either the monomeric or the polymeric substrate.*
   - *The CUE for a carbon polymer was found to be lower than that for a monomeric substrate. This finding emphasizes the importance of using*

polymeric substrates rather than exclusively focusing on monomers when studying CUE.

- *The CUE was highly dependent on metabolic conditions.*

4.1 Further research

The CP MAS-NMR analyses reported in Papers II and III, together with findings in the literature, indicate that chemical contents of highly degraded SOM from the O-horizon include around 40-50% of polymeric carbohydrates. In the literature carbohydrates are regarded as fairly readily decomposable, but they still constitute a major fraction of the carbon stored in the soil, posing two questions. What (if any) are the mechanisms that preserve polymeric carbohydrates in soil, or are they more chemical recalcitrant than we believe? Answering these questions would improve both our understanding of the processes related to carbon sequestration and predictions of how carbon in the soil will be affected by climate changes.

The results presented in Paper III indicate that nitrogen may strongly influence feedback on climate change mediated by saprotrophic decomposition of the global soil carbon pool. Therefore, further investigation is warranted on effects of nitrogen (N) on the temperature responses of decomposition of organic material from other types of sites in boreal forests, e.g. spruce-dominated and mixed spruce-pine stands. Identification of the mechanisms linking increases in nitrogen content, changes in organic chemical composition of OM and the temperature sensitivity of OM decomposition would also be valuable

Regarding the CUE there are many interesting aspects to further examine. In paper IV we measured the CUE of only two substrates: glucose and cellulose. It would be interesting to extend the analysis to a wide range of substrates with differing lability. Investigation of the CUE's dependence on nutrient and carbon stoichiometry, e.g. under nitrogen and/or phosphorous nutrient limitations, and the fate of storage compounds synthesized in later stages (e.g. if they are respired or used to synthesize microbial biomass) could also be highly informative.

5 References

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Acknowledgements

Först skulle jag vilja börja med att tacka alla min handledare (Matsarna, Ulrik, Jürgen och Tobias), utan er hade denna avhandling aldrig blivit av! Mats (Pjodd) tack för att du för många år sen gav mig förtroendet att börja denna tjänst. Att jobba med dig har varit väldigt lärorikt och väldigt kul. Man har under åren lärt känna dig rätt bra och lärt sig vilka knappar man ska tycka på om man vill få igång dig (Administration, svenska läkarkåren, revewiers, länsstyrelsen Umeå ☺). Mats tack för all hjälp med manus och ditt enorma lugn har varit till stor hjälp framförallt i slutfasen av avhandlingsarbetet. Tobias, tack för allt stöd, hjälp och din enorma kunskap du delar med dig, utan dig hade jag nog kroknat halvvägs. Jürgen tack för all din kunskap inom NMR och dina mycket värdefulla kommentarer på manuskript, samt för din alltid så upplyftande och roliga humor. Ulrik tack för all hjälp med manus och statistik. Ett stort tack även till Respicondens fader Anders Nordgren för att du introducerade mig på bästa sätt i Respicondens värld. För att du väckte nu lust att lösa problem. En annan person som jag vill tack är Sören Holm, tack Sören för ditt stora tålamod och din vilja att hjälpa.

Att vara doktorand är inte alltid helt smärtfritt utan humöret går lite som en bergodalbana och för mig har en ständig källa till inspiration och glädje varit de dagliga fika och luncher. De som har gjort dessa tillfällen så givande och roliga är alla anställda på skogens ekologi och skötsel, tack alla! Ett särskilt tack till alla doktorandkollegor. Tack Daniel, Petter och Martin för all roliga stunder.

Sen skulle jag också vilja tack Ida Taberman och Mahsa Haei. Ida tack för all dokumenthantering och taxikörnade. Mahsa Haei tack för all hjälp med data, manus och jobbiga handledare☺.

Tack också till Jenny Vinsa som har introducerat mig i hundar och hästarnas värld, tack även till Platen, Rajiah och fantastiska Tindar, ni har verkligen hjälpt mig att må bättre.

Sist men inte mist vill jag tack min Lucy. Tack Lucy för allt ditt stöd, kärlek och värme. Tack för din inspiration att vilja bli en bättre människa och inte gömma huvudet i sanden☺.