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Carbon dynamics in terrestrial ecosystems

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Carbon dynamics in terrestrial ecosystems

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Abbreviations

μCi	Microcurie
μL	Microlitre
ACIA	Arctic climate impact assessment
ANOVA	Analysis of variance
AMAP	Arctic monitoring assessment programme
amoA	Ammonia monooxygenase gene
AOA	Ammonia oxidising archaea
AOB	Ammonia oxidising bacteria
C	Carbon
CH_4	Methane
cm	Centimetre(s)
CO_2	Carbon dioxide
d	Day(s)
DOC	Dissolved organic carbon
DOM	Dissolved organic matter
DON	Dissolved organic nitrogen
DNA	Deoxyribonucleic acid
EC	Electrical conductivity
EGM-4	Environmental gas monitor
FAO	Food and agriculture organisation
g	Gravitational acceleration
GLM	General linear model
Gt	Gigatonnes (10^9 tonnes)
h	Hour(s)
h^{-1}	Per hour
ha	Hectare(s)
HCl	Hydrochloric acid
HPM	Hurley pasture model
HMW	High molecular weight
HSD	Honest significant differences
IRGA	Infra-red gas analyser
IPCC	International Panel on Climate Change
kBq	Kilobecquerel
km	Kilometre(s)
kyr	Kiloyear (1000 years)
L	Litre(s)
LMW	Low molecular weight

LSD	Least significant differences
M	Molar
m	Metre(s)
MANOVA	Multivariate analysis of variance
mg	Milligram(s)
ml	Millilitre(s)
mM	Millimolar
mm	Millimetre(s)
MRT	Mean residence time
N	Nitrogen
NaOH	Sodium Hydroxide
$NaHCO_3$	Sodium bicarbonate
NH_4^+	Ammonium
NO_3^-	Nitrate
nM	Nanomole
nm	Nanometre(s)
O_2	Oxygen
P	Phosphorous
PCA	Principal component analysis
PCR	Polymerase chain reaction
Pg	Petagram (10^{15} grams)
PLFA	Phospholipid fatty acids
ppm	Parts per million
qPCR	Real-time quantitative polymerase chain reaction
RNA	Ribonucleic acid
rRNA	Ribosomal ribonucleic acid
s	Second(s)
SEM	Standard error of the mean
SIP	Stable isotope probing
SOC	Soil organic carbon
SOM	Soil organic matter
TDN	Total dissolved nitrogen
TFAA	Total free amino acids
TPTZ	6-tri pyridyl-*s*-triazine
TXRF	Total reflection x-ray fluorescence
UV	Ultraviolet
v:v	Volume to volume
WP4	Water potential meter
w/v	Weight to volume
y	Year
yr^{-1}	Per year

Abstract

The objective of this thesis was to better understand the mechanistic control of carbon (C) cycling in two terrestrial ecosystems (agricultural grasslands and Arctic tundra), with an aim to identify the contribution of microbial respiration to below-ground C cycling. Firstly, I evaluated different techniques for measuring CO_2 evolution from soil. I found that different *in-situ* chamber-based CO_2 gas analyzers gave comparable results across contrasting ecosystems. However, the addition of collars to the CO_2 chamber induces variable flux estimates due to the disturbance created upon collar insertion, severing root and mycorrhizal networks. In subsequent studies, I showed that microbial breakdown of individual dissolved organic C (DOC) components demonstrated good reproducibility when performed under either *in-situ* and *ex-situ* conditions. After validating the experimental techniques, they were then used to study C turnover in two plant-soil systems. In Arctic tundra, soil temperature was identified as the key driver initiating microbial and vegetation response to snow melt, thereby driving early season CO_2 efflux. However, as the growing season progressed, soil water content was hypothesized to become a more important regulator of C turnover with older C compounds becoming more susceptible to decomposition as soil water content increases. In a grassland soil I found that soil microbial community composition does not correlate with increased rates of mineralization across a wide pH gradient. This suggests that abiotic drivers of respiration may directly influence microbial metabolic processes independent of community structure. Further research involving advanced molecular techniques (metabolomics, proteomics, transcriptomics) will help disseminate how metabolic processes are being influenced by different respiration drivers. The application of mathematical models to respiration data provides a more quantitative and mechanistic understanding of processes involved in soil C cycling. I found the fitting of exponential models to respiration data is a reliable proxy for describing substrate mineralization; however, the correct choice of model is critically dependent on the number of measurement points and length of experiment. The modelling approach was subsequently used to quantify the turnover of functional microbial C pools. By combining modelling with experimental measures of soil solution C concentration, we estimated that the microbial contribution to total soil respiration is ca. 18%. This research provides a more detailed understanding of how C constituents are processed by the microbial decomposer community to drive soil respiration. This is crucial to accurately model global terrestrial C fluxes in different ecosystems and to predict how these fluxes are likely to respond to future changes from both natural (e.g. climate change) and anthropogenic (e.g. land-use change) sources.

Contents

List of chapters

This thesis is based on the following papers and manuscripts, which are subsequently referred to in the text by their respective Roman numerals.

I. Mills, R.,* **Glanville, H.**,* McGovern, S., Emmett, B., Jones, D.L. 2011. Soil respiration across three contrasting ecosystem types: comparison of two portable IRGA systems. ***Journal of Plant nutrition and Soil Science*** **174, 532-535**

* Authors contributed equally

II. **Glanville, H.**, Rousk, J., Golyshin, P., Jones, D.L. 2012. Mineralization of low molecular weight carbon substrates in soil solution under laboratory and field conditions.
Soil Biology and Biochemistry **48, 88-95.**

III. **Glanville, H.C.**, Hill, P.W., Maccarone, L.D., Golyshin, P., Murphy, D.V., Jones, D.L. 2012. Temperature and water regulation of soil carbon, nitrogen, vegetation and microbial dynamics during snow melt in a high Arctic tundra ecosystem.
Functional Ecology **(doi: 10.1111/j.1365-2435.2012.02056.x).**

IV. Rousk, J., Brookes, P.C., **Glanville, H.C.**, Jones, D.L. 2011. Turnover of low molecular weight dissolved organic carbon does not correlate with differences in microbial community composition or growth across a soil pH gradient.
Applied and Environmental Microbiology **77, 2791-2795.**

V. **Glanville, H.C.**, Hill, P.W., Golyshin, P., Jones, D.L. 2012. Methods to describe carbon turnover in a UK grassland soil.
In preparation for submission to Soil Biology & Biochemistry.

1. Objectives and outline of this thesis

Although soils contain almost twice as much stored C as in the atmosphere, the sensitivity of key nutrient cycling processes to climate change remains largely unknown. This is because of the multitude of complex interactions between the plant-soil-microbial interfaces, which often occur simultaneously, making it inherently difficult to discriminate between individual processes affecting nutrient cycling within the terrestrial system.

This thesis focuses on the relative contribution of different components of DOC on overall soil respiration and how sensitive this process is to changing climatic variables. This thesis should provide an overview on temporal (yearly variation) and spatial (profile to ecosystem level) effects on total soil respiration, as well as looking at potential climatic feedbacks which may be important for validating global climate models.

The overall thesis objective can be broadly summarised under the following point:

- **Identification of mechanisms influencing the mineralization rates of individual DOC components and how these contribute to overall soil respiration.**

This main objective links all the individual chapters together and can be split into further sub-sections dealing with specific mechanisms and controls which can influence soil CO_2 flux.

I. Methodological influences on soil respiration:

- ***To investigate whether using different commercially available, in-situ, CO_2 gas analyzers can reliably be compared.***

Two commonly used and commercially available portable IRGA-based CO_2 analyzers were directly compared across three contrasting ecosystems (lowland grassland, upland grassland and an upland heathland); results are presented in Chapter I.

- ***To examine whether the inclusion/absence of collars on CO_2 gas analyzers in-situ, adversely affect respiration measurements.***

In conjunction to directly comparing different CO_2 gas analyzers, the influence of collars on CO_2 measurements was also investigated across the same different ecosystems. Results of this study are also presented in Chapter I.

- ***To investigate how the mineralization rates of individual DOC components compare under field (in-situ) and laboratory (ex-situ) conditions.***

A broad range of 31 ^{14}C-labelled low MW C substrates were added to agricultural grassland soil both *in-situ* and *ex-situ*, to directly compare the effect on individual substrate turnover rates. Results are presented in Chapter II.

II. Climatological influences on soil respiration:

- ***To investigate the effect of temporal variability on mineralization rates of individual DOC components in-situ.***

In addition to assessing the effect of *in-situ* and *ex-situ* conditions on mineralization rates, the same 31 ^{14}C-labelled substrates were monitored inter-annually to investigate whether more variation is seen across different years compared with *in-situ/ex-situ* conditions. Results are presented in Chapter II.

- ***To investigate the response of vegetation and below-ground communities to soil thaw along a natural snow melt gradient, to assess the potential feedbacks to climate change.***

A detailed field study was conducted in the high Arctic tundra monitoring vegetation emergence, nutrient fluxes and below-ground microbial dynamics post snow melt. Results are presented in Chapter III.

III. Pedological and environmental influences on soil respiration:

- ***To examine the sensitivity of the decomposition of different C compounds to changing soil properties, in particular, soil temperature and soil water content.***

The influence of two key soil variables (soil temperature and soil water content) on the mineralization of ^{14}C-labelled low and high MW fractions of DOC in high Arctic tundra soils was investigated under laboratory conditions. Results are presented in Chapter III.

- ***To elucidate how changing soil properties, such as pH, can influence mineralization rates of low MW DOC components.***

The influence of pH (pH 4, 5, 6, 7) on the mineralization of 8 different low MW C substrates was investigated on arable soils taken across a natural pH gradient. Mineralization results were then compared against microbial community composition ranges to see if results correlated. Results are presented in Chapter IV.

IV. Mathematical modelling of soil respiration results:

- ***To examine methods and mathematical models currently used to determine C dynamics.***

Two common ^{14}C-labelled low MW root exudates (alanine and glucose) were added to agricultural grassland soil (*ex-situ*). Exponential decay equations were then applied to the data in order to model how these substrates are metabolised within an agricultural soil. Validation of the model was investigated by conducting a series of different extractions of known available C pools. Results are presented in Chapter V.

- ***To examine the wider implications of soil C cycling by up-scaling of fine-scale field experimental data.***

Up-scaling of modelled results, taken from Chapter II, showing the mineralization kinetics of different low MW C compounds was conducted to investigate the relative substrate contribution to overall soil respiration. In addition the amount of organic matter C loss per year was estimated for the same dataset. We discuss the wider implications of this research and also the caveats surrounding the up-scaling process.

2. Introduction

2.1. Global Carbon cycle

This thesis is focused on the terrestrial carbon (C) sink and looked at how the balance between terrestrial and atmosphere stores of C may alter with a changing climate. This introduction aims to review the literature to ascertain current knowledge of soil C cycling and soil respiration to provide a context for each individual chapter within this thesis. Above-ground photosynthesis and below-ground soil respiration are two key processes which affect the C balance of terrestrial ecosystems. Ecosystems can either be a CO_2 sink (storing C), or a CO_2 source (producing C), the balance is determined by the relationship between CO_2 production and decomposition (Pumpanen et al., 2004); both of which can be influenced by a changing climate. The Intergovernmental Panel on Climate Change (IPCC; Houghton, 2004; 2005) postulate there is more than twice as much C stored in the world's soils (2000 Gt C) than there is present in the atmosphere (760 Gt C) and vegetation (500 Gt C; Fig. 1).

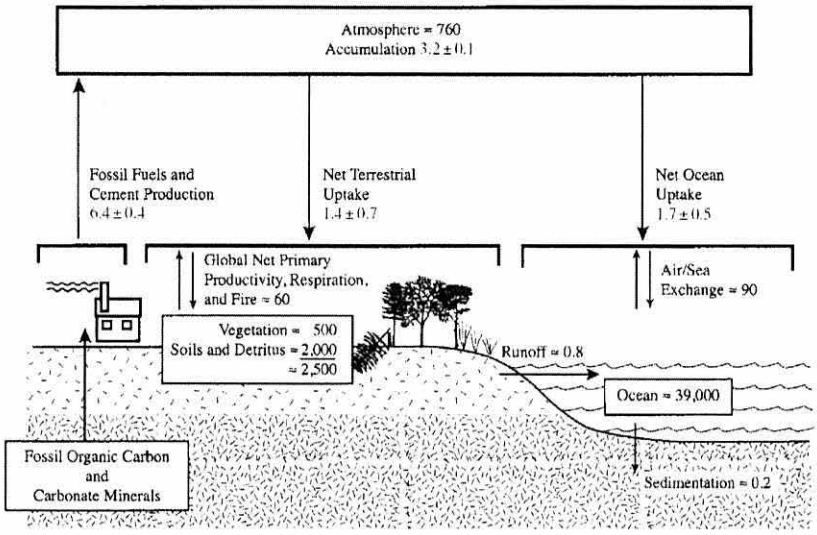

Fig.1. Schematic representation of the global C cycle, showing the amount of C stored in each reservoir (values in Gt C). The terrestrial biosphere is the second largest reservoir (Houghton et al., 2005).

Changes in this store could have profound impacts on atmospheric greenhouse gases (GHG) and global warming. Within the soil C reservoir, soil respiration is one of the largest C fluxes, exceeding all other terrestrial-atmospheric C fluxes except for gross primary productivity (Raich and Schlesinger, 1992; Rustad et al., 2000; Davidson et al., 2002). This thesis investigates the relative contribution of microbial respiration to total soil CO_2 efflux, and looks at factors involved in regulating these fluxes, such as temperature and soil water content, using two contrasting ecosystems; agricultural grasslands and Arctic tundra (Chapters II, III, IV). These ecosystems are selected because observations suggest they are both already experiencing the effects of a changing climate. Agricultural grasslands, in some parts of the world, are currently losing C from over-use and poor management practices (Conant et al., 2001). However, Bellamy et al. (2005) state that C is being lost in soils across England and Wales irrespective of land-use change and suggest climate change is having a greater influence on C loss. Increasing agricultural demands are resulting in conversion of native vegetation to grasslands for agriculture across the globe.

However, with good management practices, grasslands could potentially help offset global greenhouse gas emissions (Soussana et al., 2007). In contrast, Arctic ecosystems provide a pristine environment in which to monitor the effects of climate change. Arctic soil contains 14 % of the global soil C reservoir of organic C and while frozen and under snow cover, it is largely protected from microbial decomposition and the release of greenhouse gases. However, this vast store of C is becoming vulnerable to decomposition as soil temperatures and water regimes are altered through climate change (Post et al., 1982; Grogan and Jonasson, 2005). Combined changes to temperature and water regimes could potentially release greenhouse gases to the atmosphere as this large soil C reservoir is broken down, turning the Arctic into a CO_2 source. Using laboratory and field measurements from these two ecosystems, this thesis aims to develop a more mechanistic understanding of terrestrial C cycling and ascertain potential climate feedbacks. This is important for helping validate global climate models (GCMs), and subsequently informing climate policy makers on effective mitigation/adaptation strategies. These two ecosystems will be discussed in more detail in sections 1.6.1 and 1.6.2 respectively.

2.2. Climate change

The IPCC refers to climate change as being "any change in climate over time, whether due to natural variability or as a result of human activity" (Houghton, 2001). Relatively small changes in climatic variables could have a large impact on the delicate balance of interactions within the terrestrial C cycle (Bellamy et al., 2005; von Lützow et al., 2006). Anthropogenic perturbations combine with natural events to affect levels of atmospheric CO_2. Together these factors are producing a net increase in atmospheric CO_2 which EPICA's (European Project for Ice Coring in Antarctica) latest ice core record

(Dome Concordia) has shown to be unprecedented for the last 800 kyrs (Fig. 2; Brook, 2008).

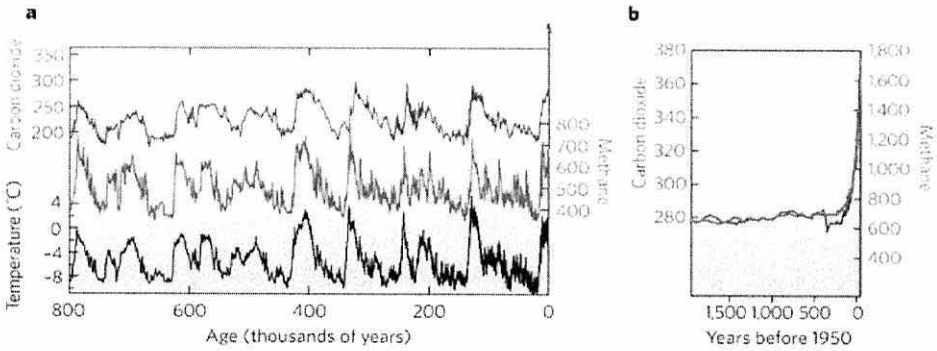

Fig. 2. Atmospheric reconstruction for the past 800 kyrs, taken from the EPICA Dome Concordia ice core (graph a), depicting temperature (°C), CO_2 (ppm) and methane (ppb) levels. The last 2 kyrs witnessed a rapid increase in greenhouse gas values, with CO_2 levels currently at 380 ppm and CH_4 approximately 1800 ppb (graph b). (Figure taken from Brook, 2008).

This period covers eight glacial-interglacial cycles (Siegenthaler et al., 2005; Denman et al., 2007; Lüthi et al., 2008; Brook, 2008) and shows that atmospheric CO_2 levels have remained relatively stable for the last 800 kyrs, with measurements of 180 ppm (glacial maximum) and 300 ppm (interglacial periods) (Denman et al., 2007). Since 1750, however, emissions have increased, with atmospheric CO_2 values being approximately 380 ppm in 2008. This is projected to reach as high as 540-970 ppm by the year 2100 (Houghton, 2001; 2005). The majority of this perturbation comes from burning of fossil fuels, with the remaining (10-30%) as a consequence of land-use change, in particular deforestation (Houghton, 2001). Concomitantly, there has been an increase in global average surface temperatures (Brook, 2008); with an estimated increase of 0.6 ± 0.2°C occurring during the 20th Century (Houghton, 2001). These perturbations cannot be explained by natural forcing mechanisms alone and thus anthropogenic factors must be influencing the earth's climate, especially during the latter part of the 20th Century (Fig. 2).

Current global rates of warming are placed at 0.17°C per decade, which exceeds the critical 0.1°C per decade range beyond which ecosystems cannot adjust (Lal, 2004). Regional warming is more varied, especially in high latitude areas, where the impacts of climate change are expected to exceed average rates of warming (Anisimov et al., 2007).

Understanding soil sensitivity to climate change is difficult due to the lack of a mechanistic understanding about how key environmental factors (e.g. temperature, soil water content and increased CO_2 levels) affect ecosystem C dynamics (Davidson and Janssens, 2006). The terrestrial biosphere currently acts as a C sink, which potentially mitigates further climate change in the short term (Janssens et al., 2005). However, it has been postulated that by 2050, the terrestrial biosphere could switch to a C source (Cox et al., 2000). This is dependent upon the sensitivity, in the long term, of soil respiration to climatic change (Cox et al., 2000; Lenton et al., 2003). Anthropogenic-induced changes in CO_2 levels and temperature occurring at a similar time to natural ecosystem changes confound our understanding of natural soil C processes (Lenton et al., 2003).

2.3. Soil Carbon cycle

Soil ecosystems are complex and dynamic with many abiotic properties influencing the soil environment (Fig. 3; Fang and Moncrieff, 1999; Bardgett, 2005; Raich and Tufekcioglu, 2000). These factors lead to wide variability in soil habitats at both spatial (soil profile to landscape) and temporal scales.

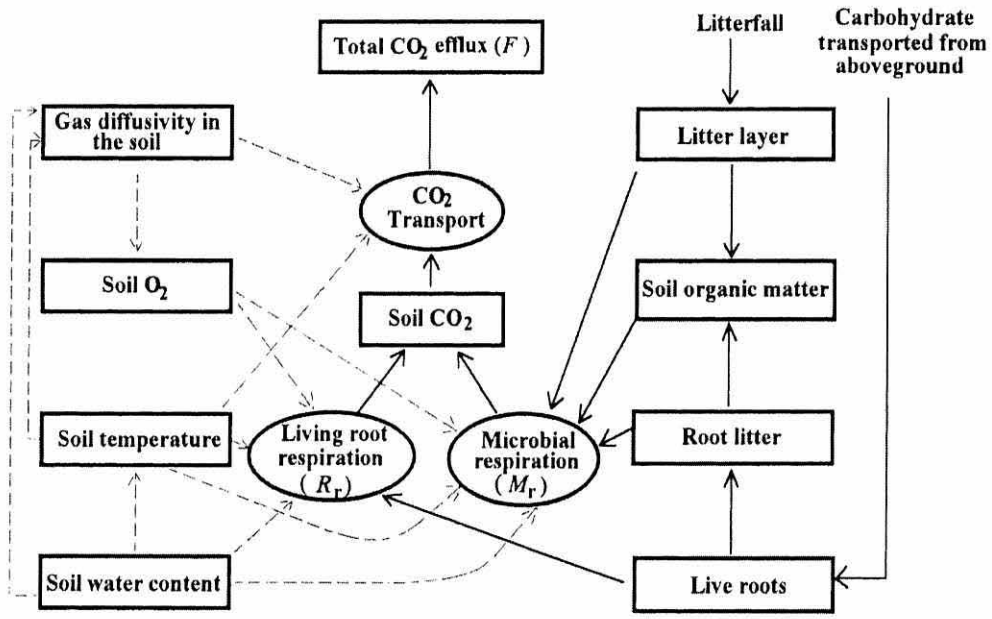

Fig. 3. Model of soil CO_2 fluxes (Fang and Moncrieff, 1999). Solid lines indicate carbon flows; dashed lines show environmental factors influencing respiration and CO_2 transport. Rectangles represent variable factors, ovals indicate processes.

Soil CO_2 efflux is controlled by CO_2 production from root (autotrophic) and soil (heterotrophic) respiration, bulk turnover of soil organic matter, and also by transport pathways which carry CO_2 from the soil to the atmosphere (Fig. 3; Fang and Moncrieff, 1996; van Hees et al., 2005; Heinemeyer et al., 2011). However, accurate measurements of soil CO_2 efflux are difficult to obtain *in-situ* due to many confounding environmental and physical soil variables (Fig. 3; e.g. soil pH, soil temperature, soil water content, C:N ratio, substrate supply, texture, porosity, oxygen levels) (Luo and Zhou, 2006). However, accurate measurements are important for determining ecosystem C budgets (Janssens et al., 2000). When conducting *ex-situ* measurements, environmental conditions can be controlled, enabling specific variables to be investigated, however, the reliability and accuracy of comparing these *ex-situ* conditions to *in-situ* conditions is subject to conjecture.

Ex-situ measurements are highly invasive with root and hyphal networks being severed, inhibiting the recharge of the dissolved organic C (DOC) pool through root and fungal hyphal exudation (Vance and Chapin, 2001; Oburger and Jones, 2009).

We selected three soil properties which are important factors influencing the soil microbial communities and are most likely to be affected by changing land-use and climate change.

I. Soil temperature (Chapter III).

Global climate models project average surface temperature to continue increase as a result of natural and anthropogenic release of GHGs (Houghton, 2001; Brook, 2008). Soil respiration and soil temperature are well correlated, with respiration increasing exponentially with rising temperatures, until an optimum temperature is reached (45-50°C), after which respiration declines (Luo and Zhou, 2006). Soil microbial communities have a broad temperature range in which they can function, thus projected changes of 0.17°C per decade may not have a significant effect on microbial communities. However, in areas where extreme climatic conditions prevail, such as the high Arctic tundra, temperature is a key driver of ecosystem function and structure. Consequently, soil microbial communities and thus soil respiration, in the high Arctic, might be more sensitive to elevated temperatures and further research is required for these areas (Raich and Schlesinger, 1992; Canadell, et al., 2004; Bardgett, 2005). The Q_{10} temperature co-efficient has been widely used as a proxy for determining how biochemical processes associated with respiration are likely to be affected by a 10°C rise in temperature. In most biological processes, it is postulated that the reaction rate doubles for every 10°C temperature increment, thus giving a Q_{10} value of 2 (Davidson and Janssens, 2006; Luo and Zhou,

2006; Yuste et al., 2004). The high Arctic tundra has reported Q_{10} values ranging from 2 – 8.8 (Bekku et al., 2003), suggesting that temperature changes could have a great impact on the biochemical processes of respiration. However, Q_{10} values vary between sites and the value is calculated based on site-specific conditions when the measurements were taken, and can fail to account for the multitude of factors (both climatological and pedological) influencing respiration annually *in-situ* (Davidson and Janssens, 2006). Davidson and Janssens, (2006) look at the temperature sensitivity of belowground soil C decomposition and the potential climatic feedbacks this can have, both positive, by means of warming-induced acceleration of soil organic matter decomposition; and also negative feedbacks, such as increasing the Net Primary Productivity (NPP) of the soil through increased rates of photosynthesis, potentially helping negate the effect of climate change (Davidson and Janssens, 2006). This study focussed on the temperature sensitivity of high Arctic-tundra soils to investigate the effects of temperature on C cycling dynamics and what feedbacks this could have on climate change (Chapter III).

II. Soil water content (Chapter III).

Water content is another very important soil property. It forms soil solution which contains important dissolved nutrients that are vital for microbial communities and growing plants (Marschner and Kalbitz, 2003; Bardgett, 2005). Soil respiration is influenced by soil water content and is often low when conditions are dry because of limited substrate supply as diffusion of nutrients is hindered. Optimal rates occur when soils are near field capacity and O_2 diffusion is not impeded. Under high water contents, respiration decreases as conditions become anaerobic and O_2 diffusion is the main limiting factor under very wet conditions (Luo and Zhou, 2006). Many soil microbial communities are well adapted to

different soil water contents by regulating their internal water potential via the production of osmolytes (e.g. simple organic compounds) under very dry conditions to prevent dehydration, these are then discarded upon re-wetting to prevent cell walls bursting through excess pressure (Schimel and Mikan, 2005; Bardgett, 2005). Precipitation patterns are projected to be influenced by climate change, with high latitude areas again expected to see a major change with an increase by up to 20 % during the 21st Century (Anisimov et al., 2007; Trenberth et al., 2007). However, uncertainty between climate models exists regarding seasonal and spatial precipitation patterns. In conjunction, temperatures are due to increase in the same area, reducing the snow cover and exposing the soil to warmer conditions, potentially drying out. There is a strong interaction between temperature and soil water content but the effect these variables could have on nutrient cycling dynamics remains poorly understood. This study focussed on the sensitivity of high Arctic-tundra soils to changing soil water contents to investigate the effects on C cycling dynamics and what feedbacks this could have on climate change (Chapter III).

III. Soil pH (Chapter IV).

Soil pH is very important for regulating chemical reactions as many enzymes are pH dependent, it is deemed as important as C and N concentrations for influencing soil microbial biomass and microbial community composition, decreasing pH can result in releasing biologically available Al^{3+}, which is toxic to plants (Wardle, 1992; Aciego Pietri and Brookes, 2008). Soil pH is determined by the concentration of H^+ ions in soil solution. Soil acidity can occur if base cations (Ca^{2+}, Mg^{2+}, K^+) are leached from the soil and replaced with H^+ and Al^{3+} ions (Bardgett, 2005). Soil acidity can occur through various processes, such as; increased acid rain either from natural events, such as volcanic

eruptions and thunderstorms which can produce sulphuric and nitric oxides which, or from atmospheric pollution from anthropogenic sources (Bardgett, 2005). In addition increased use of inorganic fertilizer use in agricultural practices can exacerbate soil acidity. This can occur via microbial oxidation (nitrification) of ammonium (NH_4^+) to nitrate (NO_3^-) which can yield H^+ ions, decreasing the pH (Bardgett, 2005). This study focussed on the sensitivity of C cycling dynamics to changing pH along a natural pH gradient (Chapter V).

2.3.1. Soil organic matter

Soil organic matter (SOM) acts as a nutrient and water reserve, helps to maintain soil structure and texture, is important for cation exchange capacity (CEC) and contributes to soil fertility (the higher the CEC, the more fertile the soil). Microbial communities require an easily accessible, readily broken down (labile) C pool to obtain nutrients needed to function. Within the SOM, the DOC pool represents the most bio-available fraction (Marschner and Kalbitz, 2003; van Hees et al., 2005). DOC is defined as being the number of organic molecules present in soil solution of differing sizes and structures that can pass through a filter with pore sizes between 0.4 - 0.6 μm (Kalbitz et al., 2000; Chantigny, 2003). Soil solutions contain differing amounts of DOC, derived from plant litter, soil humus, microbial biomass or from root exudates (Kalbitz et al., 2003). Root exudates are C compounds released from the roots of living plants into the rhizopshere, which is the unique area immediately surrounding the roots (Walker et al., 2003; Jones et al., 2004). The release of C around the roots attracts microorganisms to this area. In addition, this accumulation of C compounds results in the rhizosphere being physically, chemically and biologically different to that of the bulk soil (Walker et al., 2003; Jones et al., 2004). The majority of DOC is comprised of complex high molecular weight (MW) substances which

are stable and difficult to break down (recalcitrant) into a soluble form (e.g. proteins, cellulose, hemicellulose and lignin) (Kalbitz et al., 2000; van Hees et al., 2005). These substrates can only be assimilated by microbial communities after extra-cellular enzymes break these large (> 1000 Da), recalcitrant compounds, down into low MW subsidiary components (Fig. 4; Marschner and Kalbitz, 2003). Low MW compounds represent < 10% of the DOC pool (Kalbitz et al., 2000; Van Hees et al., 2005; 2008) and are more labile, thus easily taken up by the soil microbial community, limiting their accumulation in the soil solution (Fig. 4; Jones et al., 2012) .

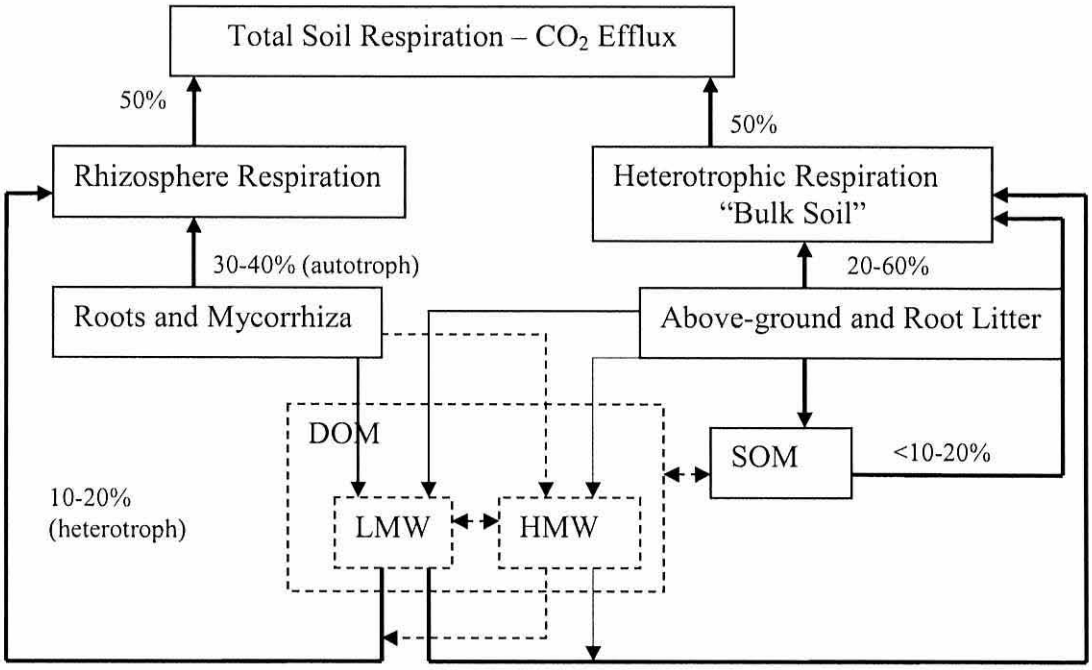

Fig. 4. Schematic diagram of soil CO_2 fluxes and C sources. Solid arrows represent major C fluxes, thin solid arrows depict small C fluxes and dashed arrows indicate minor C fluxes. All percentages convey contribution to soil respiration. Values taken from van Hees et al. (2005), with slight adaptations made to simplify the original schematic.

The low MW compounds comprise carbohydrates (50-70% of total exudates), organic acids (20-30% of total exudates), amino acids (10-20% of total exudates), amino sugars and nucleotides which are essential solutes for metabolism in most organisms (van

Hees et al., 2005). These compounds are exuded by roots in rhizodeposition and some are breakdown products of high MW compounds (e.g. polysaccharides from breakdown of cellulose) (Kuzyakov et al., 2007; van Hees et al., 2005; 2008; Farrar et al., 2012). It has been suggested that the availability of low MW compounds may dominate the total soil CO_2 flux, despite being present in low concentrations (van Hees et al., 2005). It is also important to investigate how climatic variables (e.g. temperature, CO_2 levels and soil water content), may affect individual DOC (both high and low MW) compounds and their overall contribution to soil respiration.

2.4. Soil respiration – methodological approaches (Chapter I)

For almost 90 years soil respiration has been used as measure of soil fertility as it provides an integrated measure of microbial activity in the soil (Luo and Zhou, 2006; Haney et al., 2008). Soil respiration is one way to assess the impacts of changing environmental conditions on the activity of both plant and soil microbial communities. However, there is currently no internationally recognised or standard protocol for measuring soil respiration and rarely have the different methods been validated *in-situ* or artificially (Norman et al., 1997; Pumpanen et al., 2004). Soil properties such as, pH, texture and porosity, tortuosity, precipitation and water infiltration rates influence the concentration of CO_2 within the soil (Raich and Schlesinger, 1992; Luo and Zhou, 2006; Allaire et al., 2008). The transport of CO_2 within soil occurs via diffusion flow (concentration gradients) and mass flow (pressure gradients). Diffusion through air-filled pores is a key mechanism for facilitating CO_2 transport from deep soil layers to the soil surface and subsequently to the atmosphere. This occurs via a steep concentration gradient with CO_2 moving from deeper soils containing more CO_2 to surface soils containing less

CO_2 (Luo and Zhou, 2006; Allaire et al., 2008). Mass flow can also facilitate transport of CO_2 using pressure gradients between different soil zones. This is largely influenced by changes in variables such as temperature and atmospheric pressure which can cause air within soils to expand and contract. Water infiltration and plant water uptake can also alter the pressure gradient within the soil profile. In addition, surface soils are influenced by winds which can again affect the pressure gradient within the soil profile by forcing CO_2 into the soil or removing it (Raich and Schlesinger, 1992; Luo and Zhou, 2006; Allaire et al., 2008). Accurately measuring soil CO_2 efflux *in-situ* is very challenging as most available methods are likely to induce physical and chemical disturbance of the soil environment, potentially affecting CO_2 concentration gradients. In order to overcome these challenges, a variety of methods/approaches have been developed to try and quantify CO_2 fluxes. However, results obtained from any method are contentious due to a lack of a calibrated standard and validation step by which to compare the data. Therefore, any methods calculating soil respiration can only be compared relatively (Nay et al., 1994; Widén and Lindroth, 2003). *In-situ* measurements of soil CO_2 efflux can be measured using different chamber-based techniques. Chamber-based methods are some of the most direct and accurate methods currently available to measure CO_2 flux *in-situ*. They can be classified into two main categories depending on how the CO_2 flux is calculated: (1) non-steady-state mode, and (2) steady-state mode (Davidson et al., 2002; Pumpanen et al., 2004; Hutchinson and Livingston, 2001). In non-steady-state systems the CO_2 efflux is determined by measuring the rate of increase in CO_2 concentration within an enclosed chamber shortly after its placement on the soil (Davidson et al., 2002; Pumpanen et al., 2004). Steady-state chambers measure CO_2 efflux using the difference in CO_2 concentration at the chamber inlet and outlet, once CO_2 concentration equilibrium has been

reached. The chamber is of a known volume and the flow rate of air through the chamber is also known (Davidson et al., 2002). Non-steady state chambers were used throughout this study mainly for logistical reasons as they are robust and easy to transport to the field.

2.4.1. Non-steady-state/closed chamber method for measuring soil respiration

Non-steady-state chambers (e.g. SRC-1 chamber, PP Systems Ltd, Hitchin, UK), with an associated infra-red gas analyser (IRGA), are often selected for measuring long-term gas fluxes and are widely used to measure CO_2 fluxes between soils or low level canopies and the atmosphere (Kutzbach et al., 2007; Hutchinson and Livingston, 2001). They were originally designed to measure fluxes in N_2O but have since been adapted to include detection of CO_2 fluxes (Bekku et al., 1995). Non-steady-state systems are relatively low in cost and energy consumption, simple to operate, are robust, and can be used and carried to remote locations easily, thus are ideal for use *in-situ* (Bekku et al., 1995; Kutzbach et al., 2007; Conen and Smith, 1998).

However, there are possible sources of error associated with using chambers to measure CO_2 efflux (Janssens et al., 2000), five of which are detailed below:

1) Build up of CO_2 in the chamber headspace can occur, potentially influencing Fick's first law of diffusion, whereby gases move from an area of high concentration to one of low concentration (Pumpanen et al., 2004; Davidson et al., 2002; Kutzbach et al., 2007). This could result in an under-estimation of flux if less gas is able to diffuse from the soil into the chamber due to an already high gas concentration inside the chamber head space (Rayment, 2000; Pumpanen et al., 2004). There is also the added complication of lateral diffusion of CO_2 occurring outside of the chamber base (Davidson et al., 2002). The IRGA-based equipment tries to minimise the potential impact on the CO_2 concentration gradient

by taking measurements rapidly, with chamber emplacement being < 5 min, preventing excessive build up of gas within the chamber (Davidson et al., 2002).

2) Pressure can also increase inside the chambers compared to atmospheric pressure, to combat this some chambers have a valve fitted to equalize pressure inside and outside the chamber (Rochette et al., 1997; Davidson et al., 2002; Hutchinson and Livingston, 2001);

3) Temperature and soil water content can change inside the chamber, potentially influencing decomposition and respiration rates.

4) Chamber and/or collar placement can result in soil compaction causing a loss of CO_2, in addition if chamber are inserted deeply into soil, lateral diffusion of CO_2 could occur (Davidson et al., 2002). Roots and fungal hyphal networks can also be severed upon chamber/collar placement which can influence the flux measurements.

5) Air inside the chamber needs to be well mixed in order for the measured air sample to be representative, some chambers use fans to ensure thorough mixing, however, this also has caveats with turbulence often being created by the fans which can influence the flux measurements by altering pressure gradients (Rochette et al., 1997).

Some non-steady-state chambers can be fitted with a collar to raise the chamber off the soil surface which is helpful if tall vegetation covers the soil. Collars can be inserted into the ground prior to taking measurements, reducing artificial bias that can arise from the degassing of soils, upon initial chamber placement. This is important for fine-textured soils, which can form a crust on the surface under dry conditions, upon breaking this crust, CO_2 is released which could result in an over-estimation of soil CO_2 efflux (Rochette et al., 1997). In addition, it can be difficult to insert a chamber into the soil, and so including a collar prior to measurements can help avoid this problem. They can help prevent CO_2 leakage out

of the chamber (Luo and Zhou, 2006; Heinemeyer et al., 2011). Collars also enable repeated measurements to be made on the same site over varying time periods, which can be useful for ascertaining variation in sites over time (Rochette et al., 1997). However, Pumpanen et al. (2004) argue that the addition of these collars may affect overall efflux estimations. The addition of a collar increases the volume of the system; this needs to be accurately measured to prevent an under-estimation of CO_2 efflux (Rayment, 2000). In addition, the porosity of the soil can also influence the chamber volume. Pore spaces within the soil matrix directly under the chamber can accumulate CO_2 during each measurement. This is often ignored in CO_2 calculations, mainly because it is difficult to measure, however this can affect the overall efflux measurements. It has been suggested that failure to account for this additional volume could result in an under-estimation of CO_2 efflux by up to 30 % (Rayment, 2000). The depth at which collars are inserted can influence CO_2 measurements. If the collar is inserted deep into the soil it can reduce the relative contribution to soil respiration from the rhizosphere, by limiting or inhibiting root growth under the chamber (Rochette et al., 1997). In addition, if root networks are severed during emplacement of the collar, and repeated time measurements are taken (days, weeks, and months), then root decomposition could contribute to total soil respiration measurements.

Whilst conducting field soil CO_2 efflux experiments, it is essential that ambient and stable conditions are maintained at all times. Obtaining reliable and accurate soil CO_2 measurements is difficult due to the multitude of factors influencing the flux calculations. With no standards against which to calibrate results, there is a need to improve our knowledge of soil respiration processes to aide development of techniques to account for natural variability, thereby reducing potential sampling errors.

Chapter I directly compares two commercially-available and commonly-used non-steady state chambers; the LI-COR 8100 (LI-COR Biosciences, Lincoln, NE, USA) and the PP-Systems EGM-4 coupled with a SRC-1 chamber (PP Systems, Hitchin, Herts., UK). This particular study aimed to investigate if these two chambers accurately compare with each other in terms of CO_2 efflux. In addition, the use of pre-inserted collars was investigated to assess what effect, if any, they had on CO_2 measurements. The results of which could inform researchers as to whether different IRGA systems can provide reliable and comparable results across different ecosystems, irrespective of choice of model, and whether the inclusion or absence of collars caused any bias in CO_2 measurements.

2.4.2. Isotope studies for measuring soil respiration

Isotopes can be used to trace the fate and transformation of elements through ecological processes (Luo and Zhou, 2006; Ehleringer et al., 2000; Wolfe and Chinkes, 2005). Carbon has three naturally occurring isotopes; ^{12}C and ^{13}C are stable isotopes and radioactive ^{14}C. The most abundant form is ^{12}C making up ~98.9% of the Earths C, ^{13}C has ~1.1% abundance, and ^{14}C only occurs in very trace amounts ~0.0000000001% (Farquhar et al., 1989; Ehleringer et al., 2000). Measuring CO_2 evolution using isotopically labelled substrates can provide an indication as to how microbial communities metabolise specific compounds, such as DOC components, both *in-situ* and *ex-situ* (Coody et al., 1986; Jones, 1999; van Hees et al., 2005; Glanville et al., 2012). It is important to investigate how individual C compounds are partitioned within soil microbial communities once assimilated in order to account for contributions to overall soil respiration. The use of isotopes requires two assumptions; firstly, being that different source components of soil respiration have different isotopic values; secondly, there is no significant fractionation of isotopes during

processing of C from the source to the output where the isotope sample is collected (Luo and Zhou, 2006).

The two main isotopic classifications used in soil respiration studies are; (1) Stable ^{13}C and ^{12}C, and (2) Radioactive ^{14}C (Luo and Zhou, 2006). This thesis utilises only ^{14}C-labelling because it is highly sensitive; is more economical in terms of purchasing and analysis than ^{13}C-labelling, and samples are easier to prepare compared with ^{13}C samples (Kuzyakov and Domanski, 2000; Kuzyakov, 2002).

2.4.2.1. Radioactive ^{14}C isotope tracing

Radioactive ^{14}C-labelling is commonly used to investigate metabolism of DOC components within the soil solution. In this study, radionuclides were applied in three ways; 1) by spiking soil solution directly with known low molecular weight (MW) DOC compounds which represent common root exudates (Chapters II, III, V); 2) by adding ^{14}C low MW compounds to a non-reactive media (i.e. sand; Chapter III), and 3) by adding ^{14}C-labelling high MW plant material (Chapter III). The concentration of the ^{14}C isotopes used in all experiments within this study was very low (< 10 nM) and was not expected to significantly affect the intrinsic concentrations in the soil solution. This is important to ensure that any tracer detected in the form of $^{14}CO_2$ was not derived from priming of the DOC pool with an additional C substrate. After the addition of the ^{14}C-labelled compound to the soil, it is broken down by microbial communities and assimilated and used for metabolism. As these C compounds are assimilated and oxidised by the microbial communities, the respired CO_2 will become ^{14}C-labelled ($^{14}CO_2$), which can then be quantified using liquid scintillation counting. The $^{14}CO_2$ produced is captured in a NaOH trap which is mixed with a scintillation fluid. The scintillant enables the amount of

radiation to be quantified by counting the amount of light emitted from excitation of beta rays as decay occurs (Wolfe and Chinkes, 2005). The rate of emission of beta particles decreases over time due to the number of radioactive transformations. The half-life for ^{14}C is 5730 years, which is the time taken for half of the atoms to be transformed into the stable isotope ^{14}N via radioactive decay. The longevity of ^{14}C makes it a good isotope to use as it can be stored for a long time without a significant loss of tracer through spontaneous decay. Therefore, we can be confident that the detected ^{14}C is as a result of metabolic breakdown as opposed to natural radioactive decay (Wolfe and Chinkes, 2005). However, as with chamber-based approaches, isotope tracing also has limitations, in particular it does not account for any lag phase between microbial uptake of each substrate and its subsequent mineralization and utilization (Hill et al., 2008; Jones et al., 2009). This may potentially underestimate relative substrate mineralization rates.

2.5. Mathematical modelling

Improving our understanding of C cycling has important implications for future responses of the terrestrial system to climate change. There is a wealth of literature discussing processes involved in C and nutrient cycling and we are constantly improving our understanding of the complex mechanistic plant-soil-microbe interactions. The use of mathematical modelling can assist in further developing our understanding of these complex processes by not only quantifying interactions between C pools but also including processes and fluxes that are thought to be involved in cycling of important nutrients, such as C. Mathematical models can also be used to explore how individual ecosystems currently influence climate and how this may change in the future (Roose and Schnepf, 2008). More robust and accurate mathematical modelling of C dynamics could improve our

confidence in global climate models and inform policy on the future impacts of different mitigation and adaptation strategies.

In this thesis I have used different exponential decay equations applied to soil respiration data obtained from ^{14}C-labelling studies to quantify turnover rates of specific C compounds and explore how C is partition within soil microbial communities.

The application of mathematical models, such as single, double and triple exponential decay equations, to soil respiration data obtained after ^{14}C-isotope additions, provides quantification of how specific C compounds are turned over within the soil. The production of $^{14}CO_2$ from metabolic breakdown of the ^{14}C-labelled substrates added follows an exponential decay function. The number of exponential terms (determined by either a single, double or triple equation) used in the model refers to how many pools/compartments we ascribe to the metabolic system for a particular substrate (Wolfe and Chinkes, 2005). Typically, low MW compounds are assumed to follow a biphasic pattern of mineralization (Chotte et al., 1998; Saggar et al., 1996; van Hees et al., 2005; Boddy et al., 2007) whereby C can be partitioned into two major compartments/pools. A double first-order exponential decay model is applied to the data set to best represent this biphasic pattern. Using a double exponential decay model, pool 1 is associated with immediate substrate use for catabolic processes (i.e. respiration), relating to a depletion of the DOC pool (Jones, 1999). A second, slower pool is associated with the substrate being utilized for anabolic processes (formation of new biomass, growth and repair, and cell maintenance) and eventual turnover of the soil microbial community. However, a two pool model may be too simplistic to describe how some substrates are compartmentalized during metabolism. Key criteria when selecting a model are: assessing relationships between model parameters (dependency values) and model fits (r^2 values), alongside an

understanding of how that substrate is likely to be metabolized, to avoid over-fitting of the data (Wolfe and Chinkes, 2005). Processes occurring within the rhizosphere are complex and dynamic and are difficult to measure. As a consequence, our knowledge of how microbial communities function within the rhizosphere is limited, concomitantly making it difficult to accurately model rhizosphere processes (Toal et al., 2000). Combining mathematical models with experimental data can thus help improve our understanding of the rates at which C is cycled within the soil (kinetics), providing the correct and most accurate model is selected. This thesis aims to investigate the two pool model and assess whether or not this best describes low MW C mineralization kinetics (Chapter V).

2.6. Ecosystem case studies

The main aim of this thesis is to improve our understanding of C dynamics and investigate how sensitive C turnover is to climate change. To achieve this, two contrasting ecosystems were studied; agricultural grasslands and Arctic tundra ecosystems. The relative global importance of these two ecosystems is discussed in more detail below.

2.6.1. Grassland ecosystems

Grasslands (both temperate and tropical) are one of the most wide spread vegetation types, occupying nearly 20 % of the world's land surface, with an estimated coverage of about 15 million km^2 in the tropics and 9 million km^2 in temperate regions (Ni, 2002). Grasslands are very important in terms of C storage, with up to 98 % of total C estimated to be stored below ground (Conant et al., 2001; Jones and Donnelly, 2004). Grasslands are estimated to store between 10-20 % of the global total C stocks (Scurlock and Hall, 1998; Jones and Donnelly, 2004; Glaser et al., 2006). However, grasslands are being over used

and poorly managed, leading to loss of SOM and thus C, reducing their potential for storing C in future (Conant et al., 2001). Food and forage production are increasing in order to cope with a growing population, placing greater demands on grasslands around the world, and resulting in large areas of native vegetation being converted to grasslands in order to try and cope with this increased demand (Conant et al., 2001). Between 1850 and 1990, long-term CO_2 flux of 120 Pg C has been measured from terrestrial ecosystems to the atmosphere due to changing global land use practices (Houghton, 1995). The most prevalent C loss from soils occurred in grasslands of temperate zones, with a total of 52 Pg C lost to the atmosphere (Houghton, 1995). It is estimated that if the world's permanent pastures are well-managed, then global greenhouse gas emissions could potentially be offset by 4 % because of increased C sequestration (Soussana et al., 2007). With an increased demand on grasslands for agriculture, it is important we improve our understanding of how C is cycled within this ecosystem. This has particular importance for helping implement improved management schemes which may help grasslands act as a C sink in the long term (Dugas et al., 1999; Tate et al., 1997).

Grasslands form one of the worlds terrestrial biomes along with; tropical rainforests, tropical savannas, deserts, chaparral, temperate deciduous forests, temperature boreal forests and tundra. These biomes are characterised by prevailing climatic conditions and are differentiated from each other by unique ecosystems and species which have evolved within that particular biome (Olson et al., 2001; Hoekstra et al., 2005). Raich and Tufekcioglu, (2000) depict grasslands as having higher rates of soil respiration than forests, suggesting that vegetation type may strongly influence soil respiration. Grasslands allocate most photosynthetically fixed C below ground, whereas forests allocate more C to woody biomass, which does not contribute immediately to soil respiration. Therefore, conversion

of forests to grasslands could potentially result in increased CO_2 emissions (Raich and Tufekcioglu, 2000). However, it is difficult to know whether this is related to global biomes having different vegetation types or if it is more representative of altering climatic conditions among biomes. There is no general consensus as to what effects changing climatic factors, such as elevated CO_2, temperature and soil water content, will have on soil processes (Tate et al., 1997). It is the complex interaction between climatic variables and soil processes, including photosynthesis, soil water content and plant-available nutrients within grassland ecosystems, which determine not only the composition of grasslands, but also the size and turnover of C from the soil reservoir (Tate et al., 1997; Dugas et al., 1999). Thornley et al., (1997) used the Hurley Pasture Model (HPM) to postulate the effects of climate change on grasslands. They suggested that any changes will be highly specific not only to climate but also to the intensity of grazing and nutrient supply. The HPM predicts that current conditions of increasing atmospheric CO_2 and global temperatures will continue for a few decades producing a net sink in temperate grasslands of approximately 5-15 g C m^{-2} y^{-1} (Thornley et al., 1997). This net C sink can potentially be enhanced by effective grassland management practices, such as; fertilization to increase below-ground and above-ground production; sowing of grasses to encourage below-ground C inputs; sowing legumes to increase N input, improving soil fertility and therefore above and below-ground productivity; irrigation to provide sufficient soil water content for crop growth, and addition of earthworms can help soil fertility by improving soil drainage, soil structure and aeration (Conant et al., 2001). This can lead to increased soil C content and thus net C storage. Along with land use changes; natural climatic variability, native vegetation, soil depth, time, and original soil C content will all impact on the rates of soil C change and turnover (Conant et al., 2001).

Despite the fact that grasslands occupy nearly 20 % of the worlds land surface (Tate et al., 1997; Dugas et al., 1999; Glaser et al., 2006), quantification of C exchange and interaction with the atmosphere is speculative, and highlights the need to better understand the complex processes occurring within grassland ecosystems (Rogiers et al., 2008).

2.6.2. Arctic ecosystems

Arctic tundra ecosystems occupy only 6 % of the total land area but contain 14 % of the global soil carbon (C) store (Oechel and Vourlitis, 1994) which is becoming vulnerable to decomposition (Post et al., 1982; Grogan and Jonasson, 2005). Arctic soil organic matter (SOM) contains both labile, low molecular weight (MW) organic C which can be easily decomposed and metabolised by microbial communities and also older, more recalcitrant, high MW humic C compounds which are less easily metabolised. Both low and high MW compounds are often protected from decomposition due to low temperatures, low microbial numbers and low enzymatic activity (Waldrop et al., 2010). Climate change is expected to expose more of this previously protected C to decomposition, releasing C to the atmosphere, however, the controls acting upon Arctic C dynamics are still uncertain (Dutta et al., 2006; Waldrop et al., 2010). Immediately after snow melt, labile low MW C compounds (carbohydrates, amino acids, organic acids) are thought to be released and be important for maintaining the DOC pool (Dutta et al., 2006). However, in the long term, C will also be released from more recalcitrant high MW C compounds (lignin, cellulose, and hemi-cellulose) found within old organic matter (Dutta et al., 2006). The effect of soil temperature and soil water content on the relative mineralization rates of low and high MW compounds could be important in terms of how these pools could respond to climate change.

The Arctic has warmed at twice the global average rate over the past 100 years, with mean air temperatures increasing by 5°C (Anisimov et al., 2007), with 1°C warming per decade noted over the last 30 years. This rate of warming is greatest in winter and spring (Anisimov et al., 2007; ACIA, 2004; AMAP, 2009) and has contributed to a 2 % reduction in snow cover per year and subsequent permafrost thawing (Chapin et al., 2005; AMAP, 2009). Precipitation is also projected to increase in the Arctic during the 21st Century by up to 20 % (Anisimov et al., 2007; Trenberth et al., 2007). However, uncertainty between climate models exists regarding seasonal and spatial precipitation patterns. The impacts of these changes on nutrient cycling dynamics are unclear.

Arctic vegetation and soil microbial communities drive C cycling by facilitating CO_2 and CH_4 exchange in terrestrial high latitudes. Tundra plants are uniquely adapted to their environment, showing rapid shoot growth after snow melt in spring or early summer (Billings and Mooney, 1968). As the snow pack melts, soil water content increases, and a pulse of nutrients is released which are then available for plant and microbial uptake (Lipson et al., 2002). These nutrients were previously protected by the cold conditions under the snow and were unavailable for plant and microbial uptake. High water content experienced post snow melt could improve substrate availability for plant and microbial uptake within the soil solution pool via mass transport and diffusion (Brooks et al., 1998). Immediately post snow melt, soil water content may have a more influential role in nutrient dynamics making substrates more available for uptake. However, as soils are exposed for longer they become warmer and begin to dry out at which point substrate availability again becomes a limiting factor (Lipson et al., 2002). If snow melt commences earlier in the year as a result of climate change, the growing season will be extended. This could result in increased plant productivity, leading to greater root rhizodeposition, releasing more

dissolved organic C (DOC) and N (DON) compounds into the soil. Increased nutrient availability could perpetuate a change in vegetation communities to one more dominated by vascularised plants, unless nutrient availability becomes limiting (Mack et al., 2004; Grogan and Jonasson, 2005; Hill et al., 2011).

Global climate models project temperature and precipitation in high latitude areas to increase; this is expected to result in changes in microbial community composition and nutrient flows which will also feedback on plant growth (e.g. via mycorrhizas; Schimel and Mikan, 2005; Waldrop and Firestone, 2006; Drotz et al., 2010). Predicting ecosystem responses to climate change remains difficult due to our poor understanding of fundamental soil-microbial-plant processes in polar environments. Microbial communities in polar soils are adapted to climatic extremes but their immediate response to soil thawing and snow melt is unclear (Clein and Schimel, 1995; Lipson et al., 2002; Mackelprang et al., 2011). Recent advances in metagenomic studies have enabled direct sequencing of microbial DNA from environmental samples. Combining metagenomic data with information on biological function within the microbial communities allows us to examine whole biochemical pathways and associated processes involved in metabolism (Mackelprang et al., 2011). Metagenomic studies have revealed rapid shifts in permafrost microbial communities in response to soil thaw. In particular, during thaw, permafrost metagenomes were seen to converge and become similar to those found in active soil layer samples. Phylogenetic results (study of how genetically similar populations are) results did not show the same convergence pattern, suggesting that despite different microbial communities present, the overall functional response to thaw is similar in permafrost soils and active layer soils (Mackelprang et al., 2011). The direct and indirect links between these community shifts and alterations in soil C and N cycling, however, are less well understood. Arctic systems

are nutrient limited and so it important to improve our knowledge as to how C and N are cycled by microbial communities and how these communities respond to changing soil conditions.

The Arctic tundra provides a relatively pristine environment in which to make direct studies of climate change impacts on soil C dynamics. Climate change is expected to have a great impact in polar environments and so it is imperative to improve our understanding of the many interactions that exist and how global C dynamics are likely to be influenced in the future.

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Chapter I

Mills, R.,* **Glanville, H.**,* McGovern, S., Emmett, B., Jones, D.L. 2011. Soil respiration across three contrasting ecosystem types: comparison of two portable IRGA systems. ***Journal of Plant nutrition and Soil Science* 174, 532-535.**

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H.G. and R.M conceived and developed the experiment. H.G. and R.M carried out fieldwork and laboratory and data analysis. H.G. led the writing on the results section, the methods and conclusion. R.M. led writing the introduction and abstract and prepared the figures and tables. H.G. and R.M. co-authored the discussion. S.M. performed the statistical analysis. All authors discussed results and contributed to preparation of the manuscript.

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Soil respiration across three contrasting ecosystem types: comparison of two portable IRGA systems

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Abstract

An accurate assessment of soil respiration is critical for understanding and predicting ecosystem responses to anthropogenic perturbation such as climate change, pollution, and agriculture. Infra-red gas analyzer (IRGA)–based field measurement is the most widely used technique for assessing soil-respiration flux rates. In this study, respiration rates obtained with two common IRGA systems (LI-COR 8100 and PP Systems EGM-4) were compared across three ecosystem types. Our results showed that both methods were highly comparable in their flux estimates, but the associated methodology used (notably the use or absence of a soil collar) resulted in greater uncertainty in flux rates and a greater degree of intrasite variation. Specifically, the use of collars significantly decreased the flux estimate for both IRGAs compared to the no-collar estimate. The disturbance caused by collar insertion was assumed to be a major factor in causing the differing flux estimates, with root and mycorrhizal severance likely being the main contributor. We conclude that the two IRGAs used in this study can be reliably compared for overall flux estimates but emphasis is needed to validate a common measurement methodology.

Key words: carbon / CO_2 / flux chamber / greenhouse-gas emission / methodology

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1 Introduction

Soil respiration is the major pathway of C efflux from terrestrial systems and therefore represents an important integrated reporter of ecosystem functioning. Soil respiration includes root and microbial respiration, and bulk turnover of organic matter (OM) which all contribute to the release of CO_2 (*Nay* et al., 1994; *Hill* et al., 2004). Consequently, accurate quantification of gaseous fluxes from soil remains paramount to furthering our understanding of soil C flow and ecosystem resilience (*Davidson* et al., 2002; *von Lützow* et al., 2006; *Widén* et al., 2003). The most common method for measuring soil CO_2 efflux employs infra-red gas analyzers (IRGA), which measure the increase in enclosed-chamber CO_2 concentration over a specified time (*Luo* and *Zhou*, 2006). There is currently no internationally recognized standard protocol for measuring soil respiration and rarely have the different measuring methods, which take subtly different approaches, been validated either *in situ* or in artificial media (*Norman* et al., 1997; *Janssens* et al., 2000; *Pumpanen* et al., 2004). Therefore the results obtained from any individual method can be contentious due to a lack of a calibrated standard to which to compare the data, and any method for calculating soil respiration can only be compared relatively (*Nay* et al., 1994; *Widén* et al., 2003). The two IRGA systems within the current study have not previously been directly compared in the field, and as they are two of the most widely used systems, the comparison is of significant relevance. Possible causes for differences between different IRGA-based measurements may include differences in IRGA and chamber design (*e.g.*, cuvette area and volume, the use of collars, presence or absence of chamber vents), measurement parameters (*e.g.*, enclosure time, chamber flow rate, purge parameters, *etc.*) and CO_2-flux algorithms (*e.g.*, with and without moisture and temperature correction). It is likely that these effects may also be dependent upon soil type and vegetation in which the measurements are being undertaken.

This study was devised to directly compare two commonly used and commercially available IRGA-based CO_2 analyzers; the LI-COR 8100 (LI-COR Biosciences, Lincoln, NE, USA) and the PP Systems EGM-4 equipped with a SRC-1 chamber (PP Systems, Hitchin, Herts, UK). The two main aims for this study were: (1) to assess if there was any inherent difference in measurements obtained from the two systems in the field, as supported by other comparative studies (*Bekku* et al., 1995; *Janssens* et al., 2000; *Pumpanen* et al., 2004; *Luo* and *Zhou*, 2006) and (2) to assess whether the inclusion or absence of a collar influences the measured CO_2 flux (as postulated by *Pumpanen* et al., 2004).

2 Methodology

2.1 Site description

Three contrasting ecosystems were selected for this study. Site 1 (Eutric Cambisol; 53°14′ N 4°1′ W) constituted a freely draining, intensively sheep-grazed (> 5 ewe ha^{-1}), fertilized (120 kg N ha^{-1} y^{-1}) agricultural grassland dominated by *Lolium perenne* L. and *Trifolium repens* L. Site 2 (Haplic Pod-

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Table 1: Site characteristics of the three ecosystem types. Where applicable, soil values are expressed on a dry-weight basis. Values represent mean ± SEM ($n = 3$).

	Lowland grassland	Upland grassland	Upland heathland
Soil type	Eutric Cambisol	Haplic Podzol	Orthic Podzol
Depth of organic layer / cm	15	15	7
Organic matter / g kg^{-1}	64 ± 6	713 ± 39	527 ± 84
Bulk density / g cm^{-3}	1.14 ± 0.01	0.19 ± 0.01	0.26 ± 0.04
$pH_{(H_2O)}$	5.93 ± 0.45	3.95 ± 0.04	3.71 ± 0.06
Electrical conductivity / µS cm^{-1}	123 ± 16	99 ± 36	99 ± 19
Total soil C / g kg^{-1}	38 ± 3	412 ± 13	370 ± 6
Total soil N / g kg^{-1}	3.8 ± 0.1	22.6 ± 1.6	12.3 ± 1.7
Microbial biomass C / µg g^{-1}	197.98 ± 32.87	221.39 ± 17.07	248.32 ± 3.71
Microbial biomass N / µg g^{-1}	23.06 ± 0.87	18.70 ± 1.96	13.05 ± 1.64
Exchangeable Na / meq kg^{-1}	1.4 ± 0.1	2.6 ± 0.2	2.8 ± 0.3
Exchangeable K / meq kg^{-1}	4.2 ± 1.6	3.0 ± 0.4	1.9 ± 0.4
Exchangeable Mg / meq kg^{-1}	3.5 ± 0.3	2.0 ± 0.3	8.1 ± 0.8
Exchangeable Ca / meq kg^{-1}	68.5 ± 1.3	3.4 ± 0.3	14.3 ± 0.7

zol; 53°12′ N 4°0′ W) constituted a freely draining, low-intensity sheep-grazed (< 1 ewe ha^{-1}), unfertilized agricultural grassland dominated by *Nardus stricta* L. and *Agrostis cailaris* L. Site 3 (Orthic Podzol; 53°03′ N 3°28′ W) constituted a poorly drained, ungrazed *Calluna vulgaris* L. and *Vaccinium myrtillus* L. heathland. The major characteristics of the soils are presented in Tab. 1, and represent values obtained from bulked samples of the top 15 cm of soil.

2.2 Plot preparation

In October 2009, five PVC collars (10 cm ∅, 4.4 cm depth with a 0.2 cm beveled edge at one end for easy insertion) were inserted into the ground to a depth of 2 cm at both grassland sites. Collars were distributed in a "W" shape across the site, with 20 m between each collar. At each collar, vegetation was clipped to 1 cm above the soil surface both within the collar area, and in a similarly sized area within 10 cm of the collar. This second clipped area would provide the "no-collar" respiration measurement. Collars were then left for 14 d prior to measurements being made to allow the soil and excised roots to settle after disturbance. Soil moisture and soil temperature were measured (using Delta-T ML2 theta probe and a standard digital 10 cm temperature probe respectively). These measurements yielded no spatial variation within each site, and no difference between collar and no-collar measurement points. This approach gave a total of five collared and five no-collared respiration sampling points at each site. Soil respiration was determined at each site using both a LI-COR LI-8100 and an EGM-4 with an SRC-1 chamber. Both IRGAs used a 60 s enclosure time, a 15 s purge time, and a 15 s equilibration/dead band time. Measurements were taken sequentially starting at the upland grassland, then the lowland grassland followed by the upland heathland. The upland heathland site was sampled 2 d after the two grasslands, all sampling being carried out within an hour of midday. The later sampling of the heathland site was due to logistical constraints. No notable change in prevailing conditions occurred during the sampling period. Data was automatically fitted to either a linear or nonlinear function by the LI-8100 software, and the linear option was chosen as the default fit for the EGM-4. The implications of this decision were investigated by comparing flux estimates with the linear and quadratic algorithm with both IRGAs. Both the linear and quadratic estimates were in very close agreement with each other (linear regression r^2 value > 0.99; $p < 0.001$) from which it was concluded that the linear flux equation was sound. IRGA accuracy was checked against standard gases routinely prior to analysis.

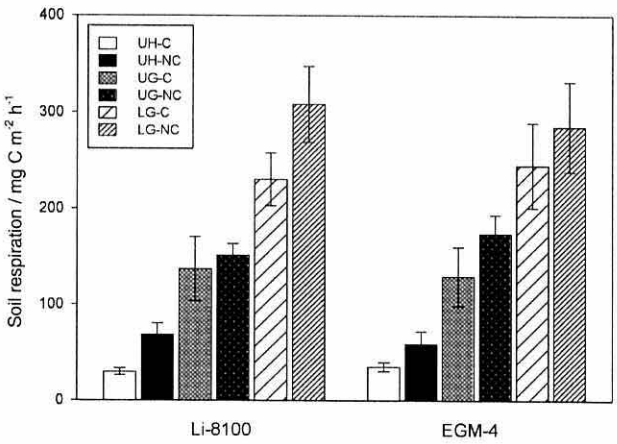

Figure 1: Mean ($n = 5$) soil-respiration rates for each site (UH = Upland Heathland, UG = Upland Grassland, LG = Lowland Grassland) and collar/no-collar treatment (C = collar, NC = no collar) grouped by IRGA type. Vertical bars show one standard error of the mean.

2.3 Data analysis

Data were visually inspected for normality using a quantile-quantile plot against a normal distribution (*Crawley*, 2007). Data were not normally distributed and were therefore log-transformed to achieve normality and equality of variances. Analysis was completed via a three-way ANOVA, with factor levels of machine, site, and treatment. Statistical procedures were carried out using the statistical package "R" v 2.8.1 (*R Development Core Team*, 2008), with $p = 0.05$ used as the upper limit for statistical significance. Data were back transformed for graphical representation using Sigma Plot 10 (Systat Software Inc., Chicago, IL, USA).

3 Results

Significant differences in mean respiration rates were observed between the three sites ($p < 0.001$) and increased in the order Orthic Podzol (heathland) < Haplic Podzol (unimproved grassland) < Eutric Cambisol (improved grassland). Overall, there was no significant difference ($p = 0.98$) in the respiration from the three sites when measured with either the EGM-4 or LI-COR-8100 IRGA (Fig. 1). This result provides confidence when comparing studies that use two different IRGA approaches. The comparison of collar and no-collar treatment did, however, raise some interesting findings. There was a significant ($p < 0.001$) difference between the collar- and no-collar-treatment estimate of soil respiration (Fig. 1). Across all sites, the soil CO_2 efflux in the presence of collars was (25 ± 11)% and (20 ± 6)% lower for the LI-COR-8100 and EGM-4, respectively, in comparison to measurements made without collars (Fig. 2).

4 Discussion

From the results presented here it is apparent that the presence of collars may result in a relatively small but systematic bias in both IRGA measurement systems. This is likely to be partly due to the effectiveness of the seal between the IRGA and the soil under the presence or absence of a collar. However, this must be balanced against the inherent variability in soil respiration over both space and time. Our results suggest that spatial variability in soil respiration was also highly site-dependent with standard errors in measurements ranging from 8% to 24% of the mean value. There are a number of biological considerations to be made when interpreting the effect collar insertion had upon measured flux rates. It would be expected that physical disturbances caused by collar insertion (*Davidson* et al., 2002) would increase the rate of soil respiration by severing roots and mycorrhizal hyphae, thus contributing a greater pool of labile C to soil solution (*Hanson* et al., 2000; *Johnson* et al., 2002). However, this labile flush caused by excision is likely to be turned over rather rapidly and as such, it would be expected that this phenomenon would be short-lived. Indeed, *Edwards* (1991) found excised roots to cause an initial increase in soil respiration compared to intact cores, but a stabilization of rates to within 30% of intact core rates was found within 2 d of root excision. However, it has been shown in other studies that severing roots from aboveground biomass causes a marked and rapid reduction in total soil respiration (*Bingham* and *Rees*, 2008). This would suggest that the rate of recovery after disturbance might be highly variable across ecosystem types, perhaps explaining some of variation in collar *versus* no-collar differences found across the sites in the present study.

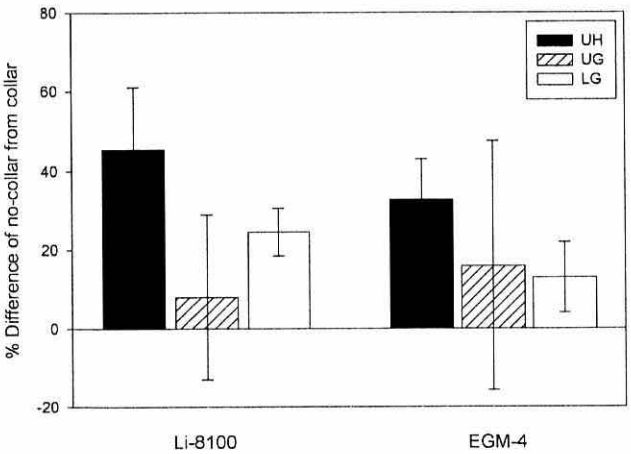

Figure 2: Respiration rates under the no-collar treatment, expressed as a percentage difference from collar treatment. Data is labeled by site (UH = Upland Heathland, UG = Upland Grassland, LG = Lowland Grassland) and grouped by IRGA type. Vertical bars show one standard error of the mean ($n = 5$).

The maintenance of a physical barrier (*i.e.*, the presence of a collar) between excised material and live roots and hyphae could present a notable obstacle for the re-establishment of homogenous steady soil conditions. It is likely that the 14 d period between collar installation and measurement would be insufficient for this process to have occurred. The lack of live, intact roots within the collar soil would have obvious implications for C transfer from plants to the microbial biomass, and therefore for total respired C. Soil-moisture modifications created by removing the transpiration demand of live roots through collar placement could cause a reduction in decomposition, as excess moisture will retard mineralization. This is likely to be most notable in soils which have a naturally impeded drainage, such as peaty soils or soils with significant clay layers. Also, the contribution of root respiration to total respiration might simply make up this difference, if we assume severance causes a complete halt in the respiration of root material not found within the collar area. This would be a sensible conclusion given that root respiration has in other studies been found to account for ≈ 50% of total soil respiration (*Ohashi* et al., 2000; *van Hees*, 2005). Although this value is highly variable and ecosystem/plant/soil-dependent, with reports ranging between 10% and 90% of total soil respiration (*Hanson* et al., 2000).

The good similarity in respiration estimates between the two IRGA types is in agreement with a previous study on forest soils by *Janssens* et al. (2000), who found a 10% difference between two IRGAs. This confirms the reliability of comparing site estimates of soil respiration flux using the two different IRGAs studied here. The application of collars to aid in flux estimates continues to be a source of variability. It is sug-

gested, however, that simple modifications to collar design taking into account soil and vegetation factors, such as moss and litter depth, keeping in mind the range of disturbances collar insertion could have on biological components should allow for a reliable, standardized approach.

5 Conclusion

In conclusion, the two IRGA systems used in this study have been shown to be reliably comparable when a common collar approach is used. However, disturbance caused by collar insertion is likely to affect both plant and microbial respiration, but the magnitude and duration of this effect is poorly understood and therefore requires further study.

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Chapter II

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H.G. and D.L.J. conceived and developed the experiment. H.G. carried out field sampling, conducted laboratory analyses for background soil chemical characteristics and ^{14}C-labelling and data analysis. J.R. assisted with sampling and laboratory data collection. All authors discussed results and contributed to preparation of the manuscript.

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Mineralization of low molecular weight carbon substrates in soil solution under laboratory and field conditions

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ABSTRACT

A more detailed mechanistic understanding of how low molecular weight (MW) carbon (C) substrates are mineralized within the rhizosphere by soil microbial communities is crucial to accurately model terrestrial C fluxes. Currently, most experiments regarding soil C dynamics are conducted *ex-situ* (laboratory) and can fail to account for key variables (e.g. temperature and soil water content) which vary *in-situ*. In addition, *ex-situ* experiments are often highly invasive, e.g. severing root and mycorrhizal networks, changing the input and concentrations of low MW exudates within soil. The aim of this study was to directly compare the mineralization rates of 31 common low MW C substrates under *ex-* and *in-situ* conditions. In addition, we also assessed the inter-annual field variability of substrate mineralization rates. We added trace concentrations of 31 individual ^{14}C-labelled common low MW C substrates into the top soil of an agricultural grassland and monitored the mineralization rates by capturing $^{14}CO_2$ evolved from the soil over 7 d. Our results showed that the contribution of low MW C components to soil respiration was highly reproducible between parallel studies performed either *in-situ* or *ex-situ*. We also found that differences in the mineralization of individual compounds were more variable inter-annually in the field than between the laboratory and the field. Our results suggest that laboratory-based C mineralization data can be used to reliably parameterize C models but that multiple experimental measurements should be made over time to reduce uncertainty in model parameter estimation.

1. Introduction

Plant and soil respiration provides the major pathway of carbon (C) efflux from terrestrial ecosystems. Soils are postulated as having a pool size of 1500 Pg C, double the size of the atmospheric pool, with an estimated global flux from soil respiration of ca. 73 Pg C y^{-1}; this exceeds all other terrestrial-atmospheric C fluxes except for gross primary productivity (Raich and Schlesinger, 1992; Schlesinger and Andrews, 2000; Rustad et al., 2000). CO_2 production can arise directly from plant roots, microbial breakdown of soil organic matter and to a lesser extent mineral carbonate dissolution. The magnitude of the soil C flux is dependent upon a wide range of soil factors (e.g. temperature, soil water content availability, pH, salinity), plant factors (e.g. root density, mycorrhizas, vegetation community composition) and anthropogenic mediated perturbations (e.g. land use change or pollution) (Raich and Tufekcioglu, 2000). Soil microbial activity and subsequent soil respiration is typically limited by the availability of labile C substrates (Aldén et al., 2001; Demoling et al., 2007), which are derived mainly from rhizodeposition which includes root exudation (Nguyen, 2009; Jones et al., 2009) and, root and mycorrhizal hyphal turnover within soil (Gill and Jackson, 2000; Wallander, 2006).

The majority of C in soil is made up of high molecular weight (MW) compounds (e.g. cellulose, protein, chitin), including recalcitrant humic substances with slow turnover rates (Van Hees et al., 2005; Vinken et al., 2005). For plants and soil microbial communities to assimilate these high MW compounds, they need to be first broken down into low MW compounds capable of transportation into the cell. These low MW compounds constitute the minority of the dissolved organic C (DOC) pool typically representing <10% of the total DOC (Van Hees et al., 2005, 2008; Fujii et al., 2010). These low MW organic compounds are derived from soil organic matter (SOM) by the action of extracellular enzymes released directly into the soil from cellular lysis and root and microbial exudation (Jones et al., 2009). Within this low MW pool, the predominant compounds present are sugars, organic acids, amino acids, amino

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sugars and nucleotides which represent the solutes central to metabolism in most organisms and the breakdown products of the dominant polymers in soil (Van Hees et al., 2005, 2008). Generally, low MW compounds are more labile and have faster turnover rates than their high MW counterparts (Van Hees et al., 2005, 2008; Fujii et al., 2010). These low MW compounds are rapidly taken up, assimilated and turned over predominantly by the soil microbial community and to a lesser extent by plant roots (Jones et al., 2004; Van Hees et al., 2005; Boddy et al., 2007). Due to this rapid flux, turnover of low MW compounds appears to dominate the total CO_2 flux from soil, despite their low concentration within soil solution (Van Hees et al., 2005; Rousk et al., 2011). Previous studies indicate the turnover of low MW compounds can be partitioned into different pools, with short term experiments often corresponding to a biphasic model (Chotte et al., 1998; Saggar et al., 1996). A first pool is ascribed to the rapid efflux of CO_2, indicated by the ^{14}C label, as the substrate is immediately used for catabolic processes (i.e. respiration), relating to the depletion of the soil solution pool (Jones, 1999). A second pool is thought to be attributable to the substrate first being utilized for anabolic processes (cell growth and maintenance), which are then subsequently broken down and respired (i.e. microbial biomass turnover).

There is still uncertainty surrounding the relative contribution of different sources of below-ground respiration (Kuzyakov, 2006; Koerber et al., 2010). Further, the efflux of CO_2 represents the summed loss of many individual C compounds which are turned over simultaneously by the soil microbial community. To gain a deeper mechanistic understanding of how soil respiration is regulated it is imperative to start to look at the processes and pathways that affect individual substrate pool sizes and their respective dynamics in both short- and long-term C flux studies.

Currently, most experiments regarding soil C dynamics are carried out *ex-situ*, and often fail to account for a multitude of factors that can influence soil respiration *in-situ* (e.g. diurnal variation in temperature and soil water content, diurnally regulated plant C inputs). *Ex-situ* sampling is often highly invasive with root and hyphal networks being severed, thus preventing recharge of the DOC pool from root and hyphal exudates (Vance and Chapin, 2001; Oburger and Jones, 2009). *In-situ* measurements offer logistical challenges, but they address the complex interactions within the terrestrial ecosystem (e.g. temperature and soil water content), which may play a vital role in mineralization of low MW C substrates. To accurately predict long-term trends, it may be necessary for *in-situ* experiments to encompass inter-annual variation, rather than taking a single time point and scaling up to approximate the total annual flux to predict long-term trends. Here, we study how inter-annual field variability and laboratory-field studies affect the mineralization rates and fates of 31 common ^{14}C-labelled low MW C substrates. We hypothesized that conditions in the laboratory (*ex-situ*) are likely to over-estimate total flux as they do not fully encompass factors (e.g. presence of roots and mycorrhizas, changing climatic variables) that are thought to control C turnover in the field (*in-situ*).

2. Materials and methods

2.1. Field site

The experimental site consisted of a freely draining agricultural grassland from a hyper-oceanic climatic region in Abergwyngregyn, Gwynedd, North Wales (53°14′N, 4°1′W). The mean annual rainfall is 1250 mm and the mean annual soil temperature at a soil depth of 10 cm is 11 °C. Soil temperature and rainfall data fluctuate monthly between 2009 and 2011 for the field site, with soil water content showing the greatest variability (Fig. 1). Field work was undertaken during the autumn of 2009 and 2010 when soil

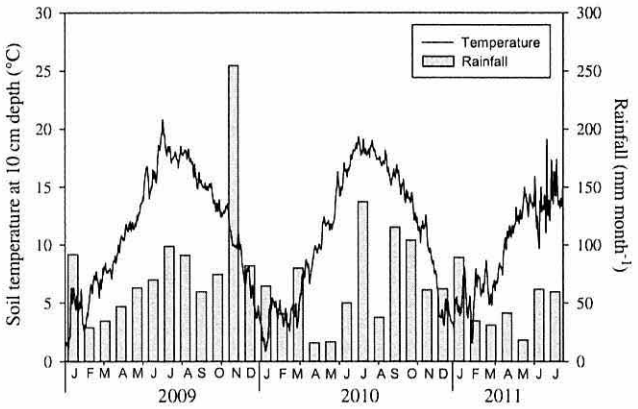

Fig. 1. Monthly soil temperature and rainfall data for the experimental field site. Experiments conducted in Autumn 2009 and 2010.

temperatures at 10 cm depth, ranged between and 14.3–18°C (2009) and 13.8–17 °C (2010) (Fig. 1). The sandy clay loam textured soil is classified as a Eutric Cambisol (FAO) or Dystric Eutrudepts (US Soil Taxonomy) and is derived from Ordovician post-glacial alluvial deposits. The vegetation at the site consists of perennial rye grass (*Lolium perenne* L.) and white clover (*Trifolium repens* L.) and is subject to intensive sheep grazing (>5 ewe ha^{-1}) and receives regular fertilizer addition (120 kg N ha^{-1} y^{-1}). Due to the high root density in the grassland (0.35 ± 0.02 kg m^{-2} in the top 10 cm), all the sampled soil was essentially classified as rhizosphere soil.

2.2. Soil characterisation

Basic soil characteristics were measured prior to conducting the experiment (Table 1). Independent samples (n = 3) were collected from a 0–10 cm depth, stored in plastic bags and brought to the laboratory for analysis. After sieving to pass 5-mm, soil water content was measured gravimetrically (24 h at 105 °C). Soil pH and electrical conductivity (EC) were determined in a 1:2.5 (w/v) soil-to-deionised water mixture using standard electrodes. Soil respiration was measured *in-situ* using an EGM-4 IRGA with a SRC-1 soil respiration chamber (PP Systems Ltd, Hitchin, UK). Total C and N were determined with a CHN2000 analyzer (Leco Corp., St Joseph, MI).

Soil solution was extracted within 12 h of soil collection from the field by the centrifugal-drainage method (3200 *g*, 15 min, 20 °C; Giesler and Lundström, 1993). The extracted soil solutions were

Table 1
Selected properties for Eutric Cambisol soil used in the mineralization studies. Values represent means ± SEM for each block (n = 3) for all measurements except for below-ground soil respiration where n = 15 for each block.

Properties	2009	2010	Significance
Soil water content (%)	28.9 ± 0.9	32.1 ± 2.3	ns
Soil organic matter (%)	7.37 ± 0.34	7.97 ± 0.52	ns
$pH_{(H_2O)}$	5.35 ± 0.04	4.77 ± 0.02	ns
EC (μS cm^{-1})	61.3 ± 5.8	203 ± 20.7	**
Below-ground respiration (g CO_2 m^{-2} h^{-1})	1.71 ± 0.09	1.67 ± 0.29	ns
Microbial biomass C (g kg^{-1})	1.99 ± 0.33	1.33 ± 0.12	ns
Microbial biomass N (g kg^{-1})	0.23 ± 0.01	0.09 ± 0.01	***
Total C (g kg^{-1})	37.7 ± 2.45	28.8 ± 1.5	*
Total N (g kg^{-1})	3.77 ± 0.11	3.09 ± 0.14	*
Soil solution organic C (mg C l^{-1})	41.8 ± 2.45	43.0 ± 5.30	ns
Soil solution organic N (mg N l^{-1})	6.07 ± 0.49	10.0 ± 1.76	ns
Soil solution NO_3^- (mg N l^{-1})	2.20 ± 0.12	1.34 ± 0.32	ns
Soil solution NH_4^+ (mg N l^{-1})	0.52 ± 0.07	0.03 ± 0.01	***
Soil solution phenolics (mg l^{-1})	0.30 ± 0.03	2.05 ± 0.37	***

further centrifuged for 5 min at 18,000 *g* to remove any suspended particles and the soil solution stored in polypropylene vials at −20 °C prior to soil solution analysis and ^{14}C-labelling.

2.3. Soil solution chemical analysis

Dissolved organic C (DOC) and total dissolved N (TDN) were determined with a Shimadzu TOC–TNV analyzer (Shimadzu Corp., Kyoto, Japan). NH_4^+ was analyzed following the salicylate–nitroprusside and hypochlorite procedure of Mulvaney (1996) whilst NO_3^- was measured using the vanadate method of Miranda et al. (2001). Total dissolved phenolics were assayed colorimetrically using the Folin–Ciocalteu reagent (F9252; Sigma–Aldrich Inc.) according to Velioglu et al. (1998) and assuming a C content of 76%.

2.4. C substrate mineralization

For the mineralization studies, 450 μl of soil solution was spiked with 50 μl of each of the 31 individual ^{14}C-labelled substrates (Table S1). The substrates selected represented common root exudates and included examples of each of the main constituents of the low MW DOC pool (sugars, amino sugars, amino acids, organic acids and nucleotides). Urea was also selected as it is a common input in grazed environments. $NaH^{14}CO_3$ was used as an inorganic control to evaluate ^{14}C capture efficiency (Boddy et al., 2007). The concentration of ^{14}C-labelled substrate added to the soil solution was very low in all cases (<10 nM) and was not expected to significantly affect the intrinsic concentrations in the soil solution.

For the field experiments, a 10 × 10 m^2 area was fenced off and three blocks (1 × 7 m) delineated in this area to provide replicates. A polypropylene cylinder (6.1 cm^2) was pushed ca. 2 cm deep into the soil. An aliquot (500 μl) of the spiked ^{14}C-labelled soil solution was then distributed onto the soil surface and the solution allowed to infiltrate into the soil (<3 s). Oburger and Jones (2009) previously measured the penetration depth of the soil solution in the field to be ca. 2–3 cm. In order to catch evolved $^{14}CO_2$, a 1 M NaOH (1 ml) trap was placed inside each cylinder and then hermetically sealed. The cylinder was then covered with tubing to ensure dark conditions and thus prevent plant re-fixation of any evolved $^{14}CO_2$. The NaOH traps were changed after 0.25, 0.5, 1, 2, 4, 8, 12, 24, 48, 72, 96, 120, 144 and 168 h. The NaOH traps were then mixed with Scintisafe 3® scintillation cocktail (Fisher Scientific, Leicestershire, UK) and subsequently analyzed using a Wallac 1404 liquid scintillation counter (Wallac EG&G, Milton Keynes, UK) to determine the amount of $^{14}CO_2$ captured. The substrates were applied to each block in a random number generated pattern.

For the laboratory experiment, representative top soil samples (0–15 cm) were collected from the same three delineated blocks used for the field experiment, and placed in plastic bags and immediately transported to the laboratory. Large roots and stone fractions (>2 mm) were removed from the soil and 5 g of soil subsequently placed in 50 ml polypropylene vials. The same amount, as per the field experiment (500 μl), of each of the ^{14}C-labelled low MW substrates were added to the soil in a polypropylene cylinder (6.1 cm^2) and hermetically sealed. The NaOH traps were placed inside the polypropylene vials and were changed at the same time intervals as described above. The samples were kept at room temperature (20 ± 1 °C).

2.5. Data and statistical analysis

To measure the efficiency of $^{14}CO_2$ capture, a single first-order kinetic equation with asymptote was fitted to the data from the $NaH^{14}CO_3$ treatment where

$$f = y_0 + \left(a \times \exp^{-kt}\right) \quad (1)$$

and where f is the amount of ^{14}C remaining in the soil, y_0 is the asymptotic value equating to the amount of $^{14}CO_2$ that is unrecoverable, a is the total amount of $^{14}CO_2$ recovered in the NaOH traps after 168 h, k is the rate-coefficient describing the turnover of pool a and t is time. The half-life of $NaH^{14}CO_3$ loss from soil ($t_{1/2}$) can then be calculated as:

$$t_{1/2} = \ln(2)/k \quad (2)$$

Incomplete $^{14}CO_2$ recovery was ascribed to upward losses to the atmosphere when the NaOH traps were changed and downward losses by diffusion from the base of the soil cylinder which were left connected to the rest of the soil profile. Data from the $NaH^{14}CO_3$ treatment provided the capture efficiency value used for the rest of the organic substrate treatments.

Previous studies indicate organic C substrate mineralization follows a biphasic pattern in soil (Chotte et al., 1998; Saggar et al., 1996). This biphasic pattern can be explained by a double first-order decay equation,

$$f = y_0 + \left(a_1 \times \exp^{-k_1 t}\right) + \left(a_2 \times \exp^{-k_2 t}\right) \quad (3)$$

where a_1 describes the amount of ^{14}C allocated to the first mineralizable pool and k_1 is the rate constant for a_1. The proportion of C partitioned to the second slower pool a_2 is described by the rate constant k_2. The asymptote value y_0 represents the capture efficiency of the traps as found in the $NaH^{14}CO_3$ treatment. The half-life of the first mineralization pool a_1 can be calculated using the following equation:

$$t_{1/2} = \ln(2)/k_1 \quad (4)$$

Calculating the half-life for the slower second phase a_2 is subject to uncertainty as the connectivity between these pools a_1 and a_2 is unknown (Saggar et al., 1999; Boddy et al., 2007).

Eq. (3) cannot be solved explicitly for finding the half-life of the individual substrates in the soil system (fast and slow pools combined). Therefore, for each substrate the amount of ^{14}C remaining in the soil at $t_{1/2}$ for both pools ($f_{1/2}$) is defined as:

$$f_{1/2} = (a_1 + a_2)/2 \quad (5)$$

After Eq. (5) is substituted into Eq. (3), the total substrate half-life for individual substrates was derived numerically by applying the Newton–Raphson iteration method presented in Oburger and Jones (2009).

All experiments were conducted in triplicate. Kinetic parameters for all substrates together from both the laboratory-field comparison and the inter-annual field experiment were compared using linear regressions. Total substrate half-lives were compared across each treatment type (laboratory vs. field, inter-annual field variation) using MANOVA (multivariate GLM), with subsequent Fisher's LSD post hoc test. Statistical procedures were carried out using the statistical package SPSS 14.0 for Windows (SPSS Inc., Chicago, IL), with $P = 0.05$ used as the upper limit for statistical significance. All graphs were created using Sigma Plot 11 (Systat Software Inc., Chicago, IL).

3. Results

3.1. $NaH^{14}CO_3$ capture efficiency

Our data for the field 2009 study showed that on average 62.4 ± 4.8% of the added $NaH^{14}CO_3$ was recovered as $^{14}CO_2$ in the

NaOH traps and in 2010 the $^{14}CO_2$ capture efficiency was similar at 68.3 ± 7.6%. A single exponential decay model with asymptote fitted well to the $NaH^{14}CO_3$ experimental data (r^2 = 0.996 ± 0.001 for all treatments). Similarly, the capture efficiency from the laboratory experiment in 2010 was not significantly different to the field treatment with a recovery of 71.5 ± 7.3% of the added $NaH^{14}CO_3$ as $^{14}CO_2$.

3.2. ^{14}C organic substrate turnover

As expected, the $^{14}CO_2$ evolution from the individual substrates showed a biphasic pattern with an initial rapid phase of evolution followed by a slower secondary phase (data not presented). Across all three experiments, all substrates fitted well to a double first-order exponential decay function for the ^{14}C mineralization data (field 2009 average r^2 across substrates = 0.992 ± 0.001, field 2010 average r^2 across substrates = 0.990 ± 0.001, laboratory average r^2 across substrates = 0.987 ± 0.001). The partitioning of ^{14}C between pools a_1 and a_2 varied significantly between substrates ($P < 0.001$) with oxalate showing the greatest allocation of ^{14}C to the first phase across all experiments (76.0 ± 9.28% of the total ascribed to pool a_1) whilst leucine showed the greatest overall partitioning of ^{14}C to the slower secondary phase (95.9 ± 1.31% of the total ascribed to pool a_2).

3.3. Laboratory vs. field comparison

To further test the relationship between the different measurement conditions (i.e. inter-annual field comparison and

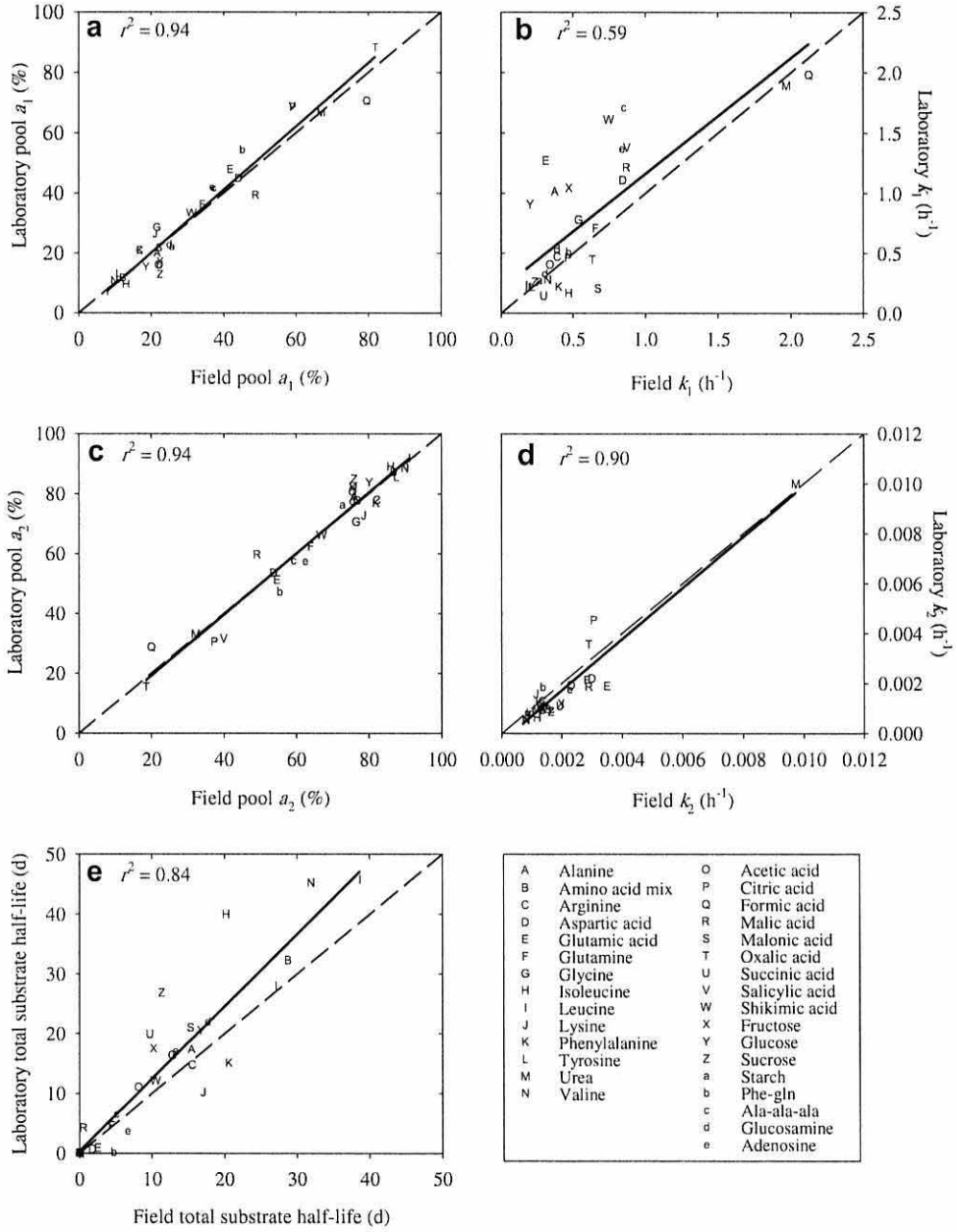

Fig. 2. Regression plots for field vs. laboratory comparison. Plots represent different kinetic parameters derived from first-order double exponential decay model (a–d), along with total substrate half-life derived from Newton–Raphson model (e). Points represent means (n = 3). Dashed line represents a 1:1 line and the solid line indicates the regression fit.

laboratory vs. field), parameters derived from the double first-order exponential decay equation and the Newton–Raphson models were subjected to linear regression analysis (Figs. 2 and 3). After visual and statistical analysis, formic acid k_2 values were omitted from both laboratory vs. field, and inter-annual field regression analyses after being identified as an outlier. Our results showed a strong linear relationship between the laboratory and field values for the amount of C partitioned into both microbial pool a_1 and a_2 (Fig. 2a and c; $r^2 = 0.94$ and 0.94 for pool a_1 and a_2 respectively, $P < 0.001$). The rate coefficients k_1 and k_2 describing the turnover rate of these two pools had a positive relationship for the laboratory-field comparison, although variation was higher for k_1 (Fig. 2b and d; $r^2 = 0.59$ and 0.90 for pool k_1 and k_2 respectively, $P < 0.001$). Overall, although the effect sizes were small, individual pool rate constants were more affected than C partitioning by the differing conditions under *ex-situ* compared to those performed *in-situ*. The Newton–Raphson model provided an indication for total substrate half-life and thus total contribution to overall respiration from each individual low MW C compound (Fig. 2e). The laboratory-field comparison again showed a significant positive relationship between estimates of total substrate half-life in soil ($r^2 = 0.84$, $P < 0.001$). It is worth noting that there were some differences noted in the total substrate half-lives between the laboratory and field experiment, but these were minimal. Only 3 out of the 31 C substrates (Fig. 4) were seen to differ significantly.

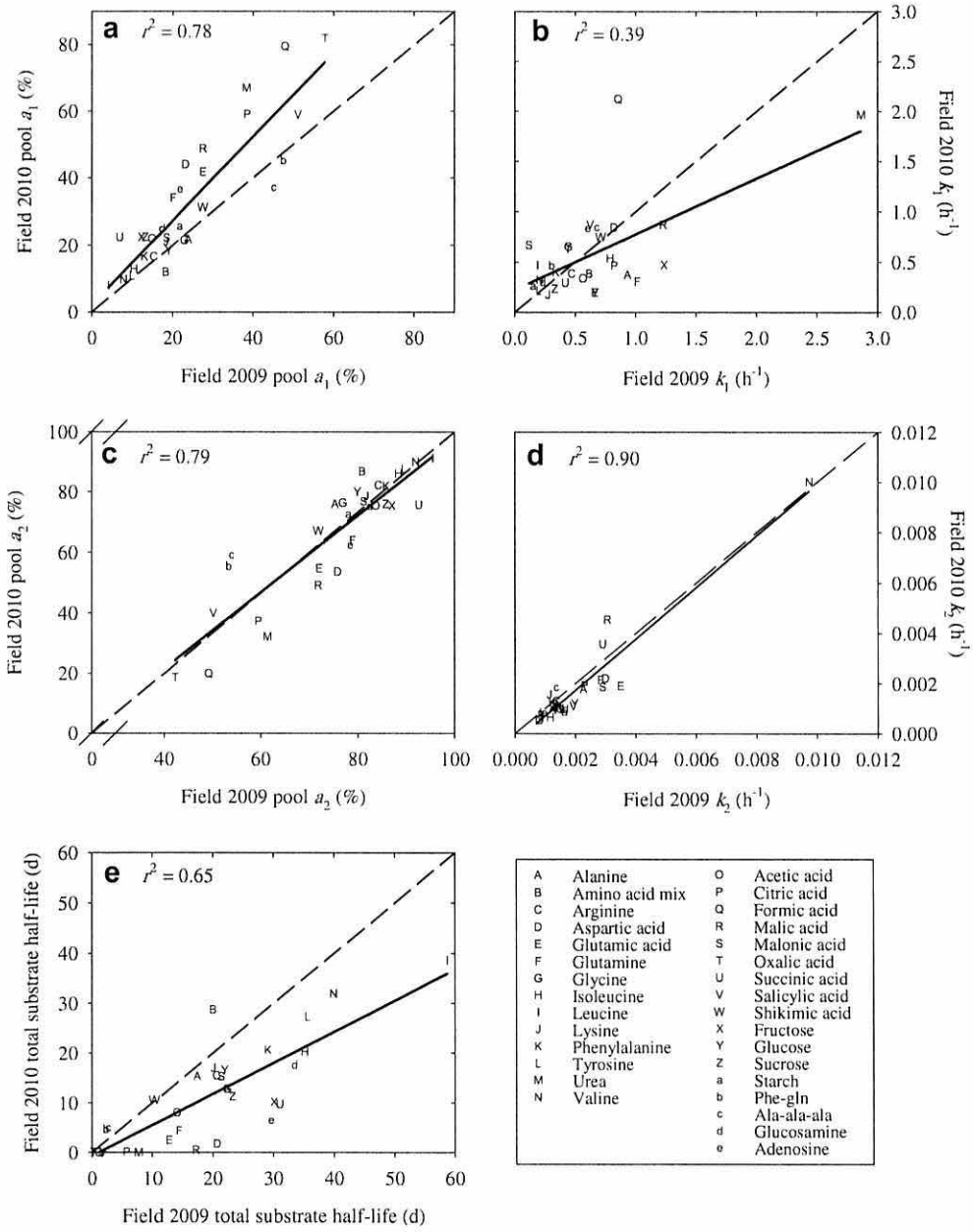

Fig. 3. Regression plots for inter-annual field comparison ($n = 3$). Plots represent different kinetic parameters derived from first-order double exponential decay model (a–d), along with total substrate half-life derived from Newton–Raphson model (e). Dashed line represents a 1:1 line and the solid line indicates the regression fit.

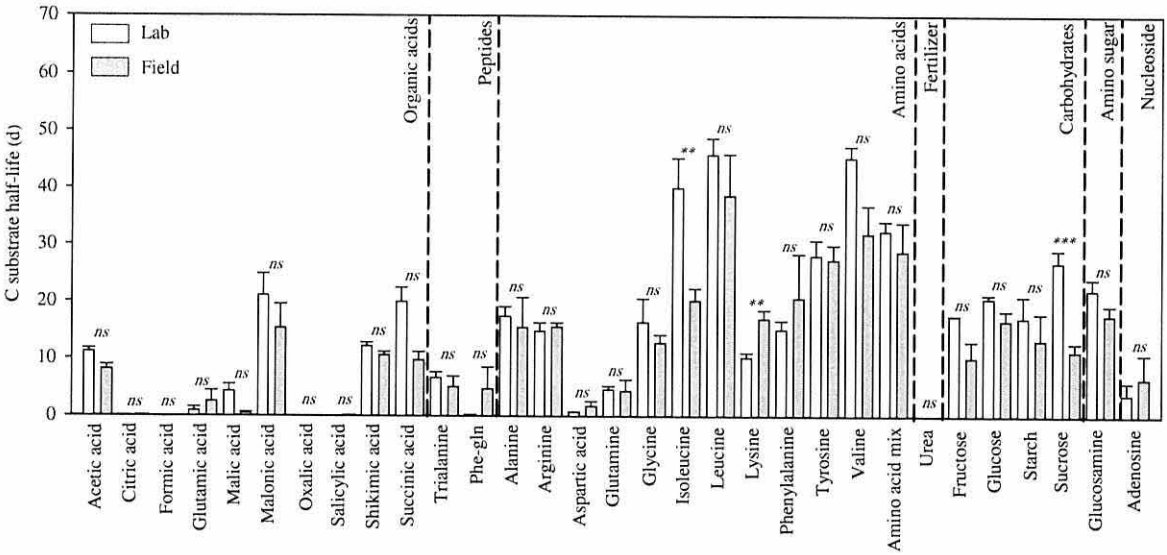

Fig. 4. Field vs. laboratory comparison for Newton–Raphson derived total substrate half-lives. Values represent means ± SEM ($n = 3$). NS, *, ** and *** represent values of, $P > 0.05$, $P < 0.05$, $P < 0.01$ and $P < 0.001$ respectively.

3.4. Inter-annual comparison

The inter-annual field comparison results also showed a strong linear relationship for the amount of C partitioned into pools a_1 and a_2 between the two measurement years (Fig. 3a and c; $r^2 = 0.78$ and 0.79 for pool a_1 and a_2 respectively, $P < 0.001$). Overall, C partitioning into these two microbial C pools was not significantly different between the two measurement years. In contrast, there was greater variation for the two rate coefficients (k_1 and k_2) over the measurement years than was observed for the variation in pool size (Fig. 3b and d; $r^2 = 0.39$ and 0.90 for pool k_1 and k_2 respectively, $P < 0.01$ for k_1, $P < 0.01$ for k_2). Overall, even though the effects were small, the total C substrate turnover rate was more affected than C partitioning within the microbial community. The Newton–Raphson model, which integrates the turnover of pools a_1 and a_2, also showed a significant positive relationship ($r^2 = 0.65$, $P < 0.001$) for overall total substrate half-life between the two measurement years, however, the differences were larger than in the laboratory-field study ($r^2 = 0.84$, $P < 0.001$). It is worth noting that there are more significant differences between individual substrates, in terms of total half-life compared with the laboratory-field study, with 13 out of the 31 substrates are identified as being significantly between the two field years (Fig. 5).

4. Discussion

4.1. Laboratory vs. field comparison

There are few systematic studies that directly compare mineralization rates of specific C substrates within the DOC pool from field and laboratory experiments. Laboratory-based studies are often preferred over field-based studies due to time and logistical constraints. Results obtained from laboratory studies are often assumed to be analogous to field results and are thus scaled up accordingly, potentially introducing experimental bias (Carlyle et al., 1998; Oburger and Jones, 2009). To better understand C

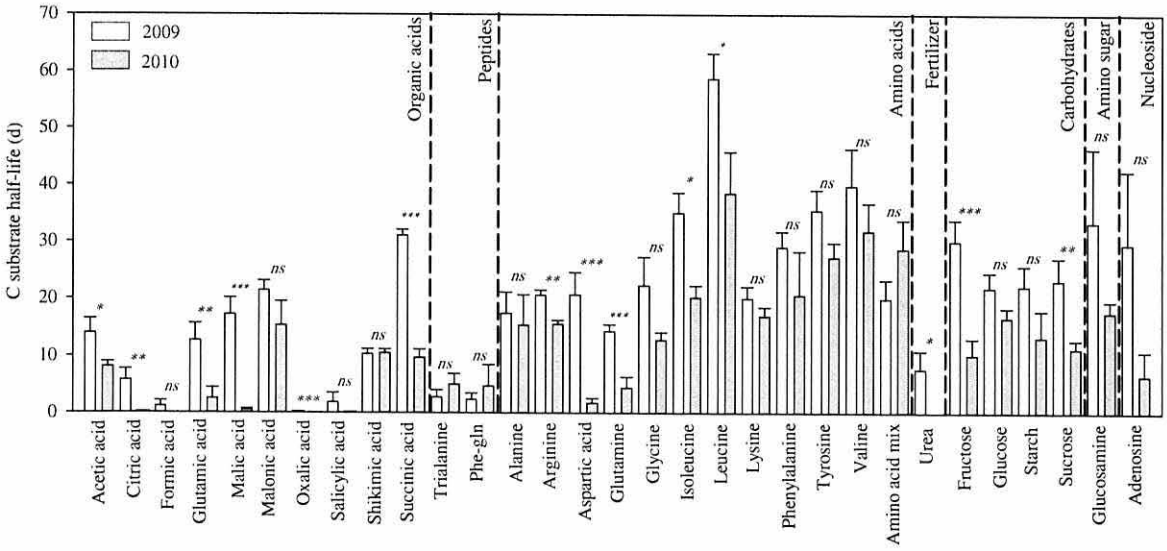

Fig. 5. Inter-annual field comparison for Newton–Raphson derived total substrate half-lives. Values represent means ± SEM ($n = 3$). NS, *, ** and *** represent values of, $P > 0.05$, $P < 0.05$, $P < 0.01$ and $P < 0.001$ respectively.

dynamics in terms of substrate turnover and contribution to respiration we need to ensure our results, whether from the field or laboratory, can realistically represent the natural system we strive to understand (Jones et al., 2004; Kuzyakov, 2006; Bengtson and Bengtsson, 2007; Strickland et al., 2011). This could then provide better and more accurate results for up-scaling and subsequent modelling of whole system dynamics, therefore improving our understanding of the mechanisms involved in terrestrial C cycling.

In this particular experiment we were primarily interested in looking at the overall relationship between laboratory-field and inter-annual field studies. Oburger and Jones (2009) found ^{14}C partitioning within the soil microbial community differed significantly between the laboratory and the field for ^{14}C-labelled citrate. However, these studies lacked the systematic power of the current analysis. Here we extend this analysis, and begin to develop a general understanding. We simultaneously studied the turnover of 31 C substrates to screen for effects that *ex-situ* conditions may have on the turnover of the complex mix of low MW C compounds common in the rhizosphere. In this analysis, we found minimal differences in overall C pool partitioning for all substrates in either the laboratory-field comparison, and also for the yearly field comparison. This emphasized how robust and reproducible estimates of low MW C substrate mineralization are. Further, it validates the use of laboratory-based studies, to estimate turnover of low MW C substrates in natural soil systems.

Mineralization rate constants showed the greatest variation between experimental conditions. Rate values (k_1) for the C substrates in soil solution were significantly correlated between field and laboratory repetitions, but we also note the highest variation in this data. This suggests that even though C pool partitioning does not seem to alter, regardless of experimental conditions, there are minor effects on individual rates. Consequently, changing microbial activity, rather than changing the size or composition of the microbial community, is likely to generate these differences, suggesting a disconnection between microbial biomass concentrations and functional contribution (Kemmitt et al., 2008; Rousk et al., 2009, 2011). Simfukwe et al. (2011) found a similar trend in their analysis of a broad range of UK soil types, with no significant differences seen in C substrate use of the soil microbial community, regardless of soil type. They suggest that there is evidence for significant functional redundancy in the soil microbial population with plant residue quality, soil water content and temperature being the well known key regulators of C storage. Our results largely support this hypothesis. Only 3 out of the 31 individual substrates showed significant differences in mineralization rates between the field and the laboratory. This suggests that disturbances are minimal when removing soil *in-situ* and transferring it *ex-situ* in terms of its impact on substrate mean residence time. Taken together, our data suggest that despite the pronounced differences between the artificial laboratory incubation and the field conditions, the mineralization of low MW C was largely unaffected.

4.2. Inter-annual field comparison

Rate values (k_1) were also significantly correlated between field years. However, our experimental data showed that, although overall variations were small, the most pronounced variation in k_1 values were from inter-annual field data. For field-based studies, it is therefore imperative to include inter-annual measurements if the contribution from that particular ecosystem to overall CO_2 flux is of interest. A significant relationship was also identified within the Newton–Raphson derived total substrate half-lives for the inter-annual field data, but with higher variation. This is consistent with the individual parameters obtained from the double first-order exponential decay model. Inter-annual measurements showed the greatest difference in terms of total substrate half-lives with 13 out of the 31 substrates showing a significant difference between field years, with 2010 half-lives being shorter than 2009. In particular, some key root exudates such as organic acids (citric, oxalic, malic and succinic acid) appear to have a significantly shorter half-life in 2010. We propose this could be due to low rainfall at the onset of the growing season (April and May). This could potentially delay the onset of the growing season and extend it further into autumn which may affect the patterns of root exudates which are linked to plant development (Jones et al., 2004). Temperature remained fairly consistent between years but diurnal fluctuations could also have an impact upon substrate half-lives. It is difficult to speculate as to why individual substrates are significantly different between years until their roles in metabolic functioning are better understood, which involves further transcriptomic, proteomic, and metabolomic research.

5. Conclusions

We show that the contribution of low MW DOC components to soil respiration is highly reproducible between parallel studies *in-situ* (intact soils systems in the field) and *ex-situ* (in laboratory microcosms), and that differences between the relative contribution of individual compounds to soil respiration are more variable between years in the field than between field and laboratory of the same year. This means that laboratory-based estimates of low MW participation to soil respiration accurately reflects *in-situ* processes and that laboratory measurements can further our understanding of intricate soil C fluxes in natural systems. This promises enormous potential for a more detailed understanding of how C constituents are processed by the microbial decomposer community to drive soil respiration, which is crucial to accurately model global terrestrial C fluxes.

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Appendix. Supplementary material

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.soilbio.2012.01.015.

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Appendix II

Supplementary material to accompany Chapter II.

Supplementary material

Table S1. Summary of the ^{14}C-labelled substrates used for laboratory-field study and inter-annual field comparisons

Substrate group	Low MW ^{14}C-labelled substrates	Activity	Supplier
Organic acids	Acetic acid	<10nM, 0.4 kBq ml^{-1}	ICN Pharmaceuticals Inc., USA
	Citric acid	<10nM, 0.4 kBq ml^{-1}	Amersham Biosciences, UK
	Formic acid	<10nM, 0.2-0.5 kBq ml^{-1}	Sigma-Aldrich Company Ltd., UK
	Malic acid	<10nM, 0.4 kBq ml^{-1}	Amersham Biosciences, UK
	Malonic acid	<10nM, 0.2-0.4 kBq ml^{-1}	ARC Inc., USA
	Oxalic acid	<10nM, 0.2-0.5 kBq ml^{-1}	Sigma-Aldrich Company Ltd., UK
	Salicyclic acid	<10nM, 0.2-0.4 kBq ml^{-1}	ARC Inc., USA
	Shikimic acid	<10nM, 0.2-0.4 kBq ml^{-1}	ARC Inc., USA
	Succinic acid	<10nM, 0.2-0.5 kBq ml^{-1}	Sigma-Aldrich Company Ltd., UK
Peptides	Ala-ala-ala	<10nM, 0.2-0.4 kBq ml^{-1}	ARC Inc., USA
	Phe-gln	<10nM, 0.2-0.4 kBq ml^{-1}	ARC Inc., USA
Amino acids	Alanine	<10nM, 0.2-0.3 kBq ml^{-1}	Amersham Biosciences, UK
	Arginine	<10nM, 0.2-0.3 kBq ml^{-1}	Amersham Biosciences, UK
	Aspartic acid	<10nM, 0.2-0.3 kBq ml^{-1}	Sigma-Aldrich Company Ltd., UK
	Glutamic acid	<10nM, 0.2-0.3 kBq ml^{-1}	Amersham Biosciences, UK
	Glutamine	<10nM, 0.2-0.3 kBq ml^{-1}	Amersham Biosciences, UK
	Glycine	<10nM, 0.2-0.3 kBq ml^{-1}	ARC Inc., USA
	Isoleucine	<10nM, 0.2-0.3 kBq ml^{-1}	Amersham Biosciences, UK
	Leucine	<10nM, 0.2-0.3 kBq ml^{-1}	Amersham Biosciences, UK
	Lysine	<10nM, 0.2-0.3 kBq ml^{-1}	ICN Pharmaceuticals Inc., USA
	Tyrosine	<10nM, 0.2-0.3 kBq ml^{-1}	Amersham Biosciences, UK
	Valine	<10nM, 0.2-0.3 kBq ml^{-1}	Amersham Biosciences, UK
	Amino acid mix (15 equimolar labelled amino acids)	<10nM, 0.2-0.3 kBq ml^{-1}	ARC Inc., USA
Fertilizer	Urea	<10nM, 0.2-0.3 kBq ml^{-1}	Sigma-Aldrich Company Ltd, UK
Carbohydrates	Fructose	<10nM, 0.2-0.5 kBq ml^{-1}	ICN Pharmaceuticals Inc., USA
	Glucose	<10nM, 0.3 kBq ml^{-1}	Sigma-Aldrich Company Ltd., UK
	Starch	<10nM, 0.2-0.5 kBq ml^{-1}	ICN Pharmaceuticals Inc., USA
	Sucrose	<10nM, 0.2-0.5 kBq ml^{-1}	ICN Pharmaceuticals Inc., USA
Amino sugar	Glucosamine	<10nM, 0.5 kBq ml^{-1}	ARC Inc., USA
Nucleoside	Adenosine	<10nM, 0.1 kBq ml^{-1}	ARC Inc., USA
Control	Sodium bicarbonate	<10nM, 0.6 kBq ml^{-1}	Sigma-Aldrich Company Ltd., UK

Chapter III

Glanville, H.C., Hill, P.W., Maccarone, L.D., Golyshin, P., Murphy, D.V., Jones, D.L. 2012. Temperature and water regulation of soil carbon, nitrogen, vegetation and microbial dynamics during snow melt in a high Arctic tundra ecosystem.

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H.C.G., D.V.M., and D.L.J. conceived and developed the experiment. H.C.G., D.V.M., and D.L.J carried out the fieldwork. H.C.G. carried out laboratory analyses characterising soil chemistry data, ran the ^{14}C-labelling experiments, extracted DNA from soil, carried out data analyses. L.D.M. carried out the qPCR analysis on the extracted DNA. D.V.M. facilitated the DNA analyses to be carried out in Western Australia. P.W.H. provided assistance with interpretation and presentation of the data. All authors discussed results and contributed to the preparation of the manuscript.

Temperature and water controls on vegetation emergence, microbial dynamics, and soil carbon and nitrogen fluxes in a high Arctic tundra ecosystem

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ABSTRACT

Arctic tundra ecosystems contain 14% of the global soil carbon (C) store which is becoming vulnerable to decomposition. Arctic soil organic matter (SOM) contains large amounts of old, recalcitrant, high molecular weight (MW) C compounds which are protected from decomposition whilst soils remain frozen. Climatic change alters soil temperature and water regimes in the Arctic, however, the impact of these changes on C decomposition and storage is poorly understood. We investigated vegetation emergence, microbial dynamics, and nutrient fluxes in response to snow melt on the high Arctic Svalbard archipelago using field and laboratory studies. Using bacterial and archaeal genetic material (16S rRNA) and ammonia-oxidising genes, microbial communities were quantified in transects across the active snow melt front. The effects of soil temperature and water content on SOM decomposition rates were measured using ^{14}C-labelled low and high MW compounds. Vegetation and below-ground microbial communities, in the field, responded rapidly with peaks in nutrient availability and soil respiration observed within 72 h of snowmelt. Temperature strongly drives early growing season C dynamics in the Arctic. We suggest the nutrient peaks following snowmelt, coupled with higher levels of DNA in the subniveal zone is due to the decomposition of bacteria and archaea from previous years. We show, in the laboratory, when soils thaw, mineralization of recalcitrant C (high MW) compounds was sensitive to soil water but not to increasing temperatures. In contrast, low MW compounds exhibited sensitivity to both temperature and soil water. We suggest that if future soil water content increases under climate change, high MW compounds could become more susceptible to decomposition, releasing more C to the atmosphere.

Keywords: Ecosystem function; Climate change; Nutrient resources; Polar; Functional ecology.

1. Introduction

Arctic tundra ecosystems contain 14 % of the global soil carbon (C) store which is becoming vulnerable to decomposition (Post et al., 1982; Grogan and Jonasson, 2005). Arctic soil organic matter (SOM) contains both labile, low molecular weight (MW) organic C (e.g. carbohydrates, amino acids, organic acids) which can be easily decomposed and metabolised by microbial communities and also older, more recalcitrant, high MW humic C compounds (e.g. lignin, cellulose, hemi-cellulose) which is less easily metabolised. Both low and high MW compounds are often protected from decomposition because of low temperatures, low microbial numbers and low enzymatic activity (Waldrop et al., 2010). Climatic change is postulated to expose more of this previously protected C to decomposition, releasing C to the atmosphere, however, the controls acting upon Arctic C dynamics are still uncertain (Dutta et al., 2006; Waldrop et al., 2010). Immediately after snow melt, labile low MW C compounds are thought to be released and be important for maintaining the DOC pool (Dutta et al., 2006). However, in the long term, C will also be released from more recalcitrant high MW C compounds found within old organic matter (Dutta et al., 2006). The effect of soil temperature and soil water content on the relative mineralization rates of low and high MW compounds could be important in terms of how these pools respond to climate change.

Arctic temperature and precipitation regimes are being affected by climate change, but the effect these variables could have on nutrient cycling dynamics remains poorly understood. The Arctic has warmed at twice the global average rate over the past 100 years, with mean air temperatures increasing by 5°C (Anisimov et al., 2007), with 1°C warming per decade noted over the last 30 years. This rate of warming is greatest in winter and spring (Anisimov et al., 2007; ACIA, 2004; AMAP, 2009) and has

contributed to a 2 % reduction in snow cover per year, since 2005, and subsequent permafrost thawing (Chapin et al., 2005; AMAP, 2009). Precipitation is also projected to increase in the Arctic during the 21st Century by up to 20 % (Anisimov et al., 2007; Trenberth et al., 2007). However, uncertainty between climate models exists regarding seasonal and spatial precipitation patterns.

Arctic vegetation and soil microbial communities are key drivers for C cycling by facilitating CO_2 and CH_4 exchange in terrestrial high latitudes. Tundra plants are uniquely adapted to their environment, showing rapid shoot growth after snow melt in spring or early summer (Billings and Mooney, 1968). As the snow pack melts, soil water content increases leading to a pulse of nutrients being released (Lipson et al., 2002) which were previously protected by cold conditions under the snow and thus unavailable for plant uptake. High water content experienced post snow melt could improve substrate availability within the soil solution pool via mass transport and diffusion (Brooks et al., 1998). Immediately post snow melt, soil water content may have a more influential role in nutrient dynamics making substrates more available for uptake. However, as soils are exposed for longer they become warmer and begin to dry out at which point substrate availability again becomes a limiting factor (Lipson et al., 2002). If snow melt commences earlier in the year because of climate change, then growing season will be extended. This could potentially result in increased plant productivity, leading to greater root rhizodeposition, releasing more dissolved organic C (DOC) and N (DON) compounds into the soil. Increased nutrient availability could perpetuate a change in vegetation communities to one more dominated by vascularised plants, unless nutrient availability becomes limiting (Mack et al., 2004; Grogan and Jonasson, 2005; Hill et al., 2011a). In contrast, other studies manipulating environmental stresses (e.g. increasing temperature, precipitation and soil nutrient

availability), found an increase in bryophyte colonization and "immigrant" angiosperm species in areas of bare ground at the same high Arctic tundra location, Ny-Ålesund (Robinson et al., 1998; Madan et al., 2007).

Global climate models project temperature and precipitation in high latitude areas to increase; this is expected to result in changes in microbial community composition and nutrient flows which will also feedback on plant growth (e.g. via mycorrhizas; Schimel and Mikan, 2005; Waldrop and Firestone, 2006; Drotz et al., 2010). Predicting ecosystem responses to climate change remains difficult because of our poor understanding of fundamental soil-microbial-plant processes in polar environments. Microbial communities in polar soils are adapted to climatic extremes but their immediate response to soil thawing and snow melt is unclear (Clein and Schimel, 1995; Lipson et al., 2002; Mackelprang et al., 2011). Further, recent metagenomic studies have revealed rapid shifts in permafrost microbial communities in terms of phylogeny and function in response to soil thaw (Mackelprang et al., 2011). The direct and indirect links between these community shifts and alterations in soil C and N cycling, however, are less well understood. Arctic systems are nutrient limited and so it important to improve our knowledge as to how C and N are cycled by microbial communities and how these communities respond to changing soil conditions.

To help unravel the impacts of projected climate change, we conducted a field-based study to quantify the response of a tundra ecosystem to snow melt and the influence of water availability and temperature on this process. In addition, we investigated both vertical and lateral nutrient resource allocation to assess whether depth and distance away from the active snow melt front would result in notable differences in nutrient resources. Field data for temperature and soil water content were then used to design laboratory studies to investigate how these two factors affect low and high

molecular weight (MW) organic matter mineralization rates. The field and experimental laboratory data were used to test the following hypotheses: i) microbial and vegetation emergence will both respond equally rapidly to snow melt, ii) nutrient resources would increase with increasing distance away from the active melt front with top soils containing a greater proportion of nutrients, iii) increasing temperature and soil water content will have a greater influence on high MW C substrate mineralization rates.

2. Materials and methods

2.1. Field site

The experimental site was an alluvial moraine at Zeppelinfjellet, Ny-Ålesund, Svalbard (78°59'N, 11°58'E). The site has a mean annual air temperature of -6.5°C (min. -24.8°C, max. 12.1°C) and annual precipitation of 197 mm with *ca.* 65 % falling as snow (Fig. 1).

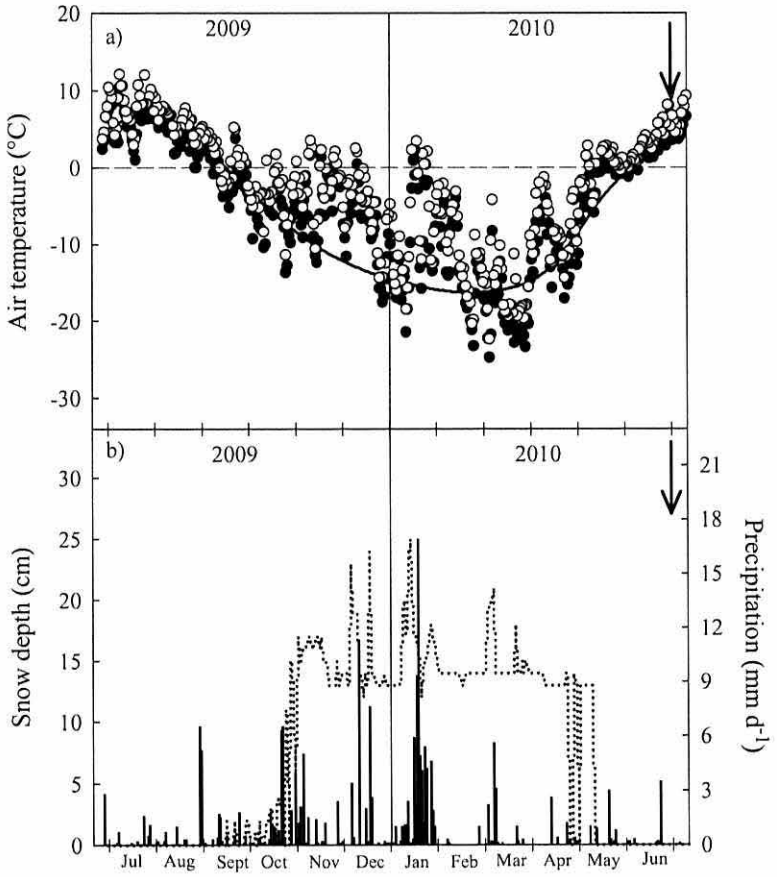

Fig. 1. Annual climatic data taken from Kings Bay Meteorological Station, Ny-Ålesund. Panel a) depicts maximum (open circles) and minimum temperature (solid circles) for 2009-2010 while the solid black line marks the long term 10 year average temperatures. The arrow represents the field sampling time point for 2010. Panel b) shows annual (from 2009-2010) snow depth (dotted line) and precipitation levels (solid lines).

The site has a mean summer air temperature of 4.4 ± 0.2 °C and summer precipitation of 0.3 ± 0.1 mm. The moraine is dominated by quaternary alluvial deposits consisting of calcareous limestone together with metamorphosed mica schists from Permian and Carboniferous epochs as soil parent material. The soil type is classified as a Gelisol (Soil Survey Staff, 1999) (4 ± 2 % stone: 60 ± 0.2 % sand; 15 ± 1.5 % silt: 16 ± 1.5 % clay). Zeppelinfjellet plant community is classified as Kartlyngsonen (*Cassiope tetragona* zone) mid-arctic vegetation (Rønning, 1996). The terrain consisted largely of bare ground (> 70 %). Plant species found on the moraine included; *Cerastium arcticum*

Lange coll., *Cerastium regelii* Ostenf., *Oxyria digyna* (L.) Hill, *Luzula confusa* Lindeb., *Polygonum viviparum* (L.) S.F. Gray, *Cassiope tetragona* L. D.Don. ssp. *tetragona* L., *Silene acaulis* (L.) Jacq., *Carex bigelowii, Saxifraga hieracifolia* Waldst. & Kit. ex Willd. *ssp. hieracifolia*, *Saxifraga cernua* L., *Saxifraga hirculus* L. ssp. *compacta* Hedberg, *Saxifraga oppositifolia* L., *Dryas octopetala* L., *Puccinellia angustata* (R. Br.) Rand & Redf. ssp. Angustata, *Ranunculus hyperboreus* Rottb. ssp. *arnellii* Scheutz, and *Salix polaris* Wahlenb alongside a range of mosses and lichens.

2.2. Site sampling – field transect measurements

Field measurements were made after the bulk of the snow pack had melted. A definitive snow melt front was identified by 43 flags (across 30 m) and the rate of snow retreat measured at each point for six days. Perpendicular to the snow line, three 20 m long transects, 10 m apart, were delineated for sampling. These transects extended past the melt front, under the snow, allowing sampling of the subniveal layer. Along each transect, soil and vegetation were monitored at -1 and -0.1 m (subniveal layer), 0 m (snow line), 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1, 2, 3, 4, 5, 6, 8, 12, 16, 20 m away from the melting snow front. At -1 m, no air space was identified between the soil and snow pack, whilst at -0.1 m, the air space was *ca.* 2 to 3 cm. At each location, soil samples (*ca.* 125 cm^3) were collected from both topsoil (0 to 5 cm) and subsoil (5 to 10 cm) and stored in gas-permeable bags at 4°C to await further analysis. Top and subsoil temperatures were measured using a HI-98509 Checktemp 1 thermometer (Hanna Instruments Ltd, UK). Soil respiration measurements were taken along each transect (-0.1, 0, 0.2, 0.4, 0.6, 0.8, 1, 2, 4, 8, 12, 16, 20 m) using an EGM-4 IRGA with attached SRC-1 soil respiration chamber that was placed on the soil for 120 s for each measurement (PP Systems Ltd, UK). The presence of the 2 to 3 cm air space between the soil and snow pack at -0.1 m, enabled the snow pack to be carefully lifted off the

soil immediately prior to taking respiration measurements without additional disturbance of the soil.

Changes in plant vegetation were monitored along each whole transect by observing the presence of different plant species (including mosses and vascular plants) with increasing distance away from the active snow melt front, to give an indication of plant community development (using a 5 m wide transect 20 m in length, n = 3). In addition, leaf width of the two most prevalent plant species (*Polygonum viviparum* L. and *Salix polaris*) were measured with increasing distance away from the active snow melt front. This represented sampling leaves of all ages along each transect (n = 10 leaves per transect point) to observe the duration of time taken to achieve maximum leaf width (n = 3).

2.3. Soil analysis

2.3.1. Transect soil analysis

Along each transect, independent soil samples were collected (-0.1, 0, 0.2, 0.4, 0.6, 0.8, 1, 2, 4, 8, 12, 16, 20 m), stored in plastic bags and brought back for analysis in the laboratory at Ny-Ålesund. Two distinct soil horizons were collected; 0 to 5 cm (topsoil) which consisted of an organic-rich horizon and 5 to 10 cm (subsoil) which comprised a mineral-rich horizon. Within 12 h of sample collection, soil solution was recovered using the centrifugal-drainage (14,000 *g*, 5 min) method (Giesler and Lundström, 1993). Soil solutions were then further centrifuged for 5 min at 18,000 *g* to remove any suspended particles. The supernatant was stored in polypropylene vials and kept on ice at 4°C during transport back to the UK. The samples were then stored at -21°C prior to chemical analysis (Table 1). Gravimetric soil water content along each transect was determined by oven drying samples (*ca.* 100 cm^3) at 105°C (24 h). The

oven-dried samples were then ground and sieved (> 2 mm) to recover the stone fraction which was subsequently weighed.

Dissolved organic C and total dissolved N (TDN) in soil solution were determined with a Shimadzu TOC-TNV analyzer (Shimadzu Corp., Japan). Ammonium (NH_4^+) concentrations were analysed following the salicylate-nitroprusside and hypochlorite methodology derived from Mulvaney (1996). Nitrate (NO_3^-) was measured colorimetrically following the vanadium method (Miranda et al., 2001). Dissolved organic nitrogen (DON) was then calculated as the difference between TDN and dissolved inorganic nitrogen (NH_4^+ and NO_3^-). Total free amino acids (TFAA) were determined by fluorescence using the *o*-phthialdehyde-β-mercaptoethanol procedure (Jones et al., 2004). Total free monosaccharides were determined following the 2, 4, 6-tri pyridyl-*s*-triazine (TPTZ) spectroscopic method (Myklestad et al., 1997).

Table 1. Temperature and available C and N resources along the melt front transect, separated into three potential zones and including 0 to 5 cm (topsoil) and 5 to 10 cm (subsoil) depths.

Potential zone	Soil solution variable	Topsoil (0 to 5 cm)	Subsoil (5 to 10 cm)	Significance
Subniveal zone (-1 to 0 m)				
	Temperature (°C)	-0.35 ± 0.05	-0.48 ± 0.02	*NS*
	DOC (mg C kg^{-1})	51.7 ± 16.6	31.4 ± 0.90	*NS*
	DON (mg N kg^{-1})	4.72 ± 3.39	0.83 ± 0.22	*NS*
	Free sugars (mg C kg^{-1})	2.90 ± 0.09	1.09 ± 0.25	*
	Free amino acids (mg N kg^{-1})	0.39 ± 0.36	0.07 ± 0.02	*NS*
	Available NH_4^+ (mg N kg^{-1})	0.12 ± 0.01	0.15 ± 0.02	*NS*
	Available NO_3^- (mg N kg^{-1})	0.18 ± 0.13	0.19 ± 0.01	*NS*
Warming zone (0 to 4 m)				
	Temperature (°C)	4.60 ± 0.36	2.53 ± 0.50	***
	DOC (mg C kg^{-1})	48.4 ± 0.9	45.4 ± 1.89	*NS*
	DON (mg N kg^{-1})	3.73 ± 0.36	2.97 ± 0.31	*NS*
	Free sugars (mg C kg^{-1})	3.30 ± 0.33	0.90 ± 0.20	***
	Free amino acids (mg N kg^{-1})	0.50 ± 0.04	0.41 ± 0.03	*NS*
	Available NH_4^+ (mg N kg^{-1})	0.07 ± 0.01	0.29 ± 0.02	***
	Available NO_3^- (mg N kg^{-1})	0.42 ± 0.09	0.34 ± 0.07	*NS*
Stable thermal zone (4 to 20 m)				
	Temperature (°C)	6.62 ± 0.11	5.08 ± 0.11	***
	DOC (mg C kg^{-1})	44.4 ± 2.0	30.6 ± 1.1	*
	DON (mg N kg^{-1})	4.17 ± 0.26	2.95 ± 0.36	*
	Free sugars (mg C kg^{-1})	4.19 ± 0.56	1.10 ± 0.23	**
	Free amino acids (mg N kg^{-1})	0.58 ± 0.05	0.45 ± 0.06	*NS*
	Available NH_4^+ (mg N kg^{-1})	0.13 ± 0.04	0.61 ± 0.07	***
	Available NO_3^- (mg N kg^{-1})	0.10 ± 0.02	0.20 ± 0.05	*NS*

Values represent mean ± standard error of the mean (SEM) (n = 2 for the subniveal layer, n = 13 for the warming zone and n = 4 for the stable thermal zone). *NS*, *, ** and *** represent values of $P > 0.05$, $P < 0.05$, $P < 0.01$ and $P < 0.001$ respectively when comparing top- and sub-soil.

2.3.2. Soil properties for mineralization experiments

For the C mineralization studies, the topsoil (0 to 5 cm) horizon was collected from within 30 cm of the active snow melt front and soil chemistry was analysed as described above (Table 2).

Table 2. Soil properties from the Zeppellinfjellet field site. The soil was subsequently used for both temperature and soil water mineralization studies.

Soil properties	Topsoil (0 to 5 cm)
Soil water content (g kg^{-1})	262 ± 15
Bulk density (g cm^{-3})	1.21 ± 0.04
Total C (g kg^{-1})	34.6 ± 7.50
Total N (g kg^{-1})	1.89 ± 0.40
C:N ratio	18.3 ± 0.61
Microbial biomass (g C kg^{-1})	3.76 ± 0.28
Microbial biomass (g N kg^{-1})	0.54 ± 0.04
pH	6.85 ± 0.03
Electrical conductivity (µS cm^{-1})	50.4 ± 6.39
Water extractable organic C (mg C kg^{-1})	64.4 ± 6.10
Water extractable organic N (mg N kg^{-1})	7.21 ± 2.50
DON (mg N kg^{-1})	7.10 ± 2.69
DOC:DON ratio	11.2 ± 2.86
Free amino acids in soil solution (mg N l^{-1})	0.66 ± 0.18
Free sugars in soil solution (mg C l^{-1})	4.12 ± 0.71
Available NH_4^+ (mg N kg^{-1})	0.10 ± 0.05
Available NO_3^- (mg N kg^{-1})	0.002 ± 0.001
Specific UV absorbance units per cm (254 nm)	0.22 ± 0.02
Specific UV absorbance units per cm (400 nm)	0.07 ± 0.003
Exchangeable K^+ (meq kg^{-1})	96.8 ± 0.45
Exchangeable Ca^{2+} (meq kg^{-1})	29.0 ± 4.04
Exchangeable Al^{3+} (meq kg^{-1})	175 ± 18
Exchangeable Mn^{2+} (meq kg^{-1})	2.76 ± 0.29
Soil respiration @ 3°C (mmol CO_2 kg h^{-1})	0.01 ± 0.003
Soil respiration @ 25°C (mmol CO_2 kg h^{-1})	0.10 ± 0.02

Values represent mean ± standard error of the mean (n = 3). Results are expressed on a dry weight basis unless otherwise stated.

The soil water mineralization experiment was conducted within 12 h of soil collection at the Ny-Ålesund laboratory. The soils used for the temperature mineralization experiment were stored at 4°C during transport back to the UK prior to analysis. Soil microbial biomass was determined using the chloroform fumigation-extraction method (Vance et al., 1987). Soil pH and EC were determined using standard probes in 1:1 w/v distilled water: soil extracts. Soluble humic substance concentrations were determined using specific UV absorbance (SUVA) at 254 and 400 nm to measure aromaticity and coloured humic substance content respectively. Total C and N of soils were determined using a CHN analyser (LECO CHN 2000, Leco Corp., USA). Trace elemental analysis was carried out using a S2 Picofox TXRF spectrometer (Bruker, Germany). Basal soil respiration was determined in the UK laboratory at 3°C and 25°C using an SR1 automated multichannel soil respirometer (PP Systems Ltd, UK) and steady state CO_2 production rates recorded after 24 h. 3°C was the average soil temperature measured during the field measurements. 25°C represented the maximum temperature these soils could reach due to 24 h sunlight during summer months and the heat absorbing properties of the dark soil.

2.4. DNA extraction and qPCR quantification

Top and subsoil samples were collected from -0.1, 0, 0.3, 4 and 20 m away from the melt front zone and were frozen within 1 h at -20 °C prior to DNA analysis. From each soil sample, DNA was extracted from a 500 mg subsample using Powersoil™ DNA Isolation Kit (MoBio Laboratories Inc., USA). Functional genes encoding bacterial and archaeal 16S rRNA as well as bacterial ammonia monooxygenase (*amoA*) and archaeal *amoA* were quantified by qPCR (7500FAST qPCR machine, Applied Biosystems, UK). Each 20 μL qPCR reaction contained 10 μL of Power SYBR® Green

PCR Master Mix (Applied Biosystems, UK), 0.2 μL of the specific forward and reverse primer at a concentration of 10 μM, 2 μL BSA (Ambion Ultrapure BSA, 5mg/mL), 2 μL of template DNA and 5.6 μL of water. Thermocycling conditions for each primer pair are described in Table 3.

Table 3. Summary of primers and thermocycling conditions used for qPCR.

Primer	Primer sequence (5 to 3)	Gene	Fragment Length	Primer Reference	Thermocycling conditions
Eub338	ACT CCT ACG GGA GGC AGC AG	Bacterial 16S	180bp	Lane , 1991.	94°C for 5mins then 40 cycles of: 95°C for 1min, 53°C for 1min and 75°C for 1.5min, followed by a melt curve. Fluorescence data was collected at the 75°C stage
Eub518	ATT ACC GCG GCT GCT GG			Muyzer et al., 1993.	
Parch519F	CAG CMG CCG CGG TAA	Archaeal 16S	396bp	Ovreas et al., 1997.	95°C for 5mins then 40 cycles of: 94°C for 30sec, 63°C for 40sec and 72°C for 40sec, followed by a melt curve. Fluorescence data was collected at the 63°C stage
arch915r	GTG CTC CCC CGC CAA TTC CT			Raskin et al., 1994.	
amoA-1F	GGG GTT TCT ACT GGT GGT	Bacterial *amoA*	491bp	Rotthauwe et al., 1997.	94°C for 10min then 40 cycles of: 94°C for 60sec, 56°C for 60sec, 72°C for 60sec and 78°C for 60sec followed by a melt curve. Fluorescence data was collected at the 78°C stage
amoA-2R	CCC CTC KGS AAA GCC TTC TTC				
Arch-amoAF	STA ATG GTC TGG CTT AGA CG	Archaeal *amoA*	635bp	Francis et al., 2005	94°C for 2min then 6 cycles of: 94°C for 30sec, 54°C for 1min (decreasing by 0.5°C/cycle), 72°C 1min, then 40 cycles of: 94°C for 30sec, 52°C for 1min, 72°C for 1min and 78°C 40sec followed by a melt curve fluorescence data was collected at the 78°C stage
Arch-amoAR	GCG GCC ATC CAT CTG TAT GT				

Samples were tested over a dilution series to determine any inhibition, analysis was completed on samples diluted 1:20 (v:v) as this gave the highest gene copy number. Copy numbers of ammonia oxidizing bacteria (AOB) and ammonia oxidizing archaea (AOA) were assumed to be equivalent to actual cell numbers.

Templates determining gene copy numbers in the qPCR reactions were linearized plasmids harbouring PCR amplicons. PCR-amplified fragments of functional genes were cloned using P-GEM T-easy system (Promega, USA) and sequenced using Big Dye Terminator Kit (Life Technologies, USA) at the Australian Genome Research Facility (AGRF) Perth, Western Australia. Archaeal genes for *amoA* and 16S rRNA and bacterial 16S rRNA genes were isolated from soil DNA whilst bacterial *amoA* was cloned into the plasmid from a pure culture of *Nitrosomonas europeae*. Sequence identities were confirmed by a Blastn search on GenBank and then submitted to GenBank with the following accession numbers: archaeal *amoA* (JF905631 and JF905632) and 16S rRNA (JF905629 and JF905630). The standard curves generated in each reaction were linear over four orders of magnitude (10^2 to 10^7 gene copies) with r^2 values > 0.97. Amplification efficiencies ranged from 97.1 to 98.7 % for AOB, 54.3 to 61.1 % for AOA, 69.6 to 75.7 % for bacterial and 64.9 to 68.4 % for archaeal 16S rRNA genes. Data were then normalized against the amount of DNA extracted per sample. Bacterial *amoA* gene copies were below detection limits and not reported.

2.5. Influence of soil water content on C mineralization

This experiment investigated how varying gravimetric soil water contents, ranging from soil held at saturation point (*ca.* 56 %) through to soil held in an air-dry state (*ca.* 3 %), affected C mineralization rates. The soil water contents were then categorised into the following groups; < 10, 10-15, 15-20, 20-25, 25-30, 30-35 and > 35

% for subsequent statistical analyses. Large roots and stones were removed, and the soil was spread thinly (*ca.* 5 mm depth) on aluminium foil and allowed to progressively dry between 15 to 20°C for 12 h. These soil water ranges replicated those experienced in soil surface layers during the Arctic summer (July-Aug). Matric potentials of these soils were also measured using a WP4 Dewpoint PotentiaMeter (Decagon Devices Inc., USA).

After drying, soil (5 g) was placed into 50 cm^3 polypropylene containers and either a ^{14}C-labelled low MW (named as "root exudate mix") or high MW (plant litter) C source added to the soil. The tubes were incubated at 6.2 ± 0.3°C representing average summer soil temperatures. Samples were weighed pre- and post-experiment to monitor any change due to water loss. The low MW C source consisted of a mixture of common root exudate compounds and included: malic acid (1 mM), citric acid (2 mM), glucose (10 mM), fructose (2 mM), sucrose (1 mM), amino acid mixture (3 mM) comprising 15 equimolar ^{14}C-labelled L-amino acids (alanine, arginine, aspartic acid, glutamic acid, glycine, histidine, isoleucine, leucine, lysine, phenylalanine, proline, serine, threonine, tyrosine, valine). The ^{14}C-labelled substrates were obtained from Sigma-Aldrich (UK), ICN Pharmaceuticals Inc. (USA), Amersham Biosciences Ltd. (UK), and ARC Inc. (USA). To prepare the substrate, 20 ml of the ^{14}C mixture (5 μCi of each ^{14}C-labelled substrate; 185 kBq) was added to sterile, acid-washed quartz sand (50 g) and the water evaporated under a stream of air with continual stirring (0.48 mg C g^{-1} sand). Finally, the ^{14}C-labelled quartz sand was dried at 80°C overnight to ensure sterility. In brief, 100 mg of the ^{14}C-labelled sand was added to the surface of each 5 g soil sample. Due to the thixotropic nature of these soils, the substrate was manually mixed into the soil to give a substrate addition rate of 0.01 mg C g^{-1}. A CO_2 trap containing 1 ml of NaOH was placed in a polypropylene vial and suspended above the

soil and vial hermetically sealed. $^{14}CO_2$ evolution was monitored by replacing the NaOH trap after 0.25, 0.5, 1, 2, 4, 8, 12, 24, 48, 72, 96, 226, 366, 492, 635, 744 hours. The ^{14}C content of the 1 M NaOH traps was determined by adding Scintisafe 3® scintillation cocktail (Fisher Scientific, UK) and ^{14}C content measured using a Wallac 1404 liquid scintillation counter (Wallac EGandG, UK).

The high MW C source consisted of air-dried ^{14}C-labelled, ground *Lolium perenne* L. shoot material (12.3 kBq g^{-1}). The *Lolium perenne* was field grown on a non-fertilised site and so composition of high MW C polymers are representative of field conditions. These high MW C polymers are typical of those in all plants present at the field site (i.e. cellulose, hemicellulose, lignin and protein). To prepare the plant material, it was extracted twice with distilled water (80°C, 4 h) to remove the soluble ^{14}C component (32.9 ± 1.5 % of the total ^{14}C label). Of the high MW ^{14}C remaining, 26.7 ± 1.0 % was soluble in HCl (0.08 M), 43.8 ± 0.7 % was NaOH (1 M) soluble and 29.5 ± 3.4 % remained insoluble (Jones and Darrah, 1994; Simfukwe et al., 2011). Briefly, 100 mg of ^{14}C-labelled plant material was added to the surface of each 5 g soil sample (46 mg C g^{-1}). Again, due to the thixotropic nature of the soil, substrate was manually mixed into the soil. Production of $^{14}CO_2$ was monitored as described for the low MW substrate except sampling times were 6, 12, 24, 48, 72, 96, 224, 364, 490, 633 and 742 hours.

2.6. Influence of temperature on C mineralization

The temperature-dependent mineralization of the high and low MW C substrates was evaluated in the same field-moist soil (i.e. not dried) used in the soil water gradient experiment described above. Soil was collected from the field; roots and large stones removed and 5 g placed in 50 cm^3 polypropylene tubes. After pre-incubating the soils at

-5, 0, 5, 10, 16 and 20°C for 24 h, high and low MW substrates were added to the soil and the tubes hermetically sealed and analysed as described previously. The rate of ^{14}C-substrate mineralization to $^{14}CO_2$ was then determined for each temperature at 0.25, 0.5, 1, 2, 4, 8, 12, 24, 53, 74, 96, 120, 144, 168 hours for low MW compounds, and 6, 12, 24, 53, 74, 96, 120, 144, 168 hours for high MW compounds and then every week for an additional 8 weeks (1512 h). The temperature selected reflected surface soil temperature ranges recorded along each field transect.

2.7. Modeling ^{14}C dynamics in soil

Previous studies indicate organic low MW C substrate mineralization follows a biphasic pattern in soil (Chotte et al., 1998; Saggar et al., 1996). The first rapid phase corresponds to $^{14}CO_2$ efflux as substrates are immediately used for catabolic processes (i.e. respiration) and a depletion of the soil solution pool (Jones and Hodge, 1999). The second, slower mineralization phase is largely attributable to substrate utilization for anabolic processes (cell growth and maintenance) and their subsequent break down (i.e. microbial biomass turnover). This biphasic pattern can be explained by a double first-order decay model with an asymptote:

$$f = y_0 + \left(a_1 \times \exp^{-k_1 t}\right) + \left(a_2 \times \exp^{-k_2 t}\right) \qquad \text{(Eqn. 1)}$$

Where f is the amount of ^{14}C remaining in the soil. The asymptote value y_0 represents a recalcitrant pool which is unavailable for microbial mineralization within the time frame of the experiment, a_1 describes the amount of ^{14}C allocated to the first mineralizable pool and k_1 is the corresponding rate constant. The proportion of ^{14}C partitioned to the second slower pool is described by a_2 and $k_2$is the rate constant for this pool. The half-life of the first mineralization pool a_1 can be calculated using the following equation:

$$t_{1/2} = \frac{\ln(2)}{k_1} \quad \text{(Eqn. 2)}$$

Calculating the half-life for the slower second phase a_2 is subject to uncertainty as the connectivity between pools a_1 and a_2 is unknown (Saggar et al., 1996; Boddy et al., 2007).

Equation 1 cannot be solved explicitly for finding the half-life of the individual substrates in the soil system (fast and slow pools combined). Therefore, the amount of ^{14}C remaining in the soil at $t_{½}$ for both pools ($f_{½}$) is defined as:

$$f_{1/2} = \frac{a_1 + a_2}{2} \quad \text{(Eqn. 3)}$$

After equation 3 is substituted into equation 1, the total substrate half-life was derived numerically by applying the Newton-Raphson iteration method (Oburger and Jones, 2009).

High MW substrates are more recalcitrant and are more difficult to mineralize by microbial communities. High MW compounds therefore do not follow the same biphasic pattern and so a single first-order exponential decay model with asymptote was fitted to the data.

$$f = y_0 + \left(a \times \exp^{-kt}\right) \quad \text{(Eqn. 4)}$$

The asymptote value y_0 represents a recalcitrant pool which is unavailable for microbial mineralization, a describes the amount of ^{14}C allocated to a single mineralizable (labile C) pool and k is the corresponding rate constant. Substrate half-life can be calculated using Eqn. 2.

2.8 Statistical analysis

All experiments had three replicates per treatment and data were visually inspected for normality using quantile-quantile plots (Crawley, 2007). Any data not

normally distributed were log-transformed to achieve normal distribution and equal variances. Data from the melt front experiment were subjected to paired t-tests to evaluate differences between topand subsoil depths. Statistical analysis of the qPCR assays were carried out using paired t-tests to look for differences between topand subsoil samples, also one-way ANOVA with associated TukeyHSD post hoc tests, were performed to observe if distance away from the melt front affected mean values. Kinetic parameters obtained from the mineralization experiments were subjected to one-way ANOVA with associated TukeyHSD post hoc test. Comparisons of total substrate half-lives were carried out using linear regression models. All statistical procedures were carried out using the statistical package 'R' v 2.12.1 (R Development Core Team, 2010), with $P = 0.05$ used as the upper limit for statistical significance. All graphs were created using SigmaPlot 11 (Systat Software Inc., CA, USA).

3. Results

3.1. Melt front climate and vegetation dynamics

The weather in the months preceding and during the field monitoring period was close to the 10-year running average for the site (Fig. 1). It should be noted that the 2009-2010 winter was unusually warm in comparison to previous years. The active vegetation growing season in 2010 was *ca.* 60 d in length. At the field site a uniform rate of snow pack retreat was observed, with 17.9 ± 1.9 cm d^{-1} measured over 6 d. Soil temperatures differed for both top-and sub-soil depths ($P < 0.05$) and showed an initial lag phase, where soil temperatures were below 0°C in the subniveal layer. After the snowpack had retreated an exponential increase in soil temperature was observed. After 1 m from the melt front, temperatures began to stabilize and remained constant along the remainder of the transect (Fig. 2a). Based on the melt rate, time taken to reach half-

maximal and maximum soil heating after snow disappearance, was *ca.* 1.5 and 5 d respectively. Soil water content decreased away from the melt front up to 1 m after which it remained stable, except for isolated organic rich soil zones which possessed high soil water content (Fig. 2b). Biological indicators including; soil respiration (Fig. 3a), plant species number (Fig. 3b) and leaf width (Fig. 3c) reached maximum activity by *ca.* 4 m along the transect profile. The time to reach half-maximal and maximum activity after snow disappearance was estimated to be 2 and 14 d for soil respiration, 5 and 26 d for the number of vegetation species present, 11 to 15 d and 23 to 28 d for leaf expansion, respectively. Soil water content showed a decreasing relationship with soil respiration (linear model $r^2 = 0.27$; $P > 0.05$, Fig. S1 a). Below-ground respiration rate correlated well to soil temperature (exponential rise to maximum model; $r^2 = 0.84$, $P < 0.001$, Fig. S1 b), with *ca.* an 8-fold increase in respiration with *ca.* a 7°C temperature rise (estimated to correspond to *ca.* 14 d based on melt rate measurements).

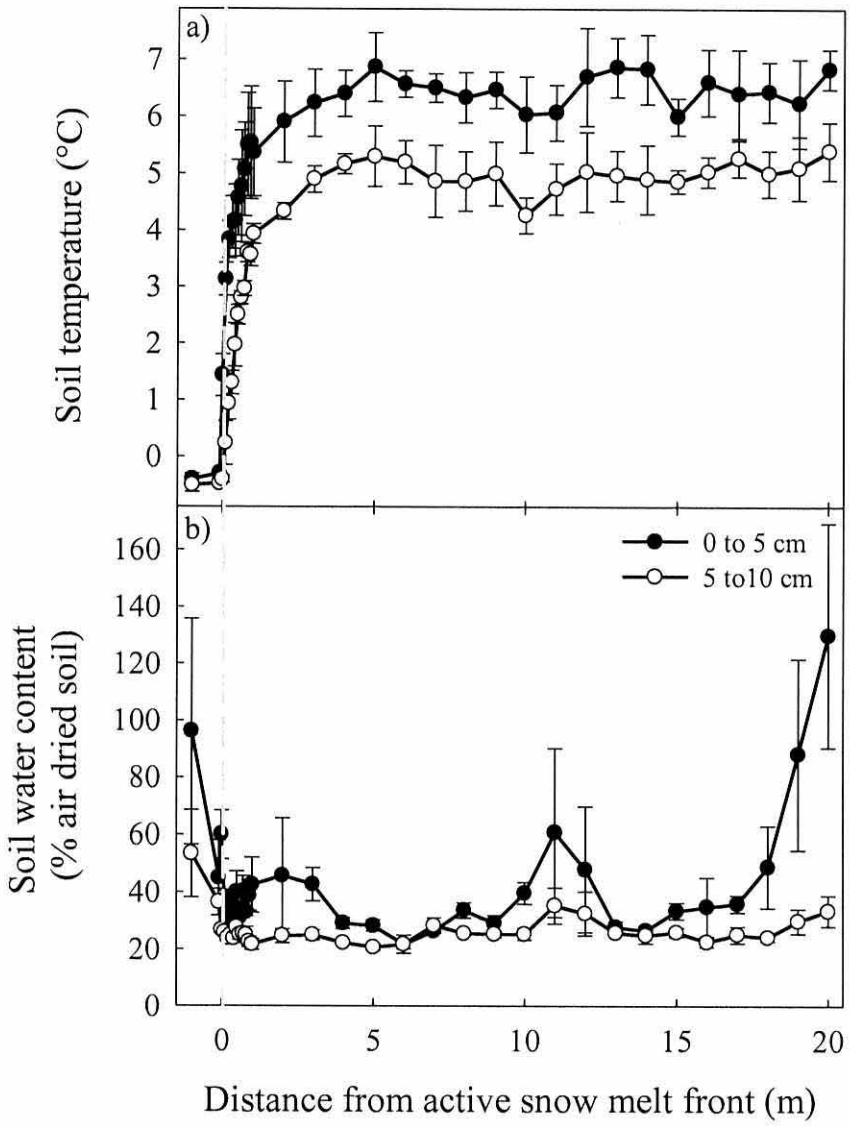

Fig. 2. Soil temperature (a) and gravimetric soil water contents (b) with increasing distance from an active snow melt front depicting both top (0 to 5 cm) and subsoil (5 to 10 cm) depths. Points represent means ± standards error of the mean (SEM), $n = 3$. The legend is the same for both panels. Dotted line represents the active snow melt front.

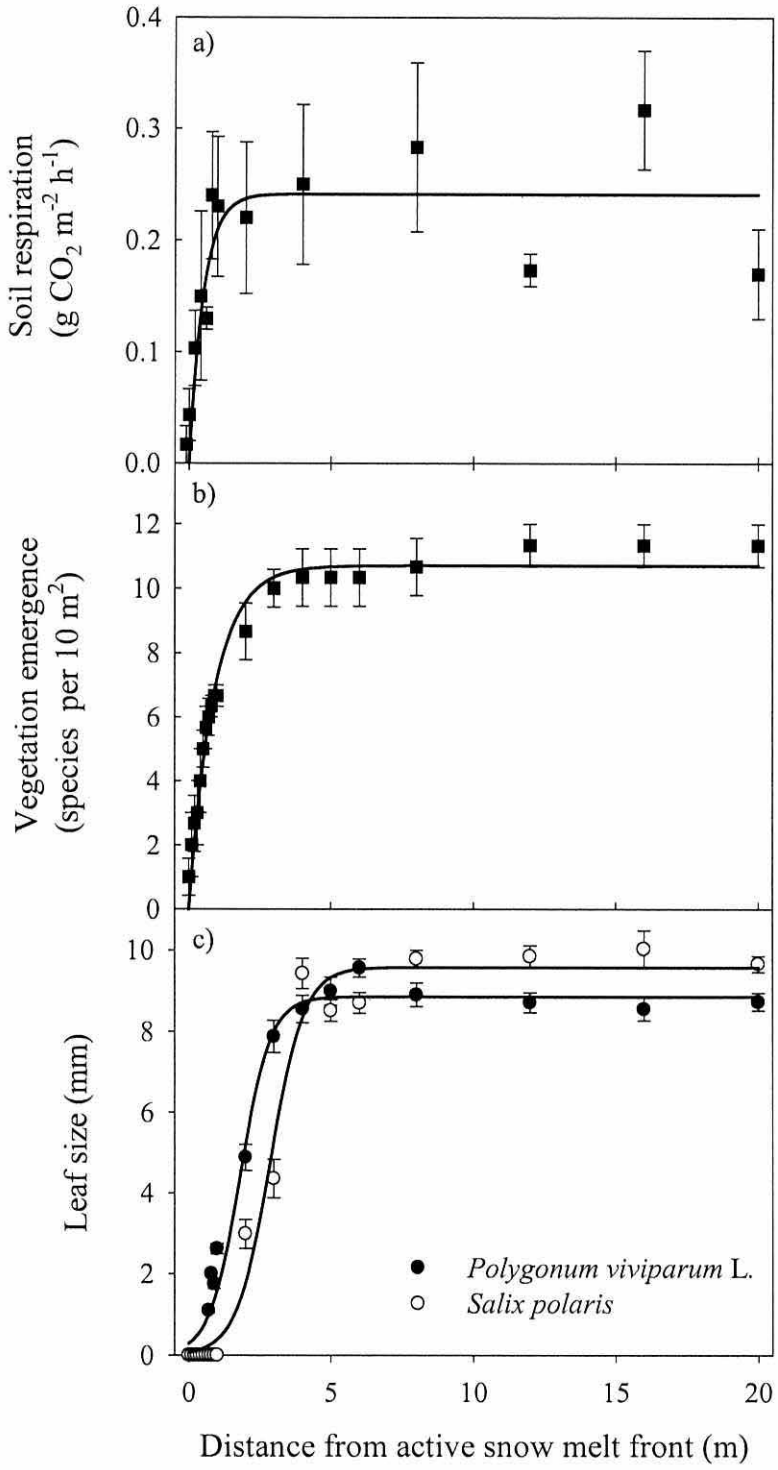

Fig. 3. Soil and vegetation field measurements taken along an active melt front transect. Points represent means ± standards error of the mean (SEM), n = 3. In all cases a 2 parameter exponential equation with asymptote was fitted to the experimental data (r^2 = 0.69 (a), 0.97 (b) and 0.93 and 0.91 (c, *Polygonum viviparum* L. and *Salix polaris*) respectively).*3.2. Transect nutrient resources*

Changes in soil solution chemistry and nutrient resources were observed within 72 h of snow melt (Fig. 4).

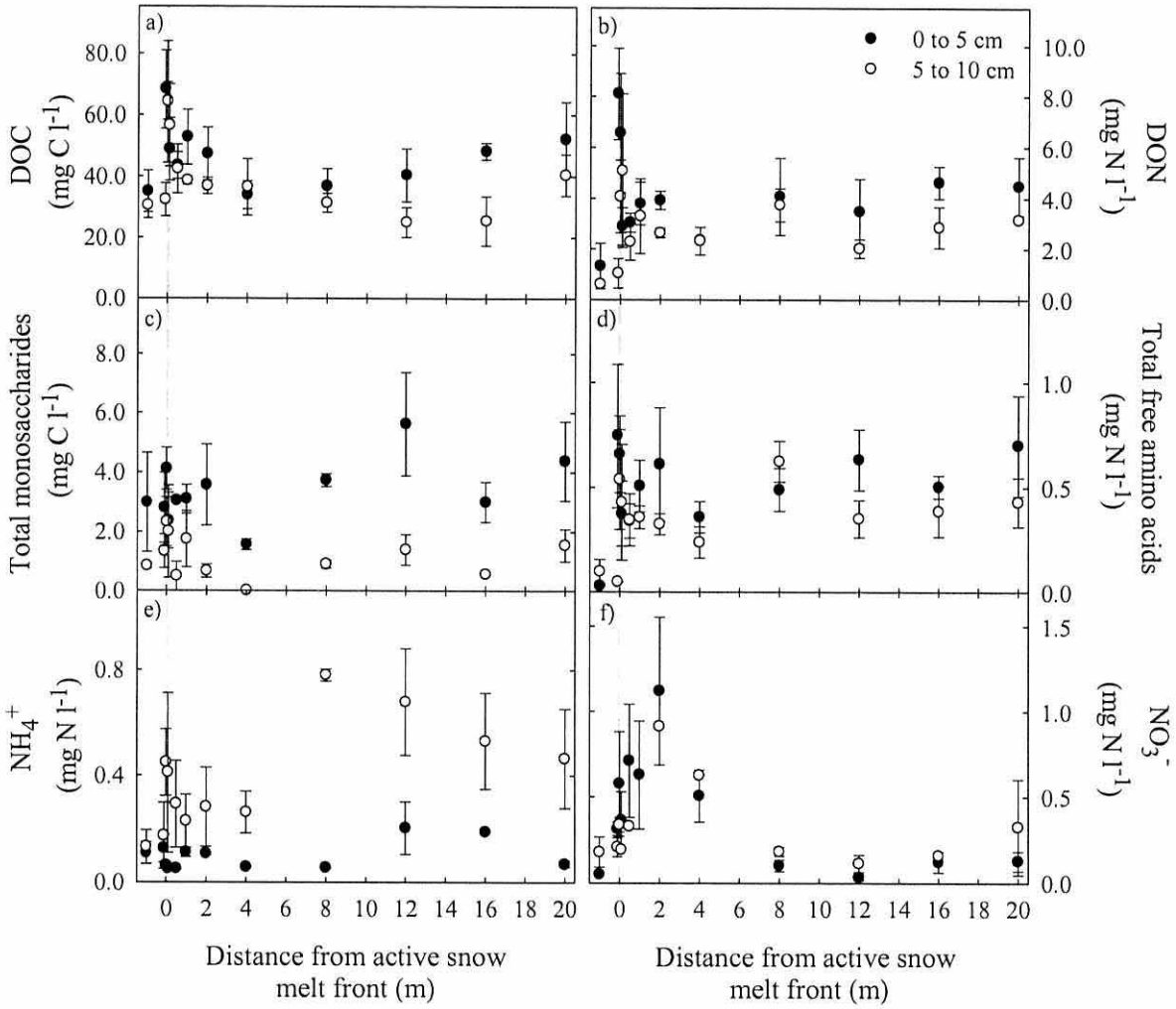

Fig. 4. Soluble C and N in soil (DOC, DON, total free monosaccharides, total free amino acids, NH_4^+ and NO_3^- (a to f respectively) with increasing distance from an active snow melt front. Points represent means ± standard error of the mean (SEM), $n = 3$. The legend applies to all panels. Dotted line represents the active snow melt front.

We identified three potential zones based on observations from the field transect data (Table 1). Temperature was the main factor which defined each potential zone ($P < 0.001$). Available C and N resources did not show any change between zones ($P > 0.05$), however patterns were observed within each individual zones. The first we

assigned to the subniveal zone (-1 to 0 m) where soil is frozen and is covered by snow, but where an air space between the soil and snow allowed above-ground primary production to commence in a limited number of species (e.g. *Polygonum viviparum L.*, *Cerastium arcticum*). In this frozen soil zone, the concentration of soluble organic and inorganic N was low.

The second is ascribed to a warming zone (0 to 4 m) where a rapid temperature shift occurred (5.1 and 3.8°C increase for top and subsoil respectively) and the number of plant species emerging increased rapidly. This zone is characterized by a transitory peak in both soluble C and N (Figs. 4a and 4b). The peak was most pronounced for NO_3^- (Fig. 4f). Generally, nutrient levels were similar between top and subsoil with only total monsaccharides and NH_4^+ concentrations being different ($P < 0.001$; Table 1).

The third is assigned to a stable thermal zone (4 to 20 m) where vegetation communities reached maximum activity and nutrient concentrations stabilised. Depth had a greater effect on soil solution properties within this zone (Table 1). Temperatures differed by 1.5°C ($P < 0.001$) between soil depths. Dissolved organic C and DON concentrations were 31 % and 29 % higher in topsoils than subsoils respectively ($P < 0.05$). Total monosaccharides were 74 % higher in topsoils than subsoils ($P < 0.001$).

3.3. Melt front soil solution chemistry

No major differences ($P > 0.05$) were observed between top and subsoil for the C mineralization studies, apart from soil microbial biomass C and basal soil respiration at 3°C ($P < 0.01$; Table 2). DON comprised > 90 % of the total water extractable N, total free amino acids made up approximately 10 % of the DON pool. Total free monosaccharides and TFAA contributed < 10 % of the DOC pool. Consequently > 90 % of both the DON and DOC pools remained unidentified within the soil.

3.4. Melt front transect qPCR trends for bacteria and archaea

3.4.1. Bacterial and Archaeal 16S rRNA

Bacterial and archaeal 16S rRNA genes were detected along the melt front transects at both top and subsoil depths (Figs. 5a and b respectively). Distance showed an effect on gene copy number for bacterial and archaeal 16S rRNA at both depths ($P < 0.05$). Copy numbers were higher for bacterial and archaeal genes at -0.1 m at both soil depths than at the melt front (0 m; $P < 0.05$; Figs. 5a and b respectively). Gene copy number rapidly decreased at the melt front and remained consistent along the melt front transect, until 20 m. At 20 m, gene copy number increased again, however, this value is associated with a high level of variation (Fig. 5).

3.4.2. Bacterial and Archaeal ammonia-oxidizers (amoA)

Bacterial *amoA* genes were below detection limits, archaeal *amoA* genes were detected along the melt front transect. Distance showed no effect in *amoA* numbers for topsoil samples ($P > 0.05$; Fig. 5c). Subsoil archaeal *amoA* showed an effect with distance ($P < 0.05$) between 0.3 m and 20 m.

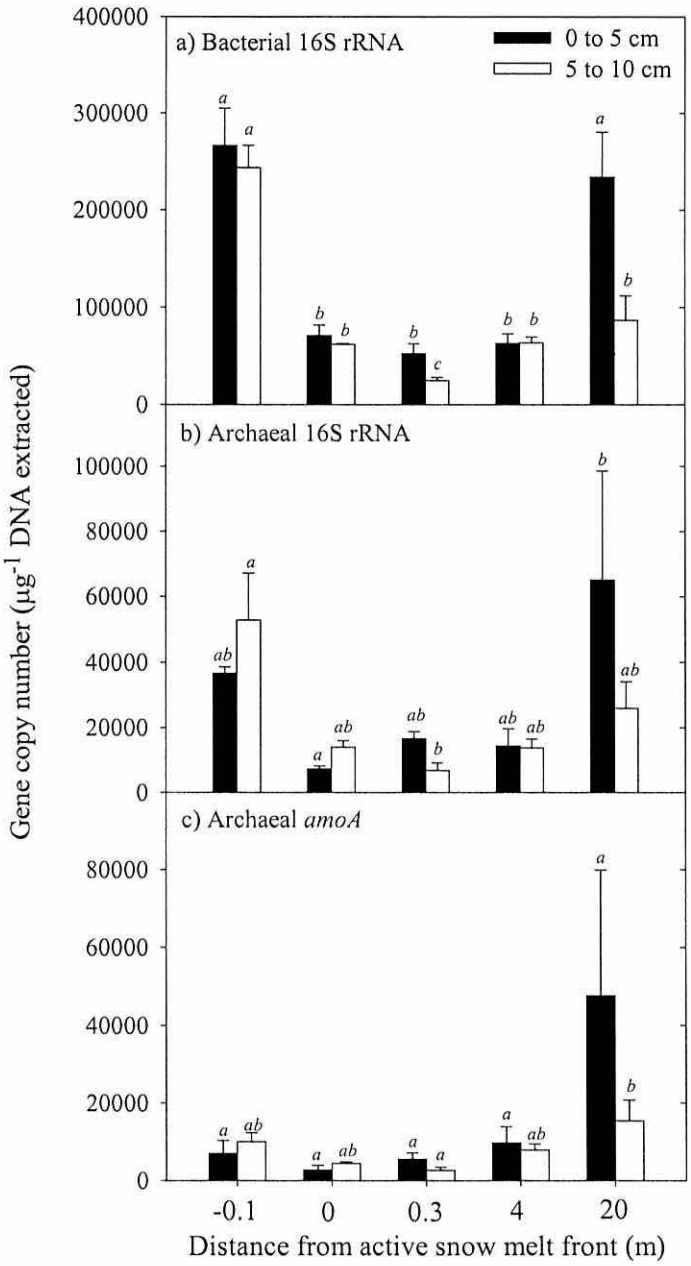

Fig. 5. Copy numbers of the 16S rRNA gene for total (a) Bacteria, (b) Archaea and (c) Archaeal *amoA*, with increasing distance from an active snow melt front at both 0 to 5 cm (topsoil) and 5 to 10 cm (subsoil) depths. Data normalized per amount of DNA extracted. The bars represent means ± standard error of the mean (SEM), $n = 3$. Letters refer to significant differences (multiple comparisons after TukeyHSD post-hoc test $P < 0.05$).

3.5. Soil water-dependent mineralization kinetics

For low MW substrates, a double first-order exponential decay model with asymptote fitted best to the mineralization data (r^2 = 0.998 ± 0.0002). High MW substrates fitted better to a single-order exponential decay model with asymptote (r^2 = 0.992 ± 0.002). Two very dry samples (~3 % soil water content or ~-30 MPa) were omitted from further analysis after influential measures tests identified these as outliers.

Individual kinetic parameters reflect different pools (i.e. y_0, a_1 and a_2) into which ^{14}C from the added substrate is partitioned (Table 4). The y_0 pool is ascribed to a large recalcitrant pool that is predominantly unavailable for microbial mineralization in the short-term (i.e. < 30 d). Low MW compounds, showed a 7 % increase in ^{14}C allocation to this recalcitrant pool (y_0) at the lowest water content group relative to the highest ($P < 0.001$). A 68 % increase in partitioning of ^{14}C to the rapidly respired pool (a_1) was noted with decreasing water content ($P < 0.01$), this corresponded to a 180 % increase in half-life ($t_{½}$) for this pool ($P < 0.01$). Allocation to the microbial pool (a_2) decreased by 35 % ($P < 0.01$); no effect was noted for the rate constant (k_2) associated with the microbial pool ($P > 0.05$). High MW data showed a 136 % increase ($P < 0.05$) in partitioning to the respired pool (a_1), coupled with a 207 % increase in half-life ($t_{½}$) for this pool ($P < 0.01$) from the lowest water content relative to the highest. No effect was noted for the allocation of ^{14}C ($P > 0.05$) to the recalcitrant pool (y_0).

Low and high MW compounds fit to different exponential models (double and single respectively). To enable a direct substrate comparison, low MW total substrate-C half-lives were calculated using the Newton-Raphson method. Low MW compounds had shorter half-lives than high MW compounds (Fig. 6a; $P < 0.001$). Low MW compounds total half-life decreased by 49 % from the highest water content group relative to the lowest ($P < 0.01$).

Table 4. Influence of soil water content on the partitioning of ^{14}C derived from low and high molecular weight (MW) carbon (C) compounds within Arctic melt front soils.

Soil water content	Pool partitioning of substrate derived ^{14}C			Half-life of pool a_1	Rate constant (k_2) for pool a_2
	Pool y_0 (%)	Pool a_1 (%)	Pool a_2 (%)	(h)	(d^{-1})
Low MW substrates					
10 – 15 %	73.5 ± 0.9^{c}	9.96 ± 1.02^{c}	16.3 ± 1.91^{a}	7.38 ± 2.44^{b}	0.09 ± 0.01^{a}
15 – 20 %	68.4 ± 1.1^{ab}	8.44 ± 0.39^{bc}	23.2 ± 0.71^{bc}	3.91 ± 0.42^{ab}	0.09 ± 0.01^{a}
20 – 25 %	64.4 ± 0.8^{a}	7.99 ± 0.53^{abc}	27.7 ± 0.33^{c}	2.52 ± 0.31^{a}	0.09 ± 0.01^{a}
25 – 30 %	72.1 ± 1.6^{bc}	7.27 ± 0.46^{ab}	20.8 ± 1.23^{ab}	2.40 ± 0.33^{a}	0.13 ± 0.01^{a}
30 – 35 %	67.1 ± 0.3^{ab}	7.99 ± 0.80^{abc}	25.2 ± 1.10^{bc}	2.09 ± 0.27^{a}	0.11 ± 0.01^{a}
>35 %	68.9 ± 0.7^{abc}	5.90 ± 0.08^{a}	25.2 ± 0.79^{bc}	2.62 ± 0.22^{a}	0.10 ± 0.01^{a}
High MW substrates					
10 – 15 %	65.3 ± 5.0^{a}	35.0 ± 5.1^{b}		1136 ± 150^{b}	
15 – 20 %	63.1 ± 9.4^{a}	37.1 ± 9.4^{b}		1256 ± 364^{b}	
20 – 25 %	75.7 ± 3.4^{a}	24.3 ± 3.0^{ab}		793 ± 186^{ab}	
25 – 30 %	79.8 ± 1.6^{a}	20.3 ± 1.6^{ab}		465 ± 57^{ab}	
30 – 35 %	81.1 ± 4.1^{a}	19.0 ± 4.3^{ab}		412 ± 82^{ab}	
>35 %	85.1 ± 1.7^{a}	14.8 ± 1.5^{a}		370 ± 81^{a}	

See materials and methods for details of the kinetic models for the high and low MW substrates. Values represent mean ± standard error of the mean (SEM) (n = 3 for all except, 15 – 20 and >35 % soil water contents where n = 4 and 2 respectively). Low MW substrates fitted well to a double first order exponential decay model with asymptote ($r^2 > 0.99$), whereas high MW substrates fitted better to a single order exponential decay model with asymptote ($r^2 > 0.99$). Letters refer to significant differences (multiple comparison after a TukeyHSD post-hoc test $P < 0.05$).

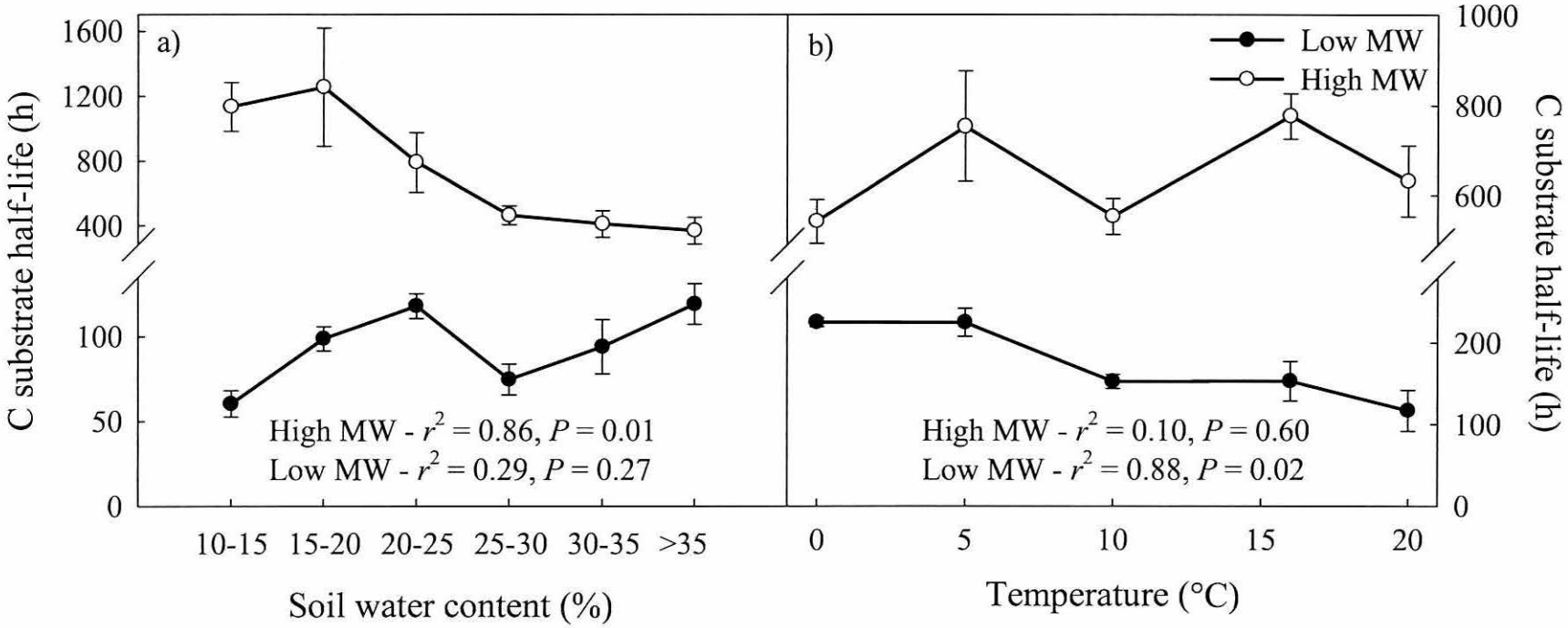

Fig. 6. Influence of soil water content (a) and temperature (b) on substrate half lives for low and high MW C substrates. Data points for temperature (a) represent means ± standard error of the mean (SEM), n = 3, for soil water content (b) means ± SEM, n = 3 (except for 15 to 20 %, n = 4 and 30 to 35 %, n = 2). Legend is the same for both panels.

High MW compounds showed the opposite effect, with a 206 % increase in total substrate half-life as water content decreased ($P < 0.01$).

Amounts of $^{14}CO_2$ evolved over time increased for both compounds, however, low MW compounds showed no change in relationship with varying soil water contents ($P > 0.05$). High MW compounds, showed positive relationships with soil water content for days 0.5 and 4 only (Fig. 7a and b; regression; r^2 = 0.91 and 0.30; $P < 0.01$ respectively). Although not included in regression analysis results from our laboratory study showed at < 5 % gravimetric water content, 14 ± 1 % of low MW substrate was captured as $^{14}CO_2$ after 31 d. High MW mineralization showed a low CO_2 flux; with only 1.1 ± 0.1 % of substrate captured as $^{14}CO_2$ after 31 d.

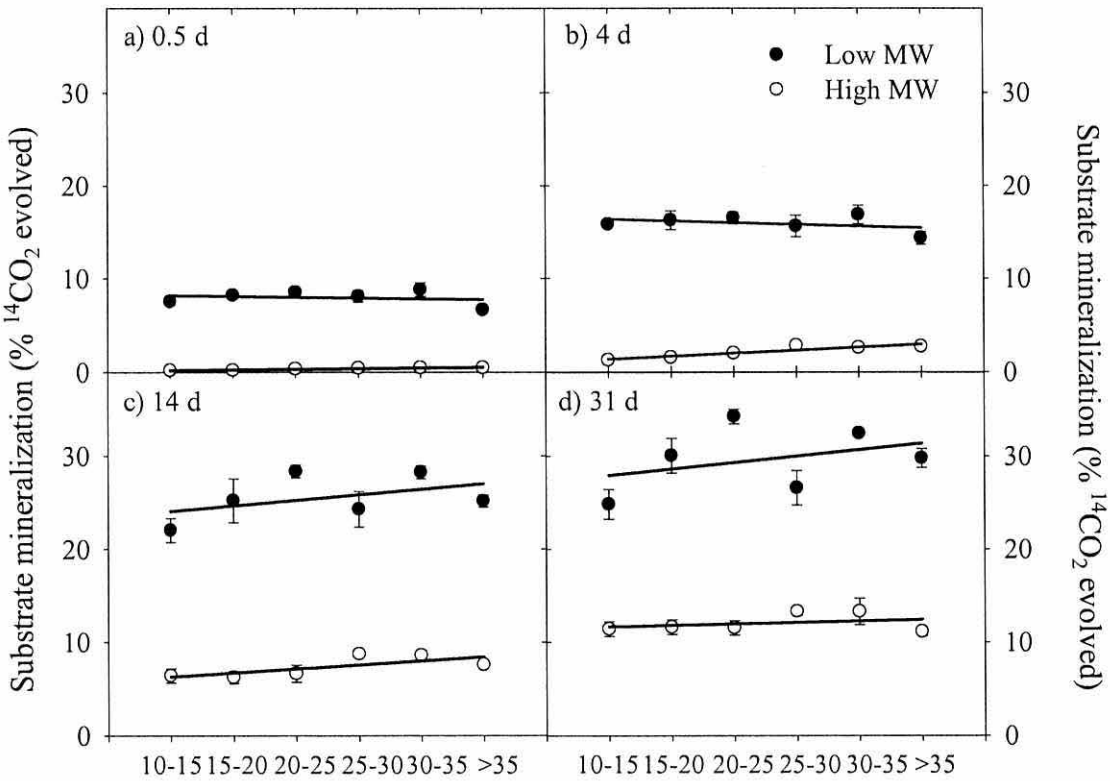

Fig. 7. Influence of soil water content on the amount of $^{14}CO_2$ evolved for low and high MW ^{14}C-labelled substrates. Data points represent means ± standard error of the mean (SEM), n = 3 (except for 15 to 20 %, n = 4 and 30 to 35 %, n = 2). Legend is the same for all panels (a to d).

3.6. Temperature-dependent mineralization kinetics

For temperature-dependent mineralization of low MW compounds, a double first-order exponential decay model with asymptote gave the best fit to the data (r^2 = 0.998 ± 0.0001). High MW substrates fitted best to a single parameter exponential decay model (r^2 = 0.992 ± 0.0003) (Table 5). Samples at -5°C were omitted from further analysis after influential measures tests proved these to be outliers.

As temperature increased, low MW compounds showed 24 % less allocation of ^{14}C to the most recalcitrant pool (y_0; $P < 0.001$). A 136 % increase in allocation ($P < 0.05$) to the rapidly respired pool (a_1) was also observed, coupled with a 207 % increase ($P < 0.01$) in the half-life ($t_{½}$) for this pool. Temperature had no effect on allocation to the microbial pool (a_2) or the rate co-efficient (k_2) associated with this pool ($P > 0.05$). High MW compounds exhibited a 29 % decrease in allocation to the most recalcitrant pool (y_0;) ($P < 0.001$). This was coupled with a 29 % increase in allocation to the rapidly respired pool (a_1). Temperature had no effect on the half-life ($t_{1/2}$) associated with this pool ($P > 0.05$).

The Newton-Raphson method was applied to the low MW data to estimate the total half-life from the combined C pools. Low MW compounds showed a negative relationship between temperature and substrate half life (regression; r^2 = 0.88, P = 0.01). Conversely, for high MW compounds, no effect in substrate half-life was noted with increasing temperature (regression; r^2 = 0.10, P = 0.60).

Total $^{14}CO_2$ evolved increased with time for both low and high MW compounds as temperatures increased (Fig. 8) particularly after 4 d (regression; r^2 = 0.82, 0.89, for low and high MW compounds respectively; $P < 0.001$).

Table 5. Influence of temperature on the partitioning of low and high molecular weight (MW) carbon (C) compounds within Arctic melt front soils.

Temperature (°C)	Pool partitioning of substrate derived ^{14}C			Half-life of pool a_1	Rate constant (k_2) for pool a_2
	Pool y_0 (%)	Pool a_1 (%)	Pool a_2 (%)	(h)	(d^{-1})
Low MW substrates					
0	70.5 ± 2.0^{c}	3.8 ± 0.6^{a}	25.6 ± 1.5^{a}	4.9 ± 0.9^{a}	0.06 ± 0.00^{a}
5	66.7 ± 0.9^{c}	6.1 ± 1.4^{ab}	26.8 ± 2.4^{a}	6.3 ± 2.1^{a}	0.05 ± 0.01^{a}
10	62.9 ± 1.5^{bc}	10.9 ± 2.6^{bc}	25.4 ± 1.4^{a}	25.8 ± 8.3^{b}	0.06 ± 0.01^{a}
16	41.8 ± 4.3^{a}	23.1 ± 1.9^{c}	33.0 ± 2.7^{a}	31.1 ± 4.0^{b}	0.03 ± 0.00^{a}
20	53.8 ± 2.7^{b}	18.8 ± 0.9^{c}	26.3 ± 1.9^{a}	20.1 ± 3.1^{b}	0.04 ± 0.01^{a}
High MW substrates					
0	89.4 ± 1.3^{d}	10.8 ± 1.2^{a}		542 ± 48^{a}	
5	72.7 ± 4.0^{bc}	27.2 ± 4.0^{bc}		754 ± 122^{a}	
10	78.8 ± 2.2^{cd}	21.0 ± 2.2^{ab}		553 ± 40^{a}	
16	55.7 ± 3.2^{a}	43.3 ± 3.0^{d}		777 ± 50^{a}	
20	63.3 ± 3.4^{ab}	35.8 ± 3.3^{cd}		632 ± 79^{a}	

See materials and methods for details of the kinetic models for the high and low MW substrates. Values represent mean ± standard error of the mean (SEM) ($n = 3$). Low MW substrates fitted well to a double first order exponential decay model with asymptote ($r^2 > 0.99$), whereas high MW substrates fitted better to a single order exponential decay model with asymptote ($r^2 > 0.99$). Letters refer to significant differences (multiple comparisons after a TukeyHSD post-hoc test $P < 0.05$).

The Q_{10} values were 1.6 ± 0.07 and 1.32 ± 0.04 ($P < 0.05$) for low MW compounds and 1.83 ± 0.04 and 1.81 ± 0.10 ($P > 0.05$) for high MW compounds for 7 d and 63 d respectively, which agree with data from Boddy et al. (2008), but are lower than reports for other Arctic soils (Q_{10} = 8.8; Bekku et al., 2003).

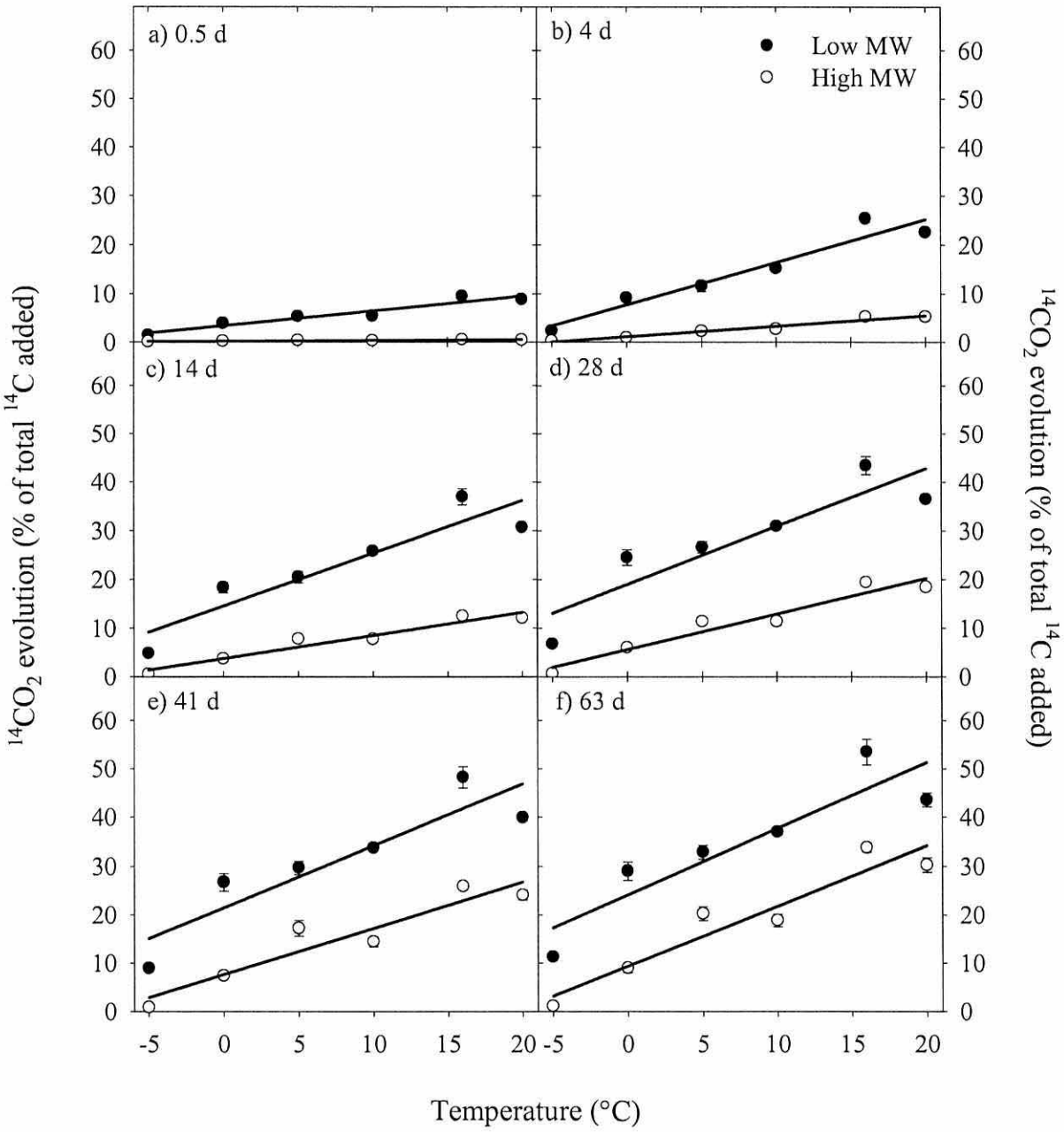

Fig. 8. Influence of temperature on the total amount of $^{14}CO_2$ evolved for low and high MW ^{14}C-labelled substrates over 31 d of sampling. Data points represent means ± standard error of the mean (SEM), n = 3. The legend is the same for all panels (a to f).

4. Discussion

4.1. Soil and vegetation dynamics in the melt front zone

We hypothesized that soil microorganisms and vegetation would respond equally rapidly to snow melt due to the high Arctic tundra having a short growing season (Grogan and Chapin, 1999; Williams et al., 2009; Wookey et al., 2009). Our field transect results corroborate this hypothesis. Once melting occurred and temperatures were above 0°C, there was a rapid increase in the number of actively growing plant species. Our results showed that the entire vegetation community had emerged and achieved complete leaf expansion after *ca.* 28 d post snow melt. Soil respiration also reached maximal activity by 28 d, with half maximal activity being quicker than plant productivity. Although this included root respiration, it suggests soil microbial communities were also able to respond rapidly once snow melt occurs. 16S rRNA studies showed gene copy numbers decreased rapidly post snow melt, between -0.1 and 0 m, correlating to 1 d post melting. Gene copy numbers then remained relatively consistent across each transect until 20 m where an increase in number was noted. Microbial biomass did not show the same rapid increase, immediately post snow melt, as soil respiration and vegetation field data. This suggests a faster response from vegetation communities, which based on our field data, could be driven by temperature change. In contrast, microbial communities may be more influenced by another factor, such as soil water content. 16S rRNA analysis indicates the presence of active and non-active cells, therefore it is likely that high numbers at -0.1 m represent a non-viable population remaining from the previous season, which is rapidly turned over upon soil thaw (Coci et al., 2010). This may also partially explain the nutrient flush upon snow melt. Metagenomic techniques indentified a rapid microbial response to thawing when looking at permafrost soils (Mackelprang et al., 2011). These authors observed rapid

shifts in microbial, phylogenetic and functional gene abundances upon thawing, with particular changes noted for multiple genes involved in C and N cycling. Bacterial *amoA* genes were below detectable limits in our study, but archaeal *amoA* genes were present at both top and subsoil depths. Ammonia-oxidizing bacterial *amoA* genes being below detection levels could indicate that in Arctic systems, AOA could be the main drivers of nitrification, supporting the hypothesis of niche separation between AOA and AOB, with AOA being better adapted to extreme conditions where nutrients are limited (Leininger et al., 2006; Schleper, 2010). However, there is conjecture surrounding the role of AOA in nitrification, irrespective of their high abundance, (Jia and Conrad, 2009).

Alongside temperature and soil water content, primary productivity in tundra ecosystems is regulated by nutrient availability. High arctic vegetation is nutrient limited especially in terms of macro-nutrients N and phosphorous (P) (Baddeley et al., 1994; Gordon et al., 2001; Madan et al., 2007). Plants were previously thought to be dependent on inorganic C for all their N requirements. Inorganic N compounds (NH_4^+ and NO_3^-) are a limited resource and soil microbial communities often out-compete plants for nutrient acquisition (Chapin et al., 1993; Schimel and Chapin, 1996; Jones et al., 2005; Hill et al., 2011a). It is now known that within terrestrial systems, organic N forms are also very important components of the N cycle and are utilised by both plant and microbial communities (Hill et al., 2011b; Hill et al., 2012). Mineralization of organic N into available inorganic forms is often slow at high latitudes due to low temperatures. Our data showed evidence for vertical separation of nutrients, which is important for N acquisition. Topsoil soluble N pools were dominated by free amino acids, whereas in the subsoil, NH_4^+ dominated. Ammonium levels remained very low along the whole transect at both depths, suggesting this inorganic form is readily

assimilated by microbial communities and bryophytes, or is being rapidly nitrified. Nitrate levels were very low across the transect at both depths, however, there is evidence for NH_4^+ conversion to NO_3^- as evidenced by a pulse in NO_3^- (Fig. 4f). This is in accordance with Buckeridge and Grogan (2010), who also showed a pulse of plant-available nutrients released during the seasonal transition from winter to spring as the snow melts. In addition, if NH_4^+ is being rapidly nitrified and then immediately leached out of the highly porous soil by snow melt, this could account for the low NO_3^- levels. As conditions became favourable for plant growth, plant available nutrients initially decreased. Microbes do not favour NO_3^-, so the decline in nutrients could be related to plant uptake rather than uptake by soil microbial communities (Hill et al., 2011a). Nutrient levels then stabilised, indicating a balance between production and consumption by soil microorganisms and plants. Our results showed DON comprised the majority of total dissolved N pool which is indicative of nutrient poor soils (Jones and Kielland, 2002; Bardgett, 2005; Farrell et al., 2011). No marked pulse was evident for amino acids, suggesting strong competition between plants and microbial communities for this N form. We speculate that initially plants are not N limited until NO_3^- levels decline and then subsoil NH_4^+ may become more important.

4.2. Soil water control of soil C mineralization

Soil water content and temperature are well documented as having an interactive effect upon decomposition and consequently C turnover, making it difficult to differentiate which factor has the greater influence on ecosystem functioning (Davidson et al., 1998; Davidson and Janssens, 2006; Schimel and Mikan, 2005). Our study looked at these two parameters independently, postulating temperature is likely to have a greater effect on mineralization rates than soil water content.

It is inferred that very dry and frozen soils are analogous to each other (Clein and Schimel, 1995; Drotz et al., 2010). Under very dry conditions, microbial activity is limited by available nutrients and their diffusion. During stressful drought conditions, soil water potential declines and cells accumulate osmolytes (e.g. simple organic compounds), reducing their internal water potential, and preventing dehydration (Schimel and Mikan, 2005). These osmolytes are discarded upon soil rewetting via respiration, polymerisation or transport across cell membranes, preventing cell walls bursting from excess pressure (Schimel and Mikan, 2005). More drying/rewetting and freeze/thaw events are projected in the tundra with a changing climate (Williams et al., 2009), therefore greater exudation of these osmolytes into the soil.

Low MW compounds showed greater allocation of ^{14}C to the recalcitrant (y_0) and rapidly respired pool (a_1), coupled with a substantial increase in the half-life ($t_{½}$) associated with this latter pool as water content decreased. This suggests that substrate decomposition rates are reduced as water content declines, resulting in larger estimated pool sizes. In addition, as soil water content reaches field capacity, microorganisms may partition more C into anabolic (maintenance processes) rather than into catabolic (CO_2 production) metabolism. High MW substrates showed the same trend with the respired pool size increasing as soil water content decreased. In addition, our data showed during dry conditions (< 15 % gravimetric water content, < -1.62 MPa), despite more ^{14}C being allocated to the rapidly respired pool (a_1), the half-life ($t_{½}$) associated with this pool increased. This suggests mineralization could be hindered by biological or chemical factors within the soil matrix.

Temperatures are projected to increase, potentially making Arctic soils vulnerable to drying out, leading to slower turnover of more recalcitrant compounds. Conversely, precipitation levels are postulated to increase, which could increase soil

water content and lengthen the half-lives of recalcitrant C compounds, until soils become saturated. Substrate decomposition can be affected by low enzyme abundance, microbial population size, and oxygen availability (Freeman et al., 2004; Schimel and Mikan, 2005; Davidson and Janssens, 2006; Waldrop et al., 2010). When soils experience high water contents and conversely very low water contents, decomposition will be negatively affected.

4.3. Temperature control of soil C mineralization

We hypothesized increasing Arctic temperatures will have a positive effect on mineralization rates, particularly for high MW compounds. Our results concur with current literature, which indicate microbial activity proceeds even when temperatures are below freezing (Elberling and Brandt 2003; Williams et al., 2009; Drotz et al., 2010). Controls on biological activity when soils are frozen are poorly understood, but they are important when looking at Arctic soil C fluxes (Hobbie et al., 2000; Drotz et al., 2010). During frozen conditions, microorganisms may break down more complex C substrates found in SOM (Clein and Schimel, 1995). However, our mineralization results showed limited respiration from high MW compounds. It is more likely that microorganisms utilize solubilised substrates in liquid water films around soil particles, or within pockets of unfrozen water within the soil (Clein and Schimel, 1995; Schimel and Mikan, 2005). A third possibility is that usable substrates are released from other microorganisms upon death, providing an influx of free amino acids and simple sugars, which are preferentially taken up by microorganisms (Skogland et al., 1988; Schimel and Clein, 1996; Schimel and Mikan, 2005).

Carbon mineralization (Table 5) showed significant shifts in C partitioning with changing temperature. Allocation of ^{14}C to the slow, recalcitrant pool (y_0) decreased

with increasing temperature for both low and high MW substrates. This occurred in conjunction with an increase in the amount of C allocated to the rapidly respired pool (a_1). We can infer there is increased breakdown of the recalcitrant pool as conditions warm. We can only speculate that substrates move from the recalcitrant pool directly into the respired pool, as the connectivity between the different pools is unknown. Total CO_2 evolution increased under warming (Fig. 8) and so it could be inferred that there is a greater allocation to the labile, respired pool with warmer temperatures.

Our results showed no increased temperature sensitivity for high MW compounds, with no change in Q_{10} values or total substrate half-life. Conversely, Q_{10} values and total substrate half-life for low MW compounds both showed an effect with temperature. Recalcitrant, high MW compounds are thought to have greater temperature sensitivity than labile, low MW substrates (Hartley et al., 2010; Briones et al., 2010), but our data suggest another factor, such as soil water content or substrate quality (Giardina and Ryan, 2000), may be more influential for decomposition of recalcitrant C compounds. Soil water content showed a greater effect on high MW total substrate half-life; suggesting these compounds become more mobile i.e. not protected by the soil matrix and thus susceptible to decomposition. Immediately post thaw, it is thought a pulse of labile C fractions will be released followed by slower, longer term release of recalcitrant C fractions (Dutta et al., 2006; Waldrop et al., 2010). This initial labile fraction may be more influenced by temperature but represents a small amount of the total C stored in Arctic soils and will be turned over quickly by the microbial community. Long term C loss will come from more recalcitrant compounds within soil organic matter which have a much slower decomposition rate. Data from permafrost loess deposits in Siberia suggest that if 10 % of the total C stored thawed, it could initially release 1 Pg C per annum (Dutta et al., 2006).

Our study combined field and laboratory experiments looking at two important drivers of ecosystem functioning; temperature and soil water content. Field transect data showed temperature and nutrient availability were the main influential drivers regulating vegetation changes during snow melt, as soil water content was not a limiting factor. Rapid changes in response to snow melt were observed in flora (plant species emergence, leaf expansion) and below-ground soil microbial communities (soil respiration) from field measurements. Quantitative analysis of the microbial communities present along the active snow melt front showed a decline in gene copy numbers immediately after the subniveal layer at the active melt front, suggesting previously un-decomposed microbial cells provide nutrients for emerging microbial communities and/or plants. Laboratory soil mineralization experiments identified soil water content as having a greater effect than temperature on mineralization rates, especially for high MW compounds. This suggests microbial decomposition of organic matter may be more sensitive to soil water changes than temperature. We propose that temperature could be the main regulator of C dynamics at the onset of the growing season, with soil water content becoming more important in regulating C cycling as the growing season progresses.

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Appendix III

Supplementary material to accompany Chapter III.

Supplementary material

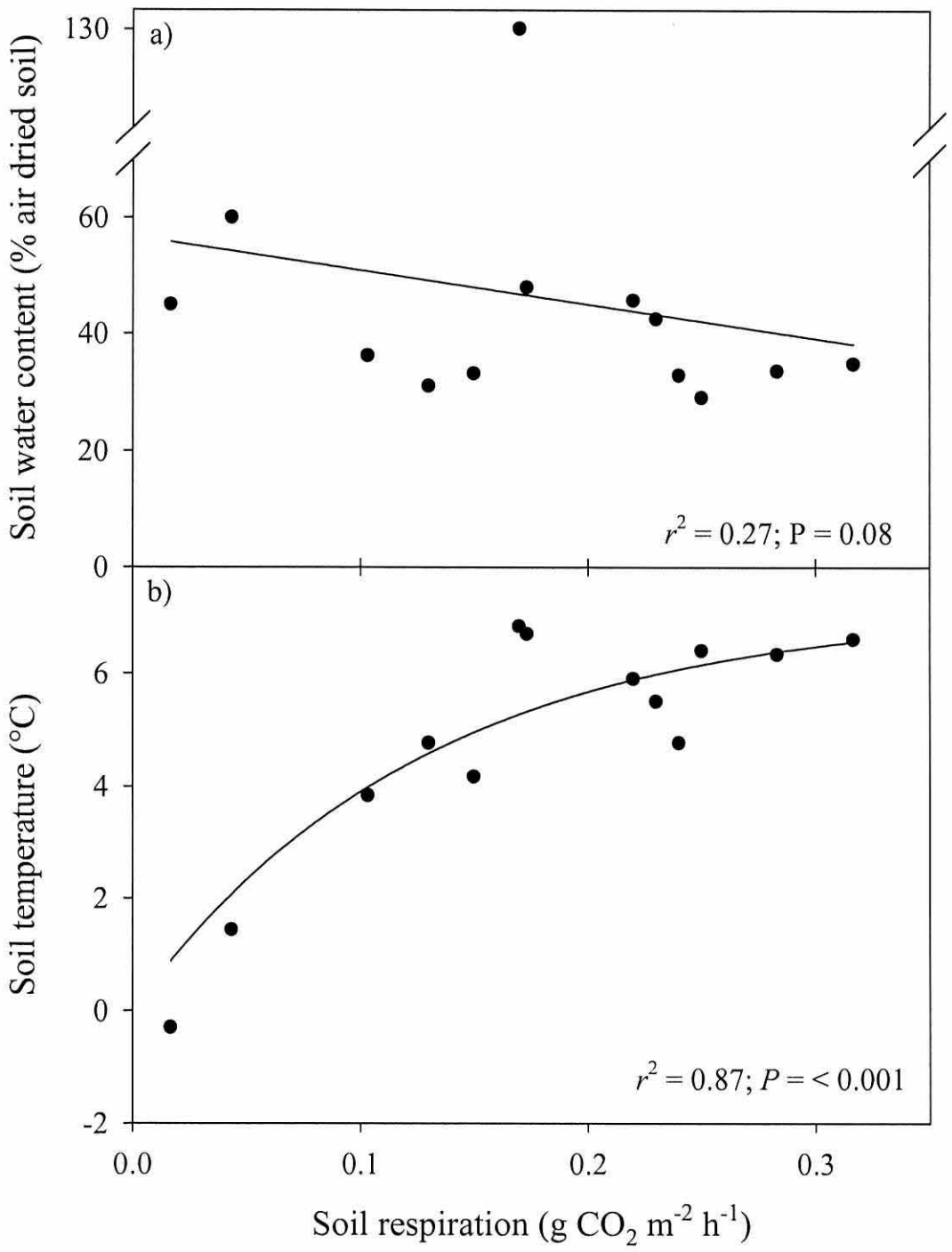

Fig. S1. Soil respiration regressed against soil water content (a) and soil temperature (b).

Chapter IV

Rousk, J., Brookes, P.C., **Glanville, H.C.**, Jones, D.L. 2011. Turnover of low molecular weight dissolved organic carbon does not correlate with differences in microbial community composition or growth across a soil pH gradient.

***Applied and Environmental Microbiology* 77, 2791-2795.**

J.R., P.B., H.C.G., and D.L.J. were involved in experimental planning and design. H.C.G. carried out sample preparation, ^{14}C-labelling work and analytical soil chemical analyses. J.R. carried out PLFA analysis, contributed to laboratory analyses, conducted data analysis. P.C.B. assisted in sample collection. All authors discussed results and contributed to manuscript preparation.

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Lack of Correlation between Turnover of Low-Molecular-Weight Dissolved Organic Carbon and Differences in Microbial Community Composition or Growth across a Soil pH Gradient[▽]†

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We studied how soil pH (pHs 4 to 8) influenced the mineralization of low-molecular-weight (LMW)-dissolved organic carbon (DOC) compounds, and how this compared with differences in microbial community structure. The mineralization of LMW-DOC compounds was not systematically connected to differences in soil pH, consistent with soil respiration. In contrast, the microbial community compositions differed dramatically. This suggests that microbial community composition data will be of limited use in improving the predictive power of soil C models.

The primary connection between soil microbial activity and carbon (C) cycling is well established (29, 46, 51); however, there has recently been a call for a more detailed understanding and integration of the microbial community into models for soil organic C (SOC) cycling to improve their predictive power (5, 6, 10, 11, 20, 29, 35, 44, 47). To accomplish this, we need to study the drivers of respiration and composition of microbial communities together (36).

Low-molecular-weight (LMW)-C compounds dominate the C used in soil respiration (13, 18, 22, 31, 48, 49, 50). Although observations regarding factors that affect LMW-DOC cycling have started to emerge (13, 23, 31), the canonical environmental factors influencing their mineralization remain unclear. This study's aims were to test how one of the most influential factors for the composition of the soil microbial community, soil pH (4, 8, 26, 32, 39, 40, 41, 42), influenced the mineralization of LMW-C compounds across a wide pH gradient and to compare our findings with microbial community composition.

Soil was obtained from the Hoosfield pH gradient at Rothamsted Research, United Kingdom (1, 2, 3, 7, 39). Thirty topsoil samples (0- to 23-cm depth) were tested along the gradient in March 2010, sieved (<2 mm), and characterized (39). The 30 individual soil samples were used for microbial and chemical analyses (e.g., organic C, total N, pH, phospholipid fatty acid [PLFA] composition, and bacterial growth), while the gradient was split into four pH levels for the soil solution analysis and C substrate mineralization assays. For these, independent replicates ($n = 3$) were used for each of the four pH levels (pH 4.1 ± 0.04, pH 5.0 ± 0.07, pH 6.0 ± 0.05, and pH 7.1 ± 0.08). Soil solution was extracted by centrifugal drainage (19, 43; see also Supplement S1 in the supplemental material), and the free amino acid and sugar concentrations of the solutions were determined (21, 30).

Soil (5 g) from each pH level was weighed into polypropylene tubes. Soil solution from each replicate (450 μl) was then individually spiked with one of eight different ^{14}C-labeled substrates (50 μl) at a trace level and added to the soil and the mineralization monitored using 1 M NaOH CO_2 traps at 22°C for 7 days (see Supplement S1 in the supplemental material). The $^{14}CO_2$ level in the NaOH traps was determined by liquid scintillation. Sorption of the added ^{14}C LMW-DOC compounds across the pH gradient was determined in sterilized soil samples (see Supplement S1). Bacterial growth was estimated using leucine incorporation (9, 25), and microbial community structure was determined from PLFA patterns (16, 17, 32).

^{14}C substrate mineralization and half-lives ($t_{1/2}$) were modeled by fitting a double-first-order decay equation to the experimental results (13, 23, 33, 48, 49; see also Supplement S1 in the supplemental material). The PLFA composition (mol% of the 26 most abundant PLFAs) was analyzed by principal-component analysis (PCA) after values were standardized to unit variance. Analysis of variance (ANOVA) with Tukey's honestly significant difference test *post hoc* comparisons were used to determine differences in variables with soil pH, while regression analyses were used to describe relationships with pH across all 30 samples. Results were compared to those for an identical sampling of the pH gradient from 2008 (40). In addition, a 454-pyrosequencing-based analysis of the bacterial community composition was also previously performed on the same 2008 samples (42; based on analyses from references 14, 27, 28, and 34). A type II major-axis regression analysis was used to investigate the connection between the sequence composition (the principal coordinate of variation of the sequence composition [see Supplement S1]) of the bacterial community with microbial PLFA composition.

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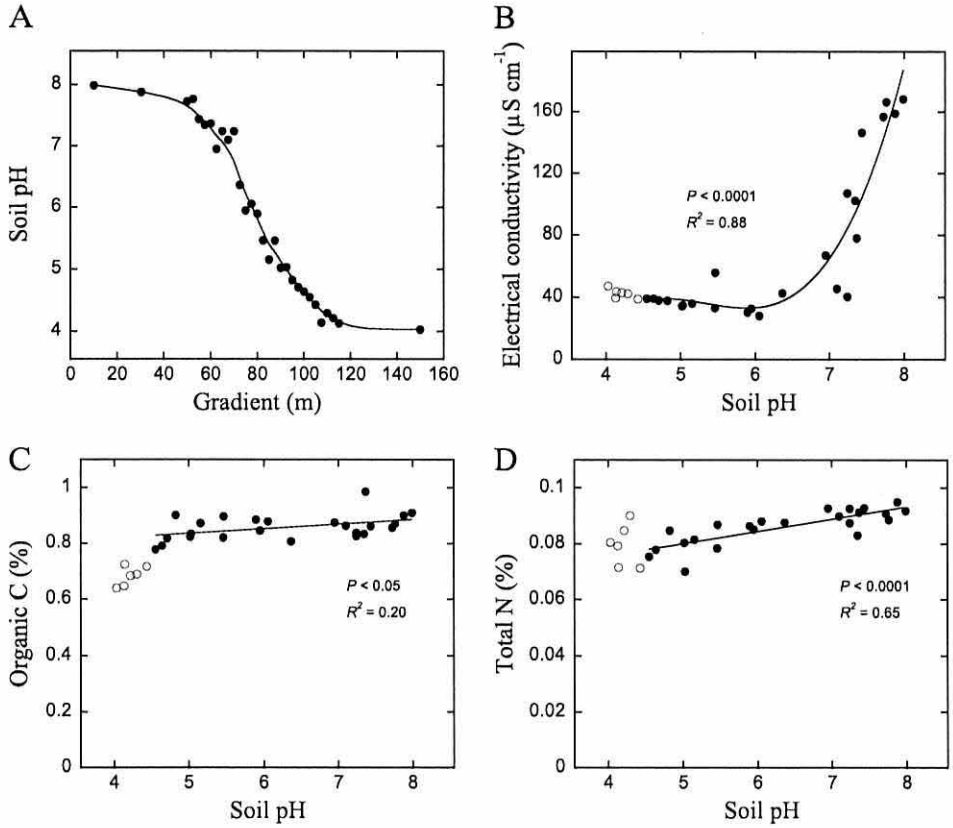

FIG. 1. Soil pH along the Hoosfield acid strip (A), the effect of soil pH on electrical conductivity (B), the organic C (C), and total N (D). Data points below pH 4.5 (open symbols) were not used in the regression analyses (see Materials and Methods).

Soil measurements showed a smooth pH gradient between pH 4 and 8 with only small differences in most other chemical variables (39) (Fig. 1). DOC and dissolved organic nitrogen (DON) concentrations did not systematically change over the pH gradient (see Table S1 in the supplemental material). The DOC and DON concentrations derived from both sugars and amino acids were similar over the gradient ($P > 0.05$) (Table S1), averaging 53.5 ± 7.1 nmol of C derived from sugar (sugar C) g^{-1} soil and 11.4 ± 2.1 nmol amino acid C g^{-1} soil. The mineralization patterns conformed well to a double-first-order kinetic model ($R^2 > 0.99$ for all substrates) (Fig. 2 and 3). The individual amino acids and sugars degraded at different rates (Fig. 2); however, this did not systematically change with pH (see Table S2 in the supplemental material).

Combining our estimates of free sugar and amino acid concentrations with estimates of their turnover rate enabled estimation of the contribution of LMW-C mineralization to soil respiration. We estimate that approximately 0.32 and 0.09 μg of C derived from CO_2 (CO_2 C) g^{-1} h^{-1} of soil respiration were derived from sugar and amino acid degradation, respectively. Taken together, this is within the range of previous assessments of the basal respiration rate at the site (0.20 to 0.50 μg CO_2 C h^{-1} g^{-1}) (3, 39, 41).

Bacterial growth increased about 6-fold between pH 4.5 and 8.0 ($P < 0.0001$) (see Fig. S1 in the supplemental material). The first component from a PCA of the microbial PLFA composition explained 40% of the variation in the data and was closely related to soil pH ($P < 0.0001$, $R^2 = 0.96$) (see Fig. S2A in the supplemental material). The variation of microbial PLFA composition across the Hoosfield site was highly reproducible, with the major component of variation from a PCA aligning identically with soil pH (Fig. 4A). There was also a very close relationship between the variation in the sequence composition of the bacterial community (42) (Fig. 4B).

The Hoosfield site effectively isolated soil pH from most other variables associated with microbial community differences. In addition, it was previously shown that excess Al (or other unidentified inhibitors) or a lack of P or N did not limit bacterial and fungal growth across the pH gradient (41). Soluble LMW-C compound concentrations were also not systematically affected by pH. Similarly, rates of turnover of individual LMW-C compounds did not change consistently with pH. In contrast, the microbial community composition radically differed along the pH gradient. Direct comparison between the microbial PLFA composition and its sequence composition (obtained by 454 pyrosequencing [42]) (Fig. 4B) indicated that the difference in microbial PLFA composition across the gradient was a consequence of a difference in bacterial species composition. The enormous pH-related differences in microbial community composition did not affect the mineralization of LMW C, the dominant source of soil respiration, across the pH gradient. Therefore, we could not find support for a connection between microbial community structure and function (the turnover of the LMW-DOC compounds that govern soil

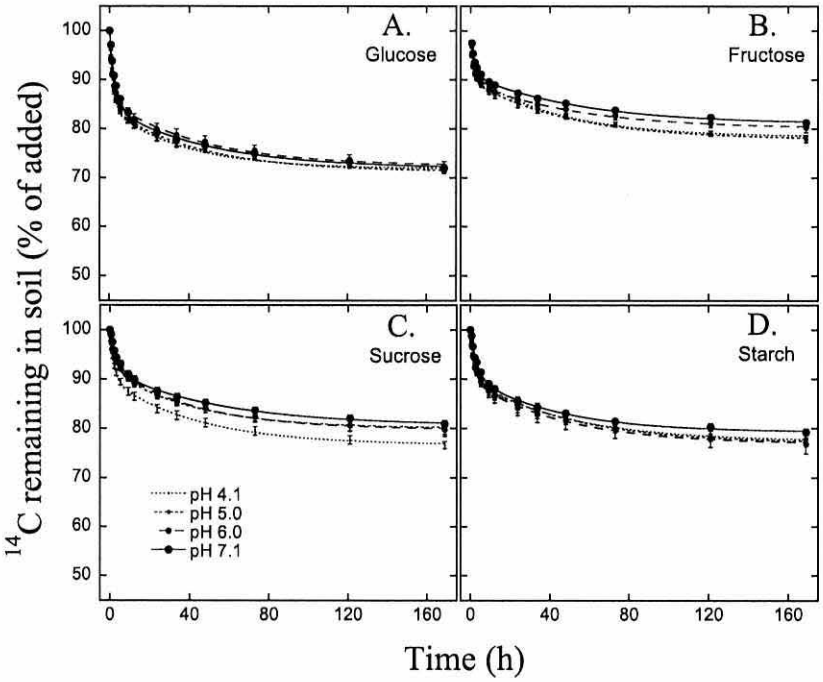

FIG. 2. Mineralization of sugars. Amounts of ^{14}C-labeled glucose (A), fructose (B), sucrose (C), and starch (D) remaining in soils of the four different pH levels after the injection of the isotopically labeled soil solution into the soil. Values represent means ± 1 SE (n = 3). The lines represent the best fits of double-first-order exponential-decay functions (R^2 > 0.99 for all curve fits).

respiration). This is consistent with conjectures derived from theoretical models of soil organic matter (SOM) turnover that have suggested high redundancy in the microbial processing of LMW C (12, 24, 45). We cannot discount the possibility that processes transforming SOM to LMW DOC are closely related to microbial community structure. However, there was only a minor discrepancy between basal respiration (30% difference across the gradient) and LMW-DOC mineralization across the

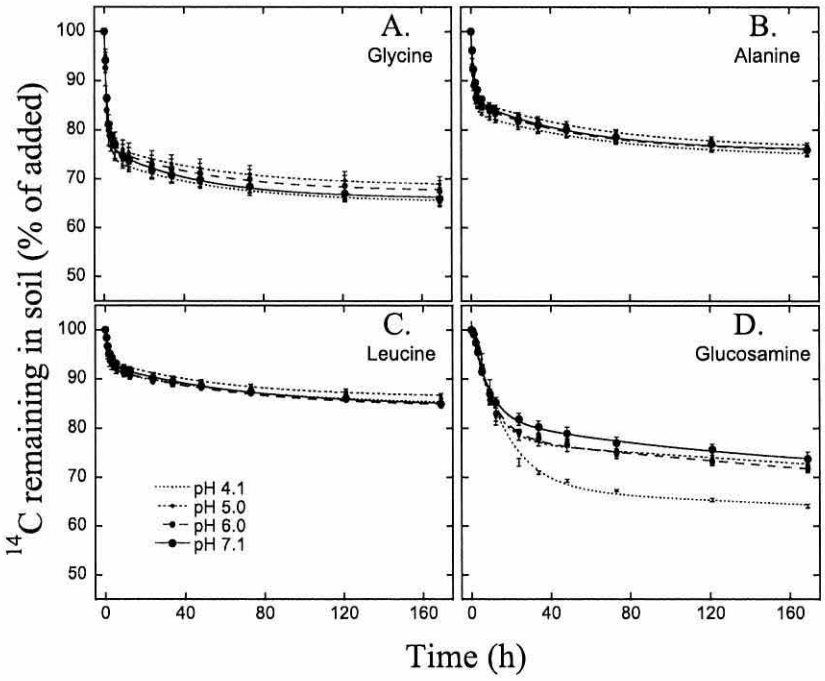

FIG. 3. Mineralization of amino acids and amino sugars. Amounts of ^{14}C-labeled glycine (A), alanine (B), leucine (C), and glucosamine (D) remaining in soils of the four different pH levels after the injection of the isotopically labeled soil solution into the soil. Values represent means ± 1 SE (n = 3). The lines represent the best fits of double-first-order exponential-decay functions (R^2 > 0.99 for all curve fits).

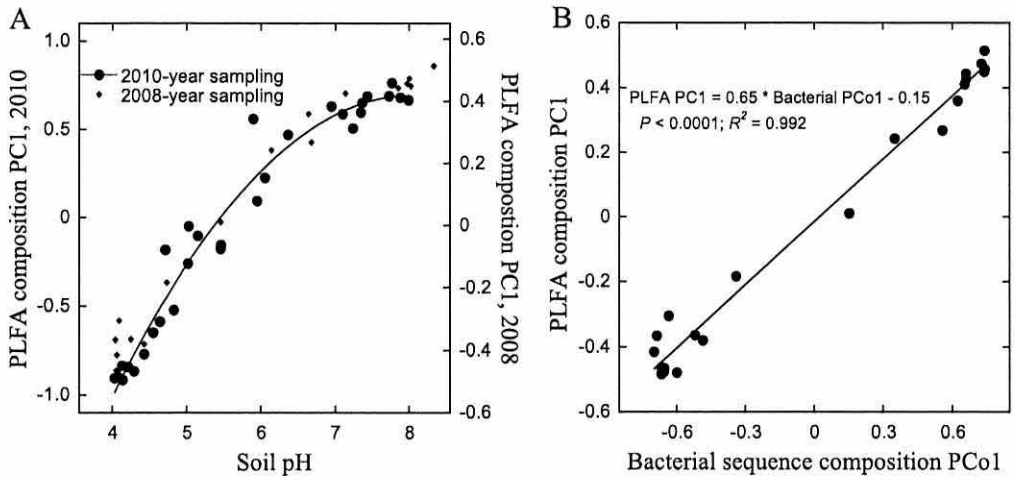

FIG. 4. (A) Comparison of the levels of pH dependence of the microbial PLFA compositions across the Hoosfield acid strips in the soil samples of the present study compared to those sampled in 2008 (data are from reference 40); (B) first principal component (PC1) of the variation in the PLFA compositions (explaining 39.6% of the variation in PLFA composition) across the Hoosfield acid strip from the 2008 sampling (data are from reference 40) compared with the first component of a principal-coordinate (PCo1) analysis (explaining 29.1% of the total sequence variation) of the compositions of bacterial sequences obtained with a 454-pyrosequencing assessment (data are from reference 42). A type II major-axis regression was used to fit the line.

gradient (no difference across the gradient), setting the upper limit for the functional relevance of the microbial community difference. This does not lessen the need to identify and incorporate the rate-limiting mechanisms in models for SOC turnover (6, 29), but it does suggest that adding information about the microbial community composition may be of limited use.

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Appendix IV

Supplementary material to accompany Chapter IV.

Supplementary material

Supplement 1.

The soil from the Hoosfield Acid strip at Rothamsted Research, UK, a Chromic Luvisol (14), was described in detail elsewhere (1, 2, 3, 39). Briefly, Hoosfield has been under arable management since before the 19th century. It probably received a single large, but uneven, application of chalk initially and has not received any further amendment including inorganic or organic fertilizers since (A. E. Johnston & P.R. Poulton, personal communication) and winter wheat has been continuously grown. By the 1950s, the uneven distribution of chalk remaining in the soil resulted in the formation of this unique pH gradient, within the 200 m strip of land, with pH decreasing smoothly with increasing distance from the original chalk pits (7).

We sampled along the first 160 m of the strip taking 5 cm diameter, 0-23 cm depth, cores at each sampling position along the gradient in late March 2010. The gradient was sampled with a resolution informed by previous assessments (2, 3, 39), resulting in sampling every 2.5 m between 50 and 115 m, with 26 samples covering the steep pH-slope of the gradient. The 30 resulting soil samples were sieved (< 2 mm) in the laboratory and water contents determined (105 °C; 24 h). The soils were at about 40 % of water-holding capacity so further moisture adjustment was not required. They were incubated at 20 °C for a week before the microbial analyses were performed. Subsamples of the sieved soil were frozen (-20°C) until PLFA analysis was performed. Other sieved samples were air-dried and ground for chemical analyses. All measurements are given as soil oven-dry weights (105 °C; 24 h).

Soil solution was extracted by adapting the centrifugal-drainage procedure described by Giesler & Lundström (19) and detailed in Supplementary material. Briefly, 1 g field-moist soil was placed in a microcentrifuge tube in which a hole had been pierced at the bottom. Distilled water (0.5 ml, 0°C) was gently pipetted on top of the

soil. The tube was then placed into another intact one, and the pair centrifuged at 18000 *g*. For each of the four pH-levels, 24 repetitions of this procedure were combined, yielding at least 6 ml of soil solution for each level, which was immediately put on ice (0°C) to avoid microbial transformations during handling and preparation (43). Aliquots of these soil extracts were subsequently used in the mineralisation assays (see below).

The soil extracts were analysed for DOC and total dissolved N (TDN) using a Shimadzu TOC-V-TN analyzer (Shimadzu Corp., Kyoto, Japan). Free amino acids in the soil extracts were measured fluorimetrically according to Jones *et al.* (21) while free sugars were measured using the 2, 4, 6-tri pyridyl-*s*-triazine (TPTZ) spectroscopic method of Myklestad *et al.* (30). Sugars in the soil extract were subjected to an oxidation reaction at alkaline pH, during which Fe^{3+} was reduced to Fe^{2+}. The Fe^{2+} was then determined colorimetrically after condensation with the chromogen TPTZ to give violet colour of the $Fe(TPTZ)_2^{2+}$. Reactions were performed using 100 µl (1:3 diluted) soil extract and reagents in 1.5 ml microcentrifuge tubes, and the absorbance was read after 30 min on a 300 µl 96-well plate at 595 nm, and a glucose dilution series was used to establish a standard curve.

Organic C and total N were analysed with a Leco CHN 200 analyser (Leco Corp., St Joseph, MI). Soil pH and electrical conductivity were analysed using a 1:1 (w/v) distilled H_2O extraction using glass electrodes.

Mineralisation assays.

Soil (5 g fresh weight) from each replicate of the four different pH-levels was weighed into eight 50 ml polypropylene centrifugation tubes, totalling in 96 tubes. The soil extract from each respective replicate (450 µl) was individually spiked with each of the 8 different ^{14}C-labelled substrates (50 µl) at a trace level (<10 nM; several orders of magnitude lower than soil solution estimates, c.f. e.g. [18, 50]), and added factorially.

Trace levels of isotopic tracers were used to maintain the intrinsic soil solution concentrations so that turnover times would reflect that naturally occurring in the soil. Immediately after the addition of the ^{14}C-labelled solutions to the soil, CO_2-traps (1 ml 1 M NaOH) were placed in the tubes, the tubes hermetically sealed and the NaOH traps subsequently changed at regular intervals over a 7 d period. The substrates used to spike the soil extracts included the four sugars glucose, fructose, sucrose and starch, the three *L*-amino acids glycine, alanine and leucine and the amino sugar, glucosamine (all <10 nM; 1 μCi ml^{-1}, Amersham Biosciences UK Ltd.). The $^{14}CO_2$ in the NaOH traps was determined by liquid scintillation counting on a Wallac 1404 liquid scintillation counter. The incubation was performed in the dark at 22°C. We investigated the sorption of the added ^{14}C LMW DOC compounds across the pH gradient in soil samples where microbial activity had been terminated (80°C for 1 h for 1 g soil samples in 1.5 ml closed microcentrifugation tubes). To do this, we repeated the procedure of the soil solution extraction described above and compared the ^{14}C content of added ^{14}C-spiked soil solution to the ^{14}C in the soil extract, compensating for the dilution of the water in the soil. The recovery rate was about 100% for the monosaccharides, about 60% for starch, and 25-60 % for the amino acids and sugars. Most importantly, the recovery was not influenced by soil pH, indicating that sorption of the added LMW DOC could not explain differences in mineralisation with regard to pH.

Microbial analyses.

Bacterial growth was estimated using leucine incorporation (25) adapted to soil (9, 37, 38) and the PLFA pattern was determined according to Frostegård *et al.* (16) with modifications (32). An internal standard (methyl nonadecanoate fatty acid 19:0) was added before the methylation step. The PLFAs chosen to indicate bacterial biomass

were i15:0, a15:0, i16:0, 16:1ω9, 16:1ω7c, 10Me16:0, cy17:0, i17:0, a17:0, 18:1ω7 and cy19:0, while PLFA 18:2ω6,9 was used to indicate fungal biomass (17).

Statistical and data analyses.

Previous assessments (e.g. [13, 23, 33, 48, 49]) have repeatedly shown and verified that the mineralisation of LMW-C compounds in soil is biphasic, and that it conforms well to a double-first order decay equation, $y = A + B*e^{-k1*t} + C*e^{-k2*t}$, where y is the amount of ^{14}C-compound remaining in the soil, A is the asymptote of y, B and C describe the sizes of the pools, k_1 and k_2 describe the rates of loss of the two pools, and t is incubations time. The first rapid phase of $^{14}CO_2$ production is attributable to the immediate use of the substrate in catabolic processes (*i.e.* respiration) and approximates to the depletion rate of LMW DOC constituents from the soil solution, which is of interest in the present context. The half-life ($t_{½}$) of the respective phases can be determined using a first order kinetic model (33) and is defined as $t_{½}= \ln(2) / k$.

The PLFA composition (mol-% of the 26 most abundant PLFAs) was analysed with a principal component analysis (PCA) after standardizing to unit variance. A 2nd degree polynomial function was used to describe the relationship between the first component of the PCA and soil pH.

Analyses of variance (ANOVAs) with Tukeys HSD posthoc comparisons were used to determine differences in variables contrasted among the four pH-levels, while regression analyses were used to describe the pH-relationship of variables measured at all 30 pH-levels across the gradient. Statistical analyses and tests were performed with JMP 7.0 for Mac (SAS Institute Inc., Cary, NC, USA), the double first-order decay functions were fitted using Kaleidagraph 4.0 for Mac (Synergy software, Reading, PA, USA), and the PCA analysis was performed using MVSP 3.1 for windows (Kovach computing services, Pentraeth, Anglesey, UK).

Table S1. Dissolved organic carbon, dissolved total nitrogen, total free sugar and total free amino acid concentrations along the Hoosfield acid strip.

	Soil pH			
	4.1	**5.0**	**6.0**	**7.1**
Dissolved Organic Carbon (DOC, nmol C g^{-1})	411 ± 30.0a	225 ± 24.0b	353 ± 13.9a	411 ± 19.4a
Dissolved Total Nitrogen (DTN, nmol N g^{-1})	218 ± 28.1a	383 ± 8.8a	339 ± 11.5a	329 ± 44.9a
Total free sugars (nmol C g^{-1})	69.1 ± 23.2a	60.8 ± 10.8a	51.2 ± 5.2a	32.9 ± 7.0a
Total free amino acids (nmol C g^{-1})	13.0 ± 6.4a	5.3 ± 1.1a	16.2 ± 4.2a	11.3 ± 2.6a

Values are the mean ± SE (n=3). Letters denote significant differences using ANOVA and Tukeys HSD post-hoc test at $P < 0.05$.

Table S2. Estimates of soil solution half-lives (h; mean ± SE) for sugars, amino acids and amino sugars, where the half life is determined as $t_{½} = \ln(2)/k_1$, and k_1 is the rate constant of the primary mineralization phase of LMW-C compounds in solution from the first order double exponential equation used to describe the biodegradation of each compound.

	Soil pH			
	4.1	**5.0**	**6.0**	**7.1**
Glucose	1.34 ± 0.18	2.09 ± 0.29	1.13 ± 0.16	1.17 ± 0.12
Fructose	0.77 ±0.07	0.79 ± 0.09	1.18 ± 0.11	1.54 ± 0.16
Sucrose	1.43 ± 0.11	1.8 ± 0.19	1.55 ± 0.15	2.73 ± 0.35
Starch	1.77 ± 0.24	1.52 ± 0.18	1.49 ± 0.17	2.33 ± 0.19
Glycine	1.10 ± 0.33	1.07 ± 0.56	0.90 ± 0.32	1.12 ± 0.33
Alanine	0.81 ± 0.15	0.99 ± 0.25	0.67 ± 0.08	1.28 ± 0.17
Leucine	0.97 ± 0.12	1.06 ± 0.17	0.99 ± 0.16	1.63 ± 0.19
Glucosamine	11.57 ± 1.45	6.61 ± 1.62	6.85 ± 2.11	5.82 ± 2.32

Supplementary figures

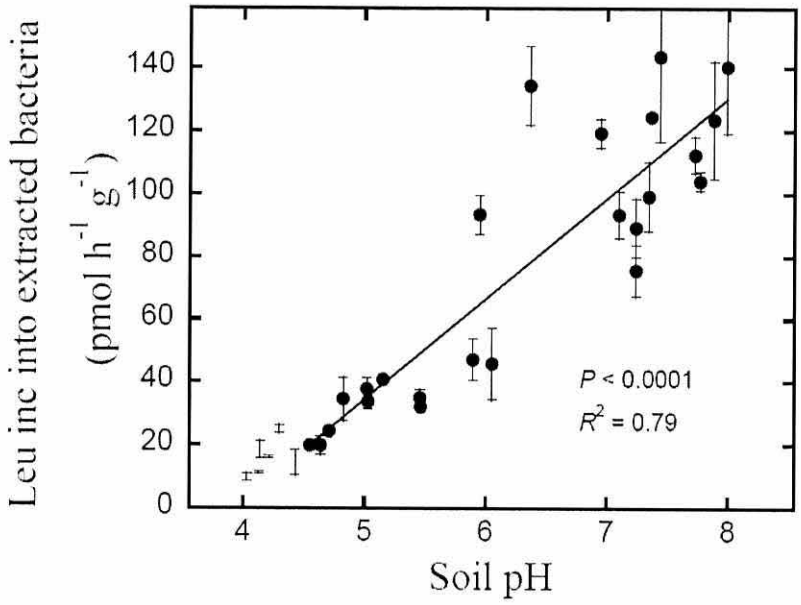

Fig. S1.

Fig. S1. The effect of soil pH on bacterial growth as measured by leucine incorporation into extracted bacteria (Panel F). Data points below pH 4.5 (open symbols) were not used in the regression analysis (see material and methods).

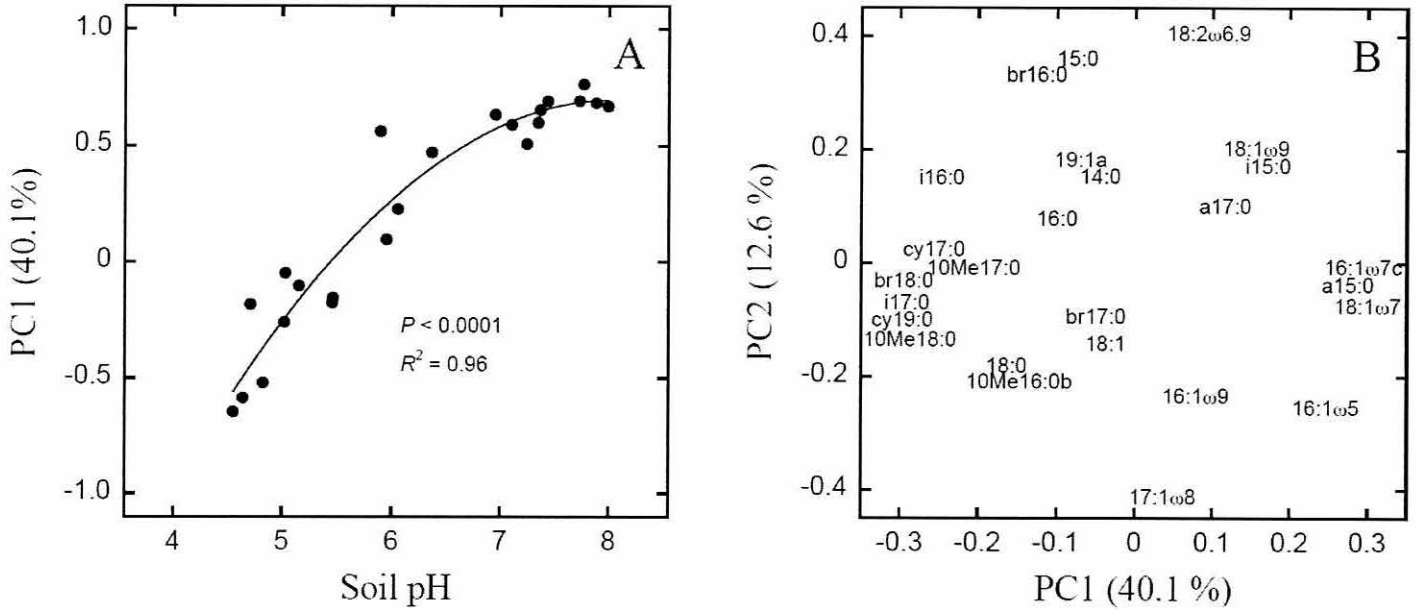

Fig. S2.

Fig. S2. The effect of soil pH on the microbial community biomass (PLFA) composition. The first component from a Principal Component Analysis (PCA) of the PLFA composition of the soil samples along the soil pH gradient regressed against soil pH (Panel A), and a loading plot of the first two components from the PCA on the PLFA composition (Panel B). A 2nd degree polynomial function was used to describe the effect of soil pH on PC1 of the soil microbial PLFA composition. Data points below pH 4.5 (open symbols) were not used in the regression analysis (see material and methods).

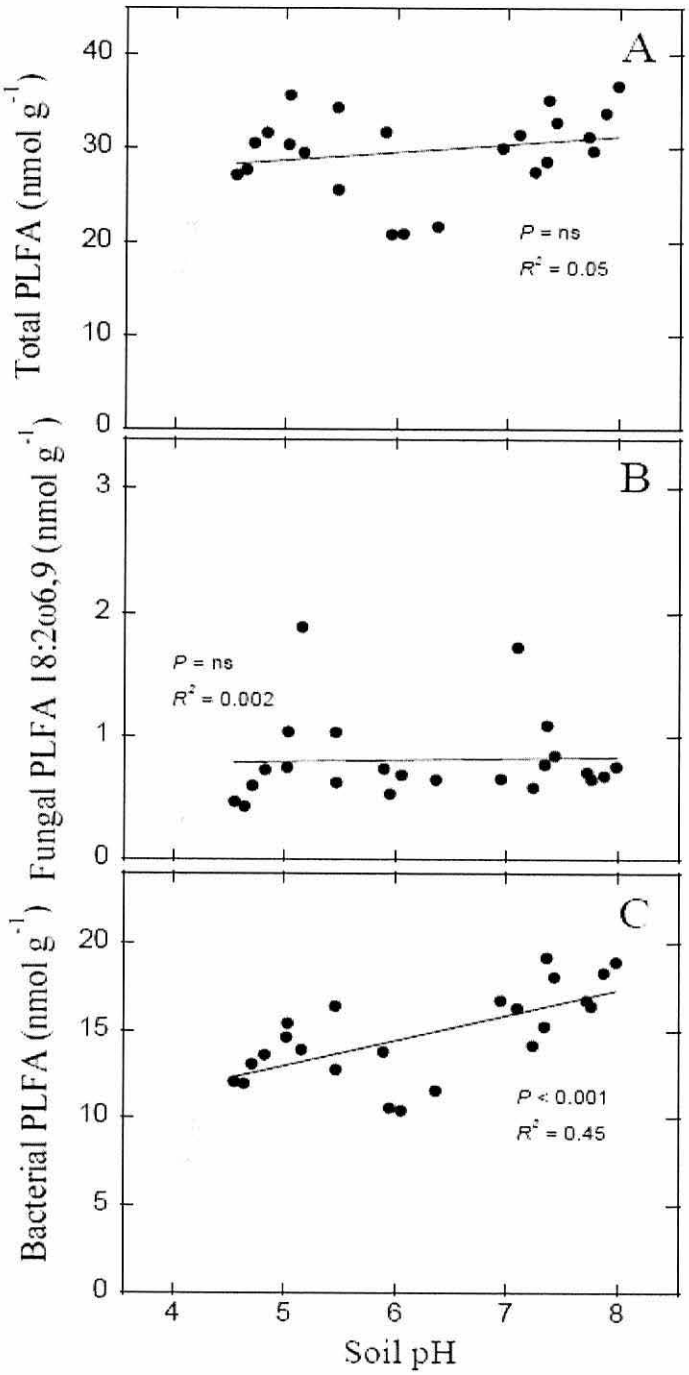

Fig. S3.

Fig. S3. The effect of pH on the concentrations of total PLFA (Panel A), fungal PLFA 18:2ω6,9 (Panel B) and Bacterial PLFA (Panel C). Data points below pH 4.5 (open symbols) were not used in the regression analysis (see material and methods).

Chapter V

Glanville, H.C., Hill, P.W., Golyshin, P., Schnepf, A., Jones, D.L. 2012. Modeling isotopically labelled carbon substrate turnover in soil.

In preparation for submission to Soil Biology & Biochemistry.

H.C.G., P.W. H., and D.L. J. conceived the experiment and investigation. H.C.G. carried out field work, laboratory experiments, and data analysis. A.S. provided assistance with mathematical modelling. All authors discussed results and contributed to the preparation of the manuscript.

Modeling isotopically labeled carbon substrate turnover in soil

In preparation for submission to Soil Biology & Biochemistry

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ABSTRACT

Soil organic carbon (SOC) dynamics and mineralization rates are currently poorly understood, despite the terrestrial soil C pool being twice the size of the atmospheric pool. Typically, C substrate turnover is measured by monitoring CO_2 evolution over time following the addition of ^{12}C, ^{13}C or ^{14}C labelled compounds to soil. The kinetics of CO_2 evolution, however, is rarely linear with substrate-C partitioned into both fast and slowly mineralizing pools. The application of mathematical models to soil respiration data therefore provides a more mechanistic understanding of how individual substrates pass through the soil microbial biomass. A biphasic pattern of mineralization is frequently assumed for low molecular weight (MW) compounds, with C partitioned into two pools. We investigated whether this two pool model best represents the mineralization of two common ^{14}C-labelled root exudates, alanine and glucose, in an agricultural soil. We show that for short term experiments (< 2 d) and with minimal monitoring points (< 8), a biphasic pattern of mineralization adequately fits the data, after which, the model parameters fit better to a triphasic pattern (i.e. three discrete mineralizable pools). Identifying the origin of each pool is currently speculative. We infer that the first mineralizable pool (half-life ca. 0.04 d for both alanine and glucose) represents substrate-C used rapidly and directly for energy production. The second mineralizable pool (half-life ca. 1.35 and 1.47 d for alanine and glucose respectively) we ascribe to turnover of soluble C held within the cytoplasm within secondary compounds (e.g. storage metabolites. nucelotides, proteins). The third mineralizable pool (half-life ca. 128.4 and 217.2 d for alanine and glucose respectively) can be attributed to the degradation of recalcitrant structural biomass compounds (e.g. cell walls) and relatively stable soil organic matter (e.g. protected material). We conducted a series of parallel extractions (soil solution, 0.5 M K_2SO_4 pre- and post-chloroform fumigation, methanol) which corroborated the use of a three pool model. The combination of measurable experimental data and mathematical modelling provides a detailed mechanistic approach to how complex biogeochemical cycles work.

Keywords: Carbon dioxide; Decomposition; Greenhouse gas emissions; DOC; SOM

1. Introduction

The amount of carbon (C) contained within the world's soils is approximately double that contained in the atmosphere (Raich and Schlesinger, 1992; Rustad et al., 2000). It is crucial that we improve our understanding of the factors regulating soil C dynamics to ascertain the potential impacts of climate and land use change on ecosystem functioning and global terrestrial C cycling. Below-ground respiration is the primary flux which mediates the passage of terrestrial C back to the atmosphere (van Hees et al., 2005). This respiration encompasses both autotrophic (plant) and heterotrophic (microbial) respiration and involves many hundreds or even thousands of mineralization processes occurring simultaneously (Hanson et al., 2000; Kuzyakov, 2006; Hill et al., 2008). A key challenge in ecosystem science is to make sense of this inherent complexity and then simplify it for mathematical modelling purposes to allow simulation of future changes in soil C storage. To realise this will require taking a compound-specific approach to the problem (Hedges et al., 2000; van Hees et al., 2005). If individual compounds show similar behavioural attributes with respect to their turnover in soil then they can be grouped within soil C models in a scientifically robust way. Before grouping can occur, however, requires that we can describe turnover of these individual compounds in a meaningful way.

Isotopically labelled substrates (e.g. ^{13}C, ^{14}C) are frequently employed to estimate the rate of substrate turnover by soil microbial communities (Coody et al., 1986; Fischer et al., 2008; Glanville et al., 2012). This remains the only approach to be able to examine the turnover times of individual compounds with relatively little alteration to the size of the substrate pool. However, despite the lack of suitable alternative and its widespread use, this approach has severe limitations. Firstly, measurements of mineralization do not account for the apparent time lag between microbial uptake of each substrate and its subsequent mineralization which can lead to a

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significant underestimation of turnover times (Hill et al., 2008). Secondly, C derived from individual substrates can be partitioned very differently once inside microbial cells (e.g. C used for making short-term storage compounds versus that partitioned into cell wall structures such as peptidoglycan or chitin). The relative amount of substrate C partitioned to these pools and their subsequent turnover may be radically different from that of the parent compound. Therefore in terms of C storage it is not only the mineralization of the substrate itself which is important but also the immobilized C derived from it.

Tracing the flow of isotopically-derived substrate C into individual metabolites within microbial cells is still in its infancy (Knowles et al., 2011). This approach is being hampered by (1) the many thousand different transformation pathways which are possible within a diverse microbial community, (2) the use of low substrate concentrations reflective of field conditions which makes quantification of isotopic enrichment with transformation products difficult, (3) the temporal dynamics of C within the community, and (4) limitations in bioinformatics to enable us to make meaningful sense of metabolomic data. Until these challenges can be resolved, a higher level approach is therefore warranted, which preferably builds on current labelling techniques.

For isotopically labelled low MW compounds, such as sugars and amino acids, a biphasic pattern of mineralization is typically observed whereby C can be partitioned into two major compartments/pools (Chotte et al., 1998; Saggar et al., 1999; van Hees et al., 2005; Boddy et al., 2007). A double first-order exponential decay model can then be used to mathematically describe the kinetics of mineralization. The first rapid phase of mineralization (Pool 1) is associated with the immediate use of substrate for catabolic processes (i.e. respiration), relating to a depletion of the soil solution (Jones, 1999). A second, slower pool of C is associated with some of the substrate-C being utilized for

anabolic processes (formation of new biomass, growth and repair, and cell maintenance) and eventual turnover of the soil microbial community (Knowles et al., 2011). However, a two pool model may be too simplistic to describe how some substrates are compartmentalized during their metabolism. When assigning a model to experimental data, the relationships between model parameters are important, alongside an understanding of how that substrate is likely to be metabolized, to avoid over-fitting of the data (Wolfe and Chinkes, 2005).

This study aims to determine how two common, ^{14}C-labelled root exudates (glucose and alanine) are mineralized within a controlled laboratory experiment. We aim to evaluate whether a biphasic or triphasic model best represents how these two compounds are mineralized. We hypothesize that the biphasic model is too simplistic and fails to account for the multifarious interactions that occur within the dynamic soil system. The duration of an experiment, and number of time points used to fit the model, will likely influence which model best fits the experimental data. Currently, laboratory ^{14}C-labelled mineralization studies are conducted over varying time frames; from minutes (Hill et al., 2008; Fujii et al., 2010), days (Coody et al., 1986; Glanville et al., 2012), weeks and years (Simfukwe et al., 2011; Farrar et al., 2012). We aim to investigate which model is most appropriate to describe mineralization over timescales ranging from 0.5 to 49 d (6 to 15 time points). In addition, we will investigate whether the pools/compartments assigned after fitting a model to the $^{14}CO_2$ evolution data correspond to different extractions of known mineralizable pools. This would provide biological justification of the chosen mathematical model.

2. Materials and methods

2.1. Field site

Soil was obtained from a hyper-oceanic, freely draining, temperate agricultural grassland located in Abergwyngregyn, Gwynedd, North Wales (53°14'N, 4°1'W). The mean annual rainfall is 1250 mm and the mean annual soil temperature at a soil depth of 10 cm is 11°C. The soil is classified as a Eutric Cambisol (FAO) or Dystric Eutrudepts (US Soil Taxonomy) and is derived from Ordovician post-glacial alluvial deposits. The vegetation at the site consists of perennial rye grass (*Lolium perenne* L.) and white clover (*Trifolium repens* L.) and is subject to intensive sheep grazing (> 5 ewe ha^{-1}) and receives regular fertilizer addition (120 kg N ha^{-1} y^{-1}). Due to the high root density in the grassland (0.35 ± 0.02 kg m^{-2} in the top 10 cm), all the sampled soil was classified as rhizosphere soil.

2.2. Soil characterisation

Independent samples were collected from a 0 to 10 cm depth (n = 3), stored in gas-permeable plastic bags and immediately transferred to the laboratory for analysis. The soil passed freely through a 2 mm sieve enabling removal of any stones, roots and macro-fauna present and ensuring soil homogeneity. Soil water content was measured gravimetrically (24 h at 105°C). Soil pH and electrical conductivity (EC) were determined in a 1:2.5 (w/v) soil-to-deionised water mixture using standard electrodes. Soil solution was extracted within 12 h of soil collection from the field by the centrifugal-drainage method (3200 *g*, 15 min, 20°C; Giesler and Lundström, 1993). The extracted soil solutions were passed through a 0.22 μm filter to remove any microbes and suspended particles and the soil solution stored in polypropylene vials at -20 °C prior to soil solution analysis and ^{14}C-labelling.

2.3. Soil solution chemical analysis

The chemical composition of the soil solution used in the experiments described below is shown in Table 1. Dissolved organic C (DOC) and total dissolved N (TDN) in soil solution were determined using a Shimadzu TOC-TNV analyzer (Shimadzu Corp., Japan). Ammonium was determined colorimetrically following the salicylate-nitroprusside/hypochlorite procedure of Mulvaney (1996). Nitrate was measured colorimetrically using the vanadium method of Miranda et al. (2001). Dissolved organic nitrogen (DON) was calculated as the difference between TDN and dissolved inorganic nitrogen. Total free amino acids (TFAA) were determined by fluorescence using the *o*-phthalialdehyde-β-mercaptoethanol procedure of Jones et al. (2002). Glucose was determined fluorometrically with an Amplex® Red glucose assay kit (Invitrogen Corp., Carlsbad, USA). Soil solution P was determined using the molybdate blue method of Murphy and Riley (1962).

Table 1. Selected soil properties for the Eutric Cambisol soil used for the mineralization studies. Values represent means ± SEM (n = 3).

Soil properties	Topsoil (0 to 5 cm)
Soil water content (g kg^{-1})	232.9 ± 3.6
Total C (g C kg^{-1})	25.0 ± 3.3
Total N (g N kg^{-1})	2.70 ± 0.35
C:N ratio	9.24 ± 0.30
Microbial biomass (g C kg^{-1})	2.26 ± 0.35
pH $_{(H20)}$	5.08 ± 0.01
Electrical conductivity (μS cm^{-1})	112.7 ± 3.1
Dissolved organic C (mg C l^{-1})	30.7 ± 3.1
Dissolved N (mg N l^{-1})	10.8 ± 2.4
DON (mg N l^{-1})	3.71 ± 0.28
DOC:DON ratio	7.61 ± 0.12
Free amino acids in soil solution (mg N l^{-1})	0.04 ± 0.01
Free amino acids in soil solution (mg C l^{-1})	0.14 ± 0.02
Free sugars in soil solution (mg C l^{-1})	0.10 ± 0.06
Available NH_4^+ (mg N l^{-1})	0.08 ± 0.04
Available NO_3^- (mg N l^{-1})	6.67 ± 2.02
Available soluble P (mg P l^{-1})	0.24 ± 0.01
Soil solution phenolics (mg l^{-1})	0.12 ± 0.04
Specific UV absorbance units per cm (254 nm)	0.31 ± 0.04
Specific UV absorbance units per cm (400 nm)	0.06 ± 0.01

2.4. Carbon partitioning

2.4.1. Substrate mineralization

To determine the rate of ^{14}C-substrate mineralization, 1.7 g of soil was placed in a 1.5 ml micro-centrifuge tube placed inside a sealable 50 ml polypropylene cylinder, and 0.35 ml of soil solution containing ^{14}C-labelled glucose or alanine was added to the soil surface. The soil solution infiltrated immediately through the soil column. Uniformly ^{14}C-labelled D-glucose (1 kBq ml^{-1}; Sigma-Aldrich Company Ltd., USA) and L-alanine (0.8 kBq ml^{-1}; Amersham Biosciences, UK) were selected for this study as they represent two common root exudates which are known to be important in soil C cycling. Glucose has no net charge and so is not sorbed to the solid soil phase in this soil (Kuzyakov and Jones, 2006), whereas alanine also exhibits no net charge at the pH of the soil but the carboxyl and amino groups allow sorption to occur. The concentration of the ^{14}C label added to the soil solution was very low (< 10 nM) and did not change the intrinsic soil solution alanine or glucose concentration by more than 5%. After soil solution addition, a NaOH trap (1 ml, 1 M) was placed alongside the soil and the cylinders hermetically sealed. NaOH traps were changed at the following time intervals; 0.5, 1, 2, 4, 8, 12, 24, 48, 168 h and then weekly up to 7 weeks. After removal, the NaOH traps were mixed with Scintisafe 3® scintillation cocktail (Fisher Scientific, UK) and subsequently analysed using a Wallac 1404 liquid scintillation counter (Wallac EG&G, UK) to determine the amount of ^{14}C activity present.

2.4.2. Substrate not mineralized to CO_2

To determine the fraction of ^{14}C that was not mineralized during the 7 weeks incubation period, the soils were completely oxidized at the end of the NaOH mineralization experiment. In brief, the 1.7 g of soil was dried (24 h, 105°C) and 0.6 g of dried soil sub-sampled for oxidation. The outflow from the OX400 biological

oxidizer (R.J. Harvey Instrument Corp., USA) was bubbled through Oxosol scintillation fluid (National Diagnostics Ltd., UK) in order to trap the $^{14}CO_2$ evolved. The amount of ^{14}C in the Oxosol was determined by liquid scintillation counting as described above.

2.4.3. Substrate uptake from soil solution

The rate of microbial uptake of substrates from the soil solution was measured using the method of Hill et al. (2008). Briefly, 1.7 g of field-moist soil was placed in a 1.5 ml micro-centrifuge tube which had a hole pierced at the bottom and the tube placed into another intact micro-centrifuge. 0.35 ml of labelled soil solution containing either ^{14}C-glucose or ^{14}C-alanine was added to the soil surface as described above. At known time periods after soil solution addition, the soils were centrifuged (4000 g, 1 min, 18°C), at which point the soil solution passed through into the lower tube from which it could be removed for analysis. The volume of soil solution recovered was measured and then mixed with Scintisafe 3® scintillation cocktail for ^{14}C determination as described above.

2.4.4. Extraction of ^{14}C-substrate sorbed to the solid phase

To determine the amount of ^{14}C in solution and retained on the soil phase after the addition of the ^{14}C-labelled substrates, 1.7 g of soil was placed in a 1.5 ml micro-centrifuge tube inside a 50 ml polypropylene cylinder, and 0.35 ml of ^{14}C-labelled glucose and alanine added to the soil as described above. The amount of ^{14}C in the solution/exchange phase was measured at each time point by performing a 1:5 (w/v) soil-to-K_2SO_4 (0.5 M) extract in which the samples were shaken (30 min, 200 rev min^{-1}), centrifuged (18,000 *g*, 5 min) and a 1 ml aliquot of the supernatant retained for ^{14}C analysis as described above.

2.4.5. *Extraction of soluble ^{14}C in the microbial biomass*

To determine the amount of ^{14}C retained within the microbial biomass after the addition of ^{14}C-labelled substrates, 1.7 g of soil was placed in a 1.5 ml micro-centrifuge tube and 0.35 ml of soil solution containing ^{14}C-labelled glucose or alanine added to the soil as described above. At known times after ^{14}C-label addition, samples were then subjected to chloroform-fumigation (Vance et al., 1987) for 24 h in the dark at 20°C. The samples were then extracted with 0.5 M K_2SO_4 (1:5 w/v) as described above for the non-fumigated samples. The extractable C fraction after fumigation (k_{ec} value of 0.45 taken from Vance et al., 1987) was used to convert the data into biomass-^{14}C (Joergensen, 1996).

2.4.6. *Methanol-soluble microbial biomass extraction*

To determine the amount of ^{14}C retained in water-insoluble fractions of the microbial biomass (e.g. lipids), the soil remaining from the $CHCl_3$-fumigation extraction was re-used. Briefly, the excess K_2SO_4 was removed from the soil and replaced with 100% MeOH. The soils were then extracted (1:5 w/v) by shaking (30 min, 200 rev min^{-1}), centrifuged (18,000 g, 5 min) and 1 ml of the supernatant recovered for ^{14}C determination as described above.

2.5. Modelling ^{14}C dynamics in soil

To assess which first order exponential model fitted best to the experimental data, the correlation between the fit of the model and the data (r^2 value) was used in conjunction with looking at the inter-dependence between each of the fitted model parameters (dependency) (Wolfe and Chinkes, 2005). For both substrates a double and triple exponential decay model was fitted to the experimental data to represent a

biphasic or triphasic pattern of mineralization. A triphasic pattern can be explained by a first-order triple exponential decay model:

$$f = (a_1 \times \exp^{-k_1 t}) + (a_2 \times \exp^{-k_2 t}) + (a_3 \times \exp^{-k_3 t}) \qquad \text{(Eqn. 1)}$$

where f is the amount of ^{14}C remaining in the soil, a_1, a_2 and a_3 describe the size of each respective pool, k_1, k_2 and k_3 correspond to the exponential decay constants for each mineralization phase, and t is time. For this model, the first rapid phase described by k_1 is thought to correspond to $^{14}CO_2$ efflux as substrates are immediately used for catabolic processes (i.e. respiration), mirroring to some extent the depletion of substrate from the soil solution (Jones, 1999; Jones and Hodge, 1999). The remaining ^{14}C-substrate is immobilized in the microbial biomass (pool a_2+a_3) via anabolic processes. The second, slower mineralization phase (k_2) is thought to be attributable to the use of this C temporarily immobilized in the biomass (e.g. storage-C, or labile cell contents mineralized after turnover of microbial biomass). The third pool (a_3) is thought to relate to the very slow breakdown (k_3) of recalcitrant microbial biomass products (e.g. lipids and cell membranes) and microbially-modified material incorporated into relatively stable soil organic matter (SOM).

A biphasic pattern can be explained by a first-order double exponential decay model:

$$f = (a_4 \times \exp^{-k_4 t}) + (a_5 \times \exp^{-k_5 t}) \qquad \text{(Eqn. 2)}$$

where f is the amount of extractable ^{14}C, a_4 and a_5 describe the size of each respective pool, k_4 and k_5 correspond to the exponential decay constants for each mineralization phase. For this model, the first rapid phase (k_4) is assumed to be similar to the first phase described in Equation 1. The second, slower mineralization phase (k_5) is thought to be attributable to the use of the remaining C immobilized in the microbial biomass (i.e. microbial biomass turnover or use of storage-C).

The substrate half-life for the first mineralization pool ($a_1$and a_4 for triple and double models respectively) can be calculated using the following equation:

$$t_{1/2=} = \frac{\ln(2)}{k_1} \qquad \text{(Eqn. 3)}$$

Calculating the half-life for the slower second and third phases is subject to uncertainty as the connectivity between these pools is unknown (Saggar et al., 1999; Boddy et al., 2007; Glanville et al., 2012).

Equations 1 and 2 cannot be solved explicitly and so the Newton-Raphson algorithm can be applied to both the double and triple exponential decay equations in order to calculate the time when the overall ^{14}C remaining in the soil is half the initial amount, this shall now be referred to as total substrate half-life. Equations 4 and 5 refer to how the Newton-Raphson algorithm was applied to the triple exponential decay model. For the double decay model, the third exponential parameter was removed from the equation.

$$f(t) = a_1 exp^{-k_1 t_{1/2}} + a_2 exp^{-k_2 t_{1/2}} + a_3 exp^{-k_3 t_{1/2}} - \frac{a_1+a_2+a_3}{2}, \qquad \text{(Eqn. 4)}$$

This equation is then substituted into the Newton-Raphson algorithm:

$$t_{n+1} = t_n - \frac{f(t)}{f'(t)} \qquad \text{(Eqn. 5)}$$

Where $f'(t)$ is the first derivative obtained for f, the algorithm is continually applied until convergence of data points is achieved.

For the extractable soluble soil solution data and the extractable phase bound to the soil, a single first-order model with asymptote fitted best to the data.

$$f = y_0 + \left(a_6 \times \exp^{-k_6 t}\right) \qquad \text{(Eqn. 6)}$$

Where y_0 represents an unrecoverable asymptotic pool, a_6 (soluble soil solution) and a_7 (extractable solid soil phase) describe the pool sizes, and k_6 and k_7 are the exponential rate constants for each pool respectively. The half-life of each substrate can then be

calculated using Eqn. (2) and substituting k_1 for k_6 and k_7 for each extraction respectively.

To describe changes in the ^{14}C-soluble microbial biomass pool ($CHCl_3$-fumigation) and the methanol extractable microbial biomass pool, a double first-order decay equation (Eqn. 2) without asymptote was found to best describe the experimental data. Here, a_4 and a_5 are replaced with a_8 and a_9 (soluble microbial biomass pool) and a_{10} and a_{11}(methanol extractable microbial biomass pool) respectively andk_8, k_9, k_{10} and k_{11} corresponding to the rate constants for each pool respectively.

2.6. Statistical and data analysis

All experiments were carried with three replicates per treatment and data visually inspected for normality using quantile-quantile plots (Crawley, 2007). Any data not normally distributed were log-transformed to achieve normal distribution and equal variances. All statistical procedures were carried out using the statistical package 'R' v 2.12.1 (2010), with P = 0.05 used as the upper limit for statistical significance. Exponential decay curves were fitted to the experimental data, and dependency values for each model parameter were calculated using Sigma Plot 11 (Systat Software Inc., USA). To critically evaluate which decay model best described the experimental data; the following criteria were implemented. An r^2 value of 0.9 was selected as an acceptable value for assessing the correlation between the fit of the model and the data. However, as the number of exponential parameters increases, the r^2 value will also increase (Wolfe and Chinkes, 2005), thus using this value alone may lead to over-fitting of the data. Dependency values were therefore also used to determine if the chosen model had too many parameters within its fit. A dependency cut-off value of 0.98 was chosen for each parameter within the model. If any parameter had a dependency value

greater than 0.98, it was deemed that particular model was too complex for the data and a lower-order, simpler model with a good r^2 value, was selected instead. In addition, to further validate the choice of model, a least-squares estimation (LSE) was calculated to evaluate the accuracy of the estimation of parameter values (Wolfe and Chinkes, 2005). We also investigated how increasing the number of time points used to fit the data would affect the model fit. We used 0.5 d (which encompassed 6 individual time points) as the first value, after which, we assessed model fits and dependency values with increasing number of time points, up to 49 d (15 time points in total).

3. Results

3.1. ^{14}C-substrate mineralization

3.1.1. Model fits to the experimental data

Both alanine and glucose fitted well to both double and triple exponential decay models with dependency levels for all parameters well below the critical 0.98 cut-off threshold (Fig. 1; Table 2). Overall, the double exponential decay model did not fit as well to the data, with lower r^2 values compared to the triple decay model for both substrates ($P < 0.001$). In contrast, the parameter dependency values were higher within the triple decay model when compared to the double decay model ($P < 0.001$).

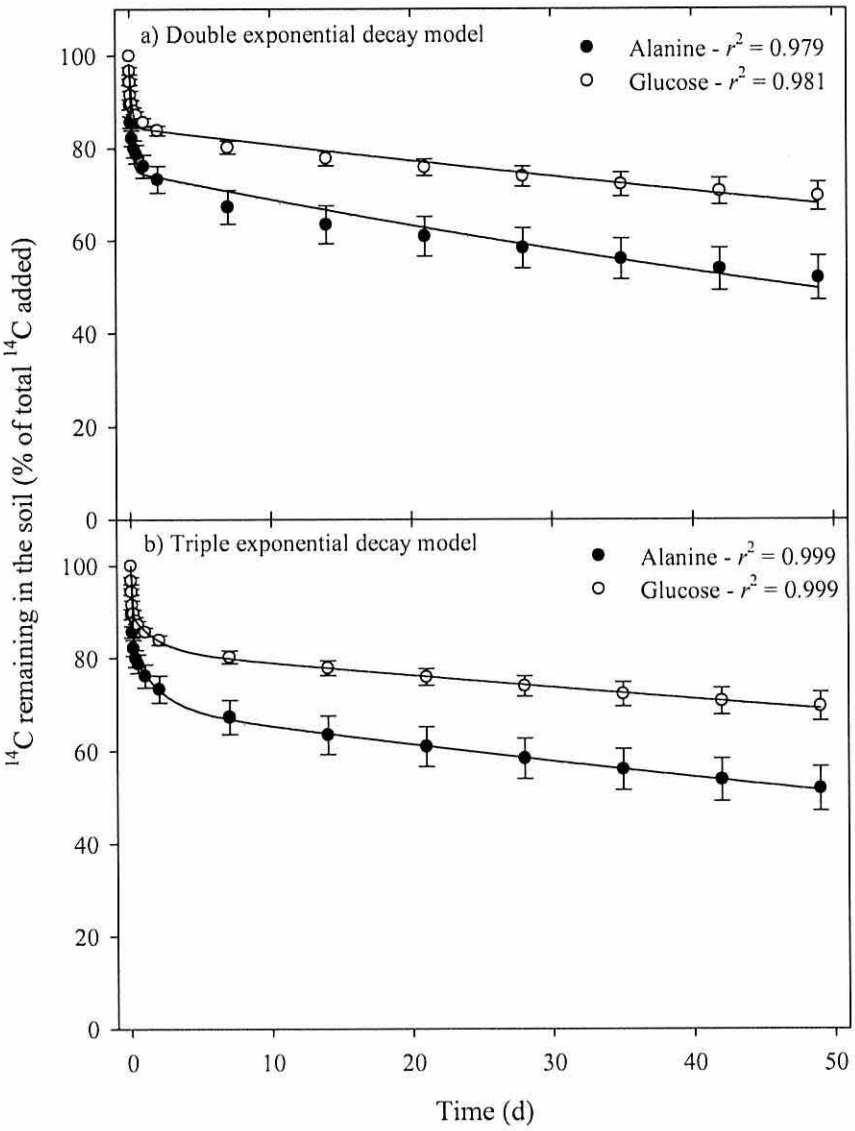

Fig. 1. Model fits for double and triple exponential decay curves describing the depletion of ^{14}C-labelled alanine and glucose in a Eutric Cambisol soil. Points represent experimental means ± SEM for each substrate (n = 3), whilst lines represent model fits to the experimental data.

Table 2. Parameter estimates and dependency values from two different exponential decay models describing the mineralization of alanine and glucose in a Eutric Cambisol soil. Values represent means ± SEM for each substrate ($n = 3$).

Alanine	Model parameters	Double exponential decay model	Dependency values	Triple exponential decay model	Dependency values
	a_1 (%)	22.6 ± 2.17	0.50 ± 0.013	18.0 ± 1.47	0.68 ± 0.017
	k_1 (h^{-1})	0.31 ± 0.04	0.54 ± 0.010	0.77 ± 0.05	0.66 ± 0.011
	a_2 (%)	75.0 ± 2.82	0.73 ± 0.015	12.6 ± 2.09	0.92 ± 0.002
	k_2 (h^{-1})	$3.7\times10^{-4} \pm 6.7\times10^{-5}$	0.50 ± 0.016	$2.1\times10^{-2} \pm 2.1\times10^{-4}$	0.75 ± 0.006
	a_3 (%)			69.4 ± 3.60	0.94 ± 0.001
	k_3 (h^{-1})			$2.3\times10^{-4} \pm 3.3\times10^{-5}$	0.81 ± 0.004
	Model r^2	0.979 ± 0.002		0.999 ± 0.0001	
Glucose	a_1 (%)	13.7 ± 0.67	0.52 ± 0.010	10.7 ± 0.61	0.69 ± 0.015
	k_1 (h^{-1})	0.24 ± 0.02	0.54 ± 0.002	0.73 ± 0.14	0.67 ± 0.009
	a_2 (%)	84.6 ± 0.74	0.77 ± 0.011	7.78 ± 0.46	0.92 ± 0.006
	k_2 (h^{-1})	$1.7\times10^{-4} \pm 3.3\times10^{-5}$	0.57 ± 0.018	$2.2\times10^{-2} \pm 4.6\times10^{-4}$	0.76 ± 0.012
	a_3 (%)			81.5 ± 0.91	0.94 ± 0.011
	k_3 (h^{-1})			$1.6\times10^{-4} \pm 4.0\times10^{-5}$	0.83 ± 0.025
	Model r^2	0.981 ± 0.001		0.999 ± 0.0001	

The type of model used to describe substrate mineralization affects how we interpret the distribution of ^{14}C within the biomass and consequently impacts on the calculation of turnover rates from individual pools. For ^{14}C-alanine, allocation of ^{14}C to the first mineralizable pool showed no significant difference ($P > 0.05$) between the double and triple exponential models (a_1 and a_4 for the triple and double model respectively). However, the double exponential decay model produced a half-life (calculated from k_4) twice as long as those for the triple decay model (calculated from k_1) for this first pool ($P < 0.01$), with values of 0.10 ± 0.01 d and 0.04 ± 0.002 d for the double and triple models respectively. For ^{14}C-glucose, attribution of ^{14}C to the first mineralizable pool a_1 and a_4 was 13 % higher in the double decay model than in the triple decay models ($P < 0.05$). However, the half-life for this pool was calculated to be three times longer from the double exponential decay model (0.12 ± 0.01 d) in comparison to the triple exponential model (0.04 ± 0.01 d; $P < 0.01$).

Pool sizes for the second mineralizable pool (a_2 and a_5) were 6- and 11-fold (glucose and alanine, respectively) larger ($P < 0.05$) for the double model than for the triple model. In contrast, the associated rate constants (k_2 and k_5) were 57- and 129-fold (glucose and alanine, respectively) larger ($P < 0.05$) for the triple model than for the double model.

The third mineralizable pool from the triple exponential model(a_3) showed no significant difference ($P > 0.05$) between any model parameters or pool half-life when compared with the second pool from the double exponential decay model (a_5).

Using the Newton-Raphson algorithm, total substrate half-life was determined for each ^{14}C-substrate with both models. Overall, there were no differences in total substrate half-life between the different models ($P > 0.05$). Alanine-derived C had a total substrate half-life of 52.7 ± 9.6 d and 62.7 ± 16.0 d for the double and triple decay

models respectively (mean ± SEM). Total substrate half-life for glucose-derived C was calculated as 151.3 ± 33.8 d and 154.4 ± 54.1 d for the double and triple decay models respectively.

3.1.2. Double exponential decay model over time

For either substrate, both of the mineralization models (Eqn. 1 and 2), showed good model fits (r^2 values) and acceptable dependency values after 49 d, however, some differences were noted as the number of individual time points used to fit the curve increased. The double decay model data for ^{14}C-alanine are shown in Fig. 2 (the ^{14}C-glucose data are shown in Supplementary on-line information Figure S1). Within this model, both substrates exhibited similar results with the best fit for the model i.e. highest r^2 values, noted after 0.5, 1 and 2 d (equates to 7, 8 and 9 measurement points respectively). After 14 d (10 measurement points), r^2 values remained relatively constant ($P > 0.05$) at ca. 0.98.

Dependency values were well below the 0.98 cut-off threshold for all model parameters (Fig. 2) irrespective of the number of measurement points used in fitting the mathematical model. As expected, dependency values for all model parameters were highest at the start of the experiment and then declined as the number of time points used in the analysis increased. Both mineralizable pools experienced notable changes in dependency values after 7 d (9 time points). For alanine, the first assigned mineralizable pool (a_4) decreased by 41% in dependency values between 0.5 and 7 d (6 and 9 measurement points; Fig. 2a; $P < 0.001$). The dependency values for the associated rate constant (k_4) also decreased by 33% over the same number of time points (Fig. 2b; $P < 0.001$). The second mineralizable pool (a_5) dependency values declined by 25% (Fig. 2c; < 0.001) with the largest decrease, 55 %, observed for the rate constant associated with this second pool (k_5), (Fig. 2d; $P < 0.001$). After 7 d (9 time points) there was no change in dependency values for all parameters, except for the k_5 rate constant, where dependency values increased after 14 d (10 time points; Fig. 2d).

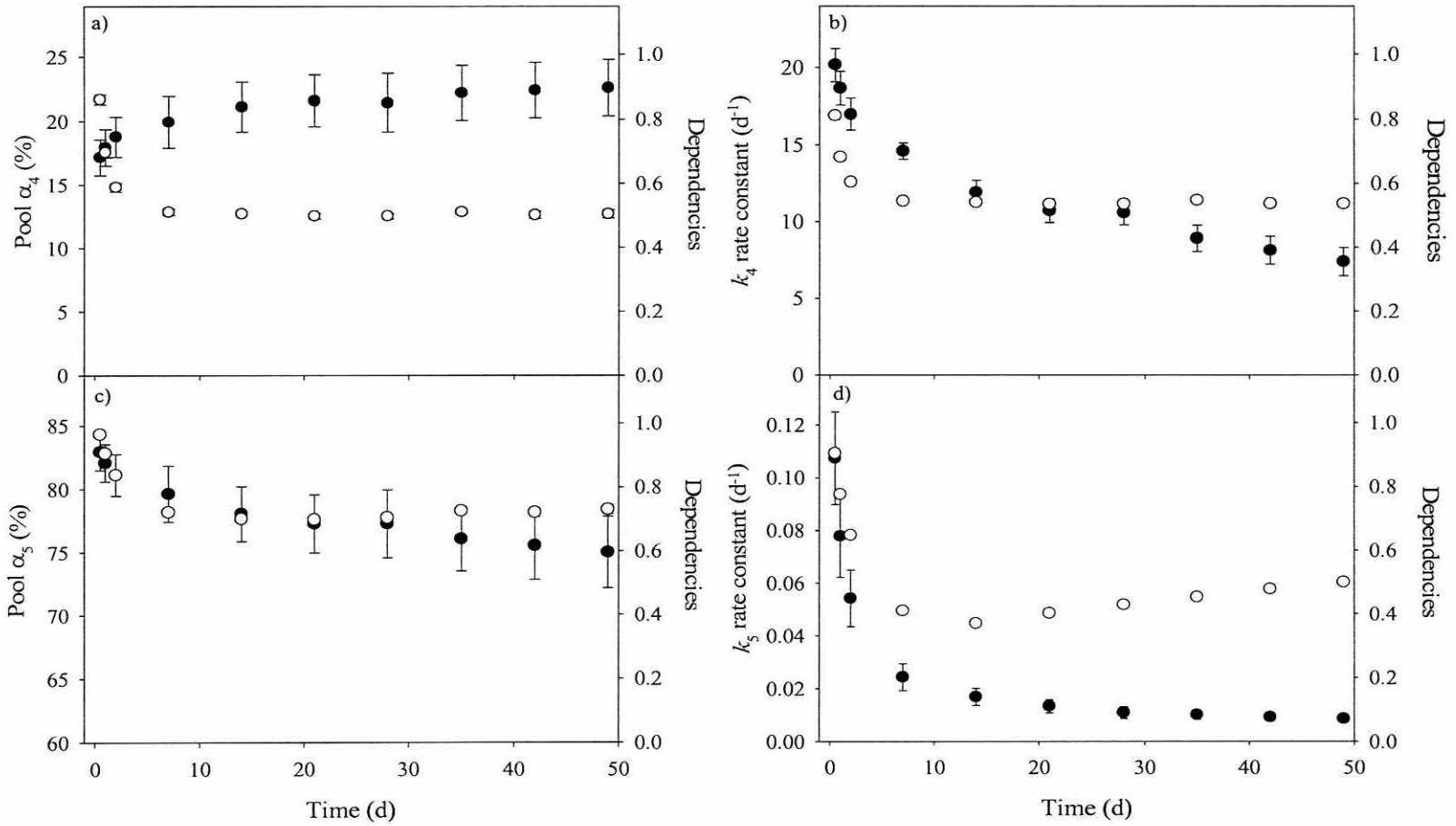

Fig. 2. Influence of the number of measurement points on model parameter estimates describing the turnover of alanine with a first-order double exponential decay model. Solid circles represent parameter values at each time point. Open circles represent dependency values associated with each parameter. Points represent means ± SEM (n = 3). For a full description of model parameters see Materials and Methods (Eqn. 2).

The amount of ^{14}C ascribed to the first mineralization pool (a_4) did not change with the increasing number of time points used to fit the curve (Fig. 2a; $P > 0.05$). Between 0.5 and 14 d (6 to 10 pints), the associated rate constant (k_4) showed a decreasing trend (Fig. 2b; $P < 0.001$), after which, values remained constant, irrespective of increasing the number of time points ($P > 0.05$). Pool a_5 showed no differences in size with an increasing number of measurement points used in the analysis (Fig. 2c; $P > 0.05$). The rate constant for this pool (k_5) showed a decreasing trend (Fig. 2d; $P < 0.001$) between 0.5 and 7 d (between 6 and 9 time points), after which values remained constant.

In order to further validate the model, LSE values were also calculated for all parameters for both alanine and glucose. All parameters proved reasonable with no values > 100%, therefore, inferring that model parameters are accurately represented within this particular model, even when a small number of time points were used to fit the model.

3.1.3. Triple exponential decay model over time

As for the double exponential decay model, the triple decay model also exhibited changes in both model fit and parameter dependency values depending upon the number of experimental data points used to fit the model. The triple decay model results are shown for alanine in Fig. 3 (glucose data presented in Supplementary On-line Information Figure S2). Within this model, again both substrates showed similar results, with consistently high r^2 values at all time points ($r^2 > 0.995$ for both glucose and alanine). No significant change in r^2 values was evident across the increasing range of data points used to fit the model ($P > 0.05$).

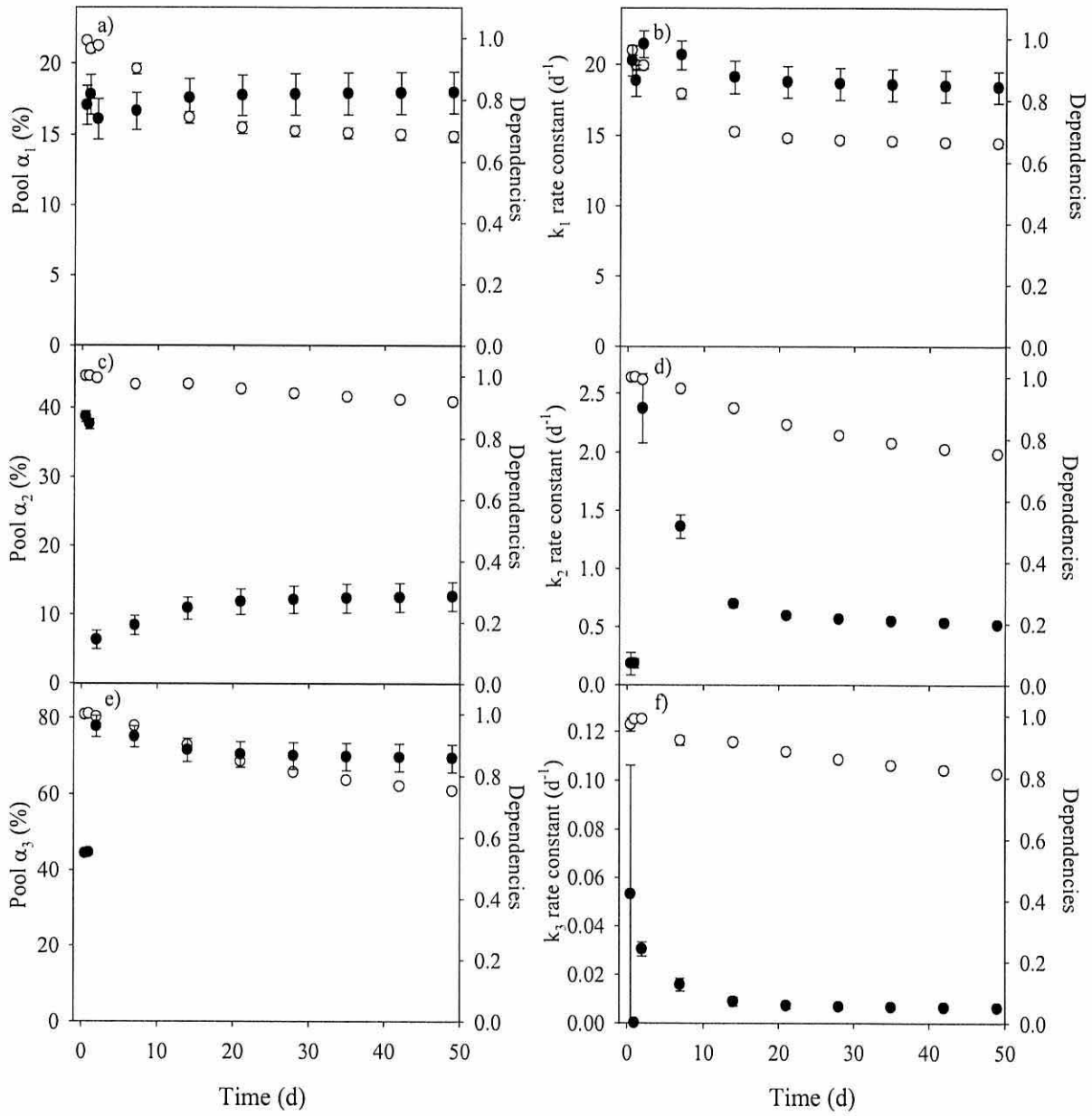

Fig. 3. Influence of the number of measurement points on model parameter estimates describing the turnover of alanine with a first-order triple exponential decay model. Solid circles represent parameter values at each time point. Open circles represent dependency values associated with each parameter. Points represent means ± SEM (n = 3). For a full description of model parameters see Materials and Methods (Eqn. 1).

Dependency values were well below the 0.98 cut-off threshold for all model parameters (Fig. 3). As seen for the double decay model, dependency values for all model parameters were highest at the beginning of the experiment and declined over time. This decline was not as pronounced as for the double decay model. The double decay model showed the greatest change in dependency values between 0.5 and 7 d (up to 9 time points). For the triple model, the greatest changes in dependency values were observed between 0.5 and 14 d (up to 10 time points) and were largest for the first mineralizable pool(a_1). Within these time intervals, dependency values for the first mineralizable pool (a_1) decreased by 25% (Fig. 3a; $P < 0.001$), and the corresponding rate constant (k_1) declined by 27% (Fig 3b; $P < 0.001$) in dependency values over the same time intervals. The dependency values for the second mineralizable pool(a_2) only reduced by 4% (Fig. 3c; $P < 0.001$) and those for the associated rate constant (k_2) decreased by 15% over the same number of time points (Fig. 3d; $P < 0.001$). The third mineralizable pool(a_3) changed the least, with only a 1% decrease (Fig. 3e; $P < 0.001$). The rate constant(k_3) associated with this pool decreased by 8% (Fig. 3f; $P < 0.01$). This triple model will be used to investigate if the three identified pools within this model correspond to the different chemical extractions.

Observed values for the size of the first mineralizable pool (a_1) and its associated rate constant (k_1) showed no change irrespective of the number of time points used to fit the model (Fig. 3a and 3b; $P > 0.05$). For the second mineralizable pool (a_2) values at 0.5 and 1 d (6 and 7 time points respectively) were much larger than at all other time points (Fig. 3c; $P < 0.001$). In addition, values for the rate constant (k_2) for this second pool at 0.5 and 1 d (6 and 7 time points) were lower than at other times (Fig. 3d; $P < 0.001$). At 2 d (8 time points) the rate constant (k_2) values showed a rapid increase followed by a decline whilst after 14 d (10 time points) the values remained

constant ($P > 0.05$). The third mineralizable pool (a_3) showed the opposite trend to the second pool with estimates made at 0.5 and 1 d (6 and 7 time points) being lower (Fig. 3e; $P < 0.001$) than the subsequent time points. The associated rate constant (k_3) had large errors and so there was no effect (Fig. 3f; $P > 0.05$) of increasing the number of time points used on this parameter.

To validate the models for both alanine and glucose, LSE values were calculated. Values were >100% for values calculated after 0.5 and 1 d (6 and 7 time points) for alanine for the third mineralizable pool (a_3) suggesting there are not enough data points used to accurately estimate the model parameters. Glucose also showed high LSE values for the rate constant (k_3) associated with the third mineralizable pool after 0.5 and 1 d (97% and 141% for 6 and 7 time points respectively). In addition, the second mineralizable pool also had high LSE values after 2 d (8 time points). These values indicate that parameters from the triple exponential decay model are not accurately estimated within this model when only a small number of time points (< 8 time points) are used to fit the model.

3.2. Substrate not mineralized to CO_2

The $^{14}CO_2$ evolution captured from the NaOH mineralization experiment accounted for approximately a third of the total ^{14}C-activity added; with 64.8 ± 2.2 and 64.5 ± 9.6% (alanine and glucose respectively) remaining in the soil (unmineralized) within the duration of this experiment and only recoverable after biological oxidation. This emphasizes that the mineralization methods provides good capture of CO_2.

3.3. Substrate uptake from soil solution

The amount of extractable ^{14}C found within the soil solution fitted well to a first order single exponential model with an asymptote (Fig. 4b; r^2 = 0.94 and 0.99 for alanine and glucose respectively). Dependency levels were <0.98 for all parameters. Levels of ^{14}C remaining in the soil solution were very low throughout the experiment for both substrates (< 1% of total activity added). The total half-life for alanine within this pool was estimated to be 0.13 ± 0.05 d, and was even faster for glucose at 0.03 ± 0.01 d (Tables 3 and 4 respectively).

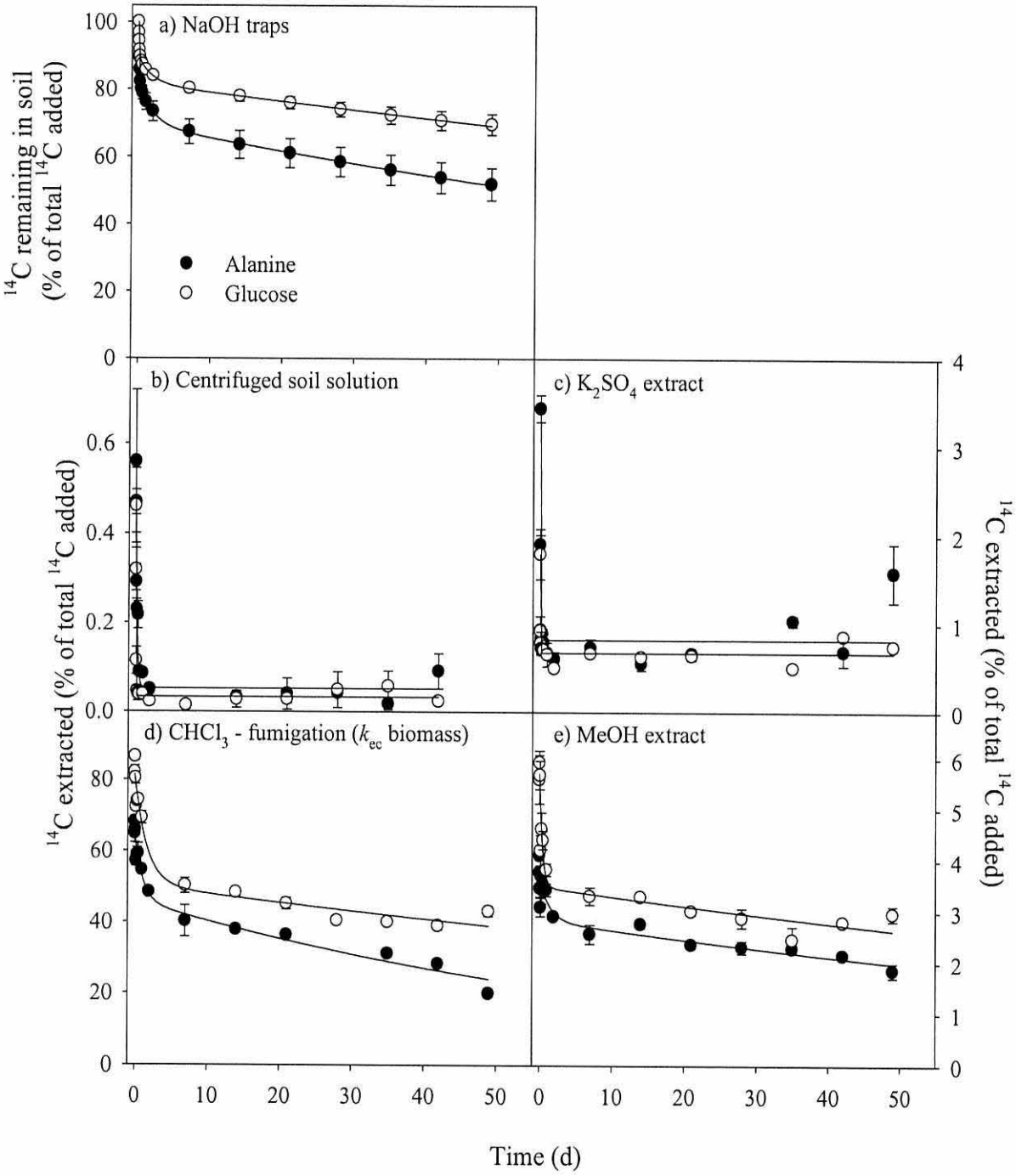

Fig. 4. Amount of ^{14}C recovered from different chemical extractions after the addition of ^{14}C-labelled alanine and glucose to an agricultural grassland soil. Values represent means ± SEM (n = 3). Lines represent model fits. The legend is the same for all panels.

Table 3. Model parameters describing the size and turnover of the extractable ^{14}C pool for alanine over time. The models are described by single, double or triple exponential decay equations. Only the single decay equations were fitted with an asymptote (asym.). Values represent means ± SEM (n = 3), n.a. indicates not applicable. Pools were assigned to each extractable ^{14}C pool, with the NaOH traps representing a respired pool, the centrifuged soil solution represents the labile soil solution pool, K_2SO_4 extracts are ascribed to the soil sorption pool, $CHCl_3$-fumigation and MeOH extracts represent the soluble and more insoluble pool of the microbial biomass pool respectively. For further clarification, see Discussion section 4.3.

	Extraction method and associated model - Alanine					
Model parameters	NaOH trap (Triple exponential decay model)	NaOH trap (Double exponential decay model)	Centrifuged soil solution (Single exponential decay model with asym.)	K_2SO_4 extract (Single exponential decay model with asym.)	$CHCl_3$ fumigation (K_{ec} biomass) (Double exponential decay model)	MeOH extract (Double exponential decay model)
Asymptote pool - (%) (y_0)	n.a.	n.a.	0.06 ± 0.02	0.83 ± 0.03	n.a.	n.a.
Pool 1 - (%) ($a_1, a_4, a_6, a_7, a_8, a_{10}$)	18.0 ± 1.47	22.6 ± 2.17	0.73 ± 0.29	6.51 ± 0.31	19.7 ± 1.02	0.89 ± 0.16
Pool 1 rate constant - (h^{-1}) ($k_1, k_4, k_6, k_7, k_8, k_{10}$)	0.77 ± 0.05	0.31 ± 0.04	0.50 ± 0.33	1.83 ± 0.06	0.04 ± 0.01	0.19 ± 0.15
Pool 1 half-life (d)	0.04 ± 0.00	0.10 ± 0.01	0.13 ± 0.06	0.02 ± 0.00	0.80 ± 0.20	0.58 ± 0.27
Pool 2 - (%) (a_2, a_5, a_9, a_{11})	12.6 ± 2.09	75.0 ± 2.82			46.0 ± 3.13	3.01± 0.02
Pool 2 rate constant - (h^{-1}) (k_2, k_5, k_9, k_{11})	$2.1\times10^{-2} \pm 2.1\times10^{-4}$	$3.7\times10^{-4} \pm 6.7\times10^{-5}$			$5.7\times10^{-4} \pm 8.8\times10^{-5}$	$4.0\times10^{-4} \pm 5.8\times10^{-5}$
Pool 2 half-life - (d)	1.35 ± 0.01	83.4 ± 12.8			58.9 ± 9.38	75.4 ± 11.2
Pool 3 - (%) (a_3)	69.4 ± 3.59					
Pool 3 rate constant - (h^{-1}) (k_3)	$2.3\times10^{-4} \pm 3.3\times10^{-5}$					
Pool 3 half-life - (d)	128.4 ± 16.0					
Total substrate half-life (d) (Newton-Raphson algorithm)	62.7 ± 16.1	52.7 ± 9.61	0.13 ± 0.05	0.02 ± 0.00	24.9 ± 1.36	47.8 ± 7.46
Assigned pool	Respired pool (triphasic pattern)	Respired pool (biphasic pattern)	Labile soil solution pool	Soil sorption pool	Soluble microbial biomass pool	Insoluble microbial biomass pool

Table 4. Model parameters describing the size and turnover of the extractable ^{14}C pool for glucose over time. The models are described by single, double or triple exponential decay equations. Only the single decay equations were fitted with an asymptote (asymp.). Values represent means ± SEM (n = 3), n.a. indicates not applicable. Pools were assigned to each extractable ^{14}C pool, with the NaOH traps representing a respired pool, the centrifuged soil solution represents the labile soil solution pool, K_2SO_4 extracts are ascribed to the soil sorption pool, $CHCl_3$-fumigation and MeOH extracts represent the soluble and more insoluble pool of the microbial biomass pool respectively. For further clarification, see Discussion section 4.3.

	Extraction method and associated model - Glucose					
Model parameters	NaOH trap (Triple exponential decay model)	NaOH trap (Double exponential decay model)	Centrifuged soil solution (Single exponential decay model with asym.)	K_2SO_4 extract (Single exponential decay model with asym.)	$CHCl_3$ fumigation (K_{ec} biomass) (Double exponential decay model)	MeOH extract (Double exponential decay model)
Asymptote pool – (%) (y_0)	n.a.	n.a.	0.03 ± 0.01	0.67 ± 0.02	n.a.	n.a.
Pool 1 - (%) ($a_1, a_4, a_6, a_7, a_8, a_{10}$)	10.7 ± 0.61	13.7 ± 0.67	0.77 ± 0.23	16.0 ± 12.1	31.2 ± 1.38	2.37 ± 0.27
Pool 1 rate constant - (h^{-1}) ($k_1, k_4, k_6, k_7, k_8, k_{10}$)	0.73 ± 0.14	0.24 ± 0.02	0.99 ± 0.25	3.86 ± 1.66	0.03 ± 0.00	0.10 ± 0.03
Pool 1 half-life (d)	0.04 ± 0.01	0.12 ± 0.01	0.03 ± 0.01	0.02 ± 0.01	1.15 ± 0.11	0.34 ± 0.09
Pool 2 - (%) (a_2, a_5, a_9, a_{11})	7.79 ± 0.46	84.6 ± 0.74			50.6 ± 2.22	3.45± 0.11
Pool 2 rate constant - (h^{-1}) (k_2, k_5, k_9, k_{11})	$2.2\times10^{-2} \pm 4.6\times10^{-4}$	$1.7\times10^{-4} \pm 3.3\times10^{-5}$			$2.3\times10^{-4} \pm 6.7\times10^{-5}$	$1.7\times10^{-4} \pm 3.3\times10^{-5}$
Pool 2 half-life - (d)	1.47 ± 0.35	192.5 ± 48.1			160.5 ± 64.2	144.4 ± 00
Pool 3 - (%) (a_3)	81.5 ± 0.91					
Pool 3 rate constant - (h^{-1}) (k_3)	$1.6\times10^{-4} \pm 4.0\times10^{-5}$					
Pool 3 half-life - (d)	217.2 ± 72.8					
Total substrate half-life (d) (Newton-Raphson algorithm)	154.4 ± 54.1	151.3 ± 33.8	0.03 ± 0.01	0.06 ± 0.02	44.3 ± 10.3	36.3 ± 6.04
Assigned pool	Respired pool (triphasic pattern)	Respired pool (biphasic pattern)	Labile soil solution pool	Soil sorption pool	Soluble microbial biomass pool	Insoluble microbial biomass pool

This suggests that the ^{14}C-labelled substrates present in the soil solution were readily assimilated by the microbial communities. Complete mixing of the added ^{14}C-labelled solution with the intrinsic (non ^{14}C-labelled) soil solution present in soil macropores was assumed after 1 min (Hill et al., 2008). The soluble soil solution component comprised 0.13 ± 0.04 and 0.04 ± 0.01% of the biologically oxidised pool after 49 d for alanine and glucose respectively (Fig. 5).

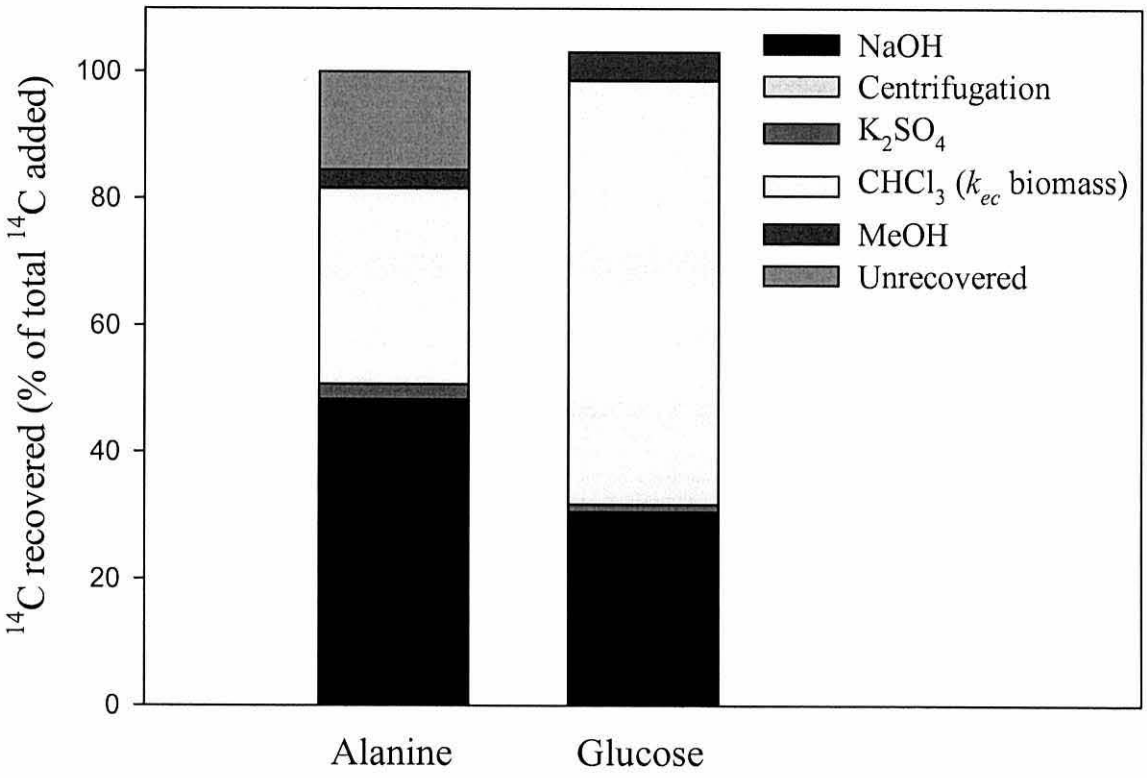

Fig. 5. Partitioning of ^{14}C into discrete pools after different chemical extractions ($n = 3$ for each separate extraction).

3.4. Extraction of substrate held on the soil's solid phase

The amount of ^{14}C-labelled substrate bound onto soil particles was extracted using 0.5 M K_2SO_4; these data also followed a single exponential decay pattern with asymptote (Fig. 4c; r^2 = 0.89 and 0.91 for alanine and glucose respectively). Dependency levels were < 0.98 for all model parameters. After 0.5 h, the amount of ^{14}C

bound to the soil was low for both substrates with < 4% of the total activity recovered using 0.5 M K_2SO_4. After 49 d the quantity of ^{14}C added as alanine was increased such that 47% more ^{14}C was bound to the solid phase compared to glucose (1.80 ± 0.28 and 3.43 ± 0.15% of the total ^{14}C added for alanine and glucose respectively). Total half-lives for substrates within this pool were very fast being 0.02 ± 0.01 and 0.06 ± 0.02 d for alanine and glucose respectively (Tables 3 and 4). The proportion of the biologically oxidized pool made up of substrate-^{14}C bound to the soil phase after 49 d was calculated to be 2.46 ± 0.56 and 2.52 ± 0.51% for alanine and glucose respectively (Fig. 5).

3.5. Water-soluble microbial biomass extraction

The amount of ^{14}C extracted from the water-soluble microbial biomass fraction fitted well to a first order double exponential decay model (Fig. 4d; $r^2 = 0.97$ for both alanine and glucose respectively). Dependency levels were < 0.98 for all model parameters. A greater proportion of ^{14}C added as glucose was extracted after the $CHCl_3$-fumigation compared to ^{14}C added as alanine after 49 d ($P < 0.001$), with 20.0 ± 0.79% and 43.1 ± 1.46% of total activity added recovered for alanine and glucose respectively. The first pool half-life for alanine was estimated to be 0.80 ± 0.20 d whilst for glucose it was 1.15 ± 0.11 d. This result is reflective of glucose having a much higher amount of ^{14}C ascribed to the first pool (a_8, 37% larger; $P < 0.01$) than alanine. The proportion of the ^{14}C recovered by the biological oxidation accounted for by the soluble microbial biomass pool was the largest of all the extractions undertaken within this study (Fig. 5). The amount of ^{14}C-added as alanine extracted from within the microbial biomass was 31.0 ± 2.2% of total activity added, whereas over double that amount (69.6 ± 9.4% of total activity added) of ^{14}C added as glucose recovered from water-soluble material from the microbial biomass.

3.6. Methanol-soluble microbial biomass

After $CHCl_3$-funigation, a MeOH extraction removed water-insoluble microbial biomass fractions (e.g. lipids). The results of this extraction undertaken over time also fitted well to a first order double exponential decay model (Fig. 4e; $r^2 = 0.90$ and 0.93 for alanine and glucose respectively). 38% more ($P < 0.01$) ^{14}C-activity recovered as glucose was recovered after 49 d compared to that added as alanine, with 1.86 ± 0.14 and 2.97 ± 0.15% (of total activity) extracted from alanine and glucose respectively. The MeOH-extractable microbial biomass fraction constituted 2.88 ± 0.28% and 4.82 ± 0.75% of the biologically oxidized pool for alanine and glucose respectively.

4. Discussion

4.1. Model selection to describe C substrate mineralization

This study aimed to identify a valid kinetic model which accurately describes the non-linear dynamics of CO_2 evolution arising from the microbial uptake, assimilation and mineralization of common C compounds found in soil solution. To address this we chose glucose and alanine as model solutes as they are abundant in root exudates and also form a major constituent of soil organic matter. The application of mathematical models (in particular, double and triple first-order exponential decay models) to describe substrate turnover provides an indication as to how C is partitioned inside the microbial community once assimilated (Paul and Clark, 1996). Knowledge of this partitioning is important as the ultimate fate of C derived from contrasting substrates may be very different after their uptake from soil, especially if the substrate is simultaneously partitioned into multiple pathways which each possess different C through flow rates. If we assume this complex scenario is the norm rather than the exception, then a simple single exponential decay model is obviously unsuitable and multiple pool models must be sought. Isotopic ^{14}C-labelling provided a robust method

to measure the fate of a particular substrate (Luo and Zhou, 2006). We adopted this method and traced the fate of ^{14}C-labelled alanine and glucose within the soil, without the presence of roots, and measured the temporal dynamics of $^{14}CO_2$ evolution. In this study the respired $^{14}CO_2$ only accounted for a third of the total ^{14}C added with the remainder remaining in the soil. Within this biologically oxidized fraction, the majority of ^{14}C from both alanine and glucose was retained within the microbial biomass. Most likely it was used to synthesize new cellular material (growth and maintenance), with some C recycled (either internally or due to cell death) and subsequently utilized as an energy source (Paul and Clark, 1996).

Mineralization of Low MW compounds is frequently assumed to follow a biphasic pattern (Chotte et al., 1998; Saggar et al., 1999; van Hees et al., 2005). Our results confirm that a double first-order exponential decay model does fit adequately to the data after 49 d (Fig. 1a; r^2 = 0.98). Importantly, however, our results showed that over the duration of the experiment (49 d), a triple exponential decay model provided a much better fit (Fig, 1b; r^2 = 1) to the experimental data. The triple model has an additional third exponential parameter which potentially accounts for the slower degradation of recalcitrant compounds (e.g. peptidogylcan in cell walls) and chemically/physically protected material. In addition, dependency values for all parameters after 49 d for the triple exponential decay were less than our 0.98 threshold, thus assuring we were not over-fitting the data by applying a triple decay model.

4.2. Influence of experimental time on model fits

Laboratory ^{14}C-labelled mineralization studies are typically conducted over different time frames, ranging in duration from minutes to years. There have been no previous studies to date, however, which have systematically evaluated the influence of experiment duration on estimates of C turnover for low MW C substrates. We had

hypothesized that the duration and number of time points used within the experiment would strongly influence the type of model used and the final parameter estimates. Our data showed within short timeframes (< 2 d), or with only a small number of individual time points (n < 8), a triple exponential decay model was too complex (i.e. failed parameter acceptance criteria) and was deemed unreliable. This occurred irrespective of the model fits having high r^2 values, as dependencies were above the 0.98 cut-off threshold, suggesting too many parameters were being used (Wolfe and Chinkes, 2005). In addition, the predicted parameter values for the slower pools (a_2, k_2, a_3 and k_3) differed greatly in their estimates (P < 0.001) between 0.5 and 1 d (6 and 7 time points), suggesting that 1 d (7 time points) is not long enough to adequately describe the microbial degradation of recalcitrant compounds and protected material, therefore providing inaccurate estimates of C turnover. Within the triple decay model, the estimated half-life for pool (a_2) is 1.35 ± 0.02 d and 1.47 ± 0.35 d (alanine and glucose respectively) which confirms that after 1 d (7 time points) we would not be accounting for the total turnover of even this first C pool. We therefore recommend that experiments be conducted for at least 7 d with at least 9 measured time points to ensure that reasonable parameter estimates are made.

The amount of extractable biomass C after $CHCl_3$-fumigation is calculated using the k_{ec} value established by Vance et al. (1987). The k_{ec} value is generally assumed to be constant. However, in our experiments looking at temporal effects on C turnover, this value changed with time. This is not surprising, as with increasing time, less ^{14}C will be extractable from the biomass as the ^{14}C becomes incorporated into insoluble material up during metabolic processes. Consequently, the use of $CHCl_3$-fumigation to estimate the incorporation of isotopically-labelled compounds into microbial biomass should be interpreted with caution.

4.3. Identification of C pools from exponential decay models

A better understanding of rhizosphere C cycling is crucial in investigating the influence of climate change on terrestrial system functioning, however, C flow in the rhizosphere is known to be highly dynamic and complex with multiple feedback loops (Jones et al., 2004, 2009). Combining mathematical models with experimental research can provide a quantitative analysis of the dynamic mechanisms involved in C cycling and may integrate across this inherent complexity (Paul and Clark, 1996; Roose and Schnepf, 2008). Ideally, however, the models should be relatively simple to enable incorporation into large scale global climate models (Todd-Brown et al., 2012). In addition, C pools within these soil models should preferably have some functional/mechanistic basis, rather than being purely descriptive, to allow experimental validation and also to improve our scientific understanding of model outputs.

In this study we were able to parameterize the turnover of multiple C pools which we assume to be largely discrete. In agreement with Hill et al., (2008, 2012), we found that a very simple model described the uptake of substrate from soil solution and that rates of microbial assimilation were extremely rapid (< 3 h) suggesting a very high flux through the soil solution. After microbial assimilation, however, the fate of alanine- and glucose-derived C was different. Our results clearly demonstrate that alanine is used for catabolic processes (i.e. respiration) and subsequently more is rapidly respired than retained within the microbial biomass. In contrast, glucose is used more for anabolic processes (e.g. formation of new biomass, maintenance) and thus disproportionately more is immobilized within the microbial biomass. This high level of immobilization was surprising given that glucose represents an important direct source of C for cellular respiration. This supports the notion that glucose represents a central hub in microbial metabolism supporting both direct assimilation pathways (e.g. respiration) and provides the C skeletons for a whole host of other indirect pathways (e.g. lipid, protein,

peptidoglycan metabolism) and implies that it is a dominant C solute passing through soil solution. Considering the high proportion of cellulose in plant litter and glucose/sucrose in root exudates this is not surprising. In contrast, we had expected that alanine would be used directly in protein synthesis with little used in respiration. Our results, however, suggest that significant amounts of alanine were converted to produce glycine, aspartate or glutamate in conjunction with pyruvate, the latter being used in respiration (Frolkis et al., 2010). This implies that the rate of supply of alanine greatly exceeds internal demands. Further work with alanine labelled in different atom C positions may help address the uncertainty.

The triple exponential model which provided the best fit to our data infers there are three compartments into which each substrate is partitioned once assimilated by microbial communities. Ascribing these three compartments to biological functional pools is inferential and difficult to validate. We investigated whether we could provide qualification as to where the ^{14}C is being allocated within the microbial populations. The first pool from the triple decay model (a_1) has a rapid half-life suggestive of immediate catabolic usage by microbial communities. The second and third mineralizable pools(a_2 and a_3) are ascribed to the turnover of different fractions of microbial biomass, with (a_2) representing degradation of more labile components (e.g. temporary C stores) and (a_3) accounting for the more recalcitrant biomass components (e.g. cell walls). Combined, these two pools constitute the majority (~80% for alanine and ~90% for glucose) of the $^{14}CO_2$ respired. Results from the $CHCl_3$-fumigation extraction showed combined pool sizes for alanine and glucose are ~70% and 80% respectively (Table 3 and 4 respectively) supporting the view that the majority of added C in used for anabolic processes rather than for respiration. The second mineralizable pool (a_2) constitutes the more labile biomass fraction and corresponds in terms of flux

rate with the first pool from the $CHCl_3$-fumigation extraction (a_8). Statistical analysis suggests no differences between the size of a_1 and a_8 for alanine ($P > 0.05$), but for glucose these two groups are marginally different ($P < 0.01$). In addition the rate constants and half-lives associated with these pools (k_1 and k_8) are also within similar ranges and show no statistical differences for either alanine or glucose ($P > 0.05$). This suggests that pool (a_2) corresponds to a cytoplasmic soluble, transitory reservoir of ^{14}C, possibly in the form of soluble proteins/peptides and nucleotides (Koch, 1991). The third mineralizable pool (a_3) ascribed to the degradation of the recalcitrant biomass fractions is the largest pool and has the longest pool half-life for both alanine and glucose. We can only speculate on how this pool is turned over, however, it could occur simultaneously by (1) internal recycling of cellular metabolites which are generally slow (Koch, 1991), (2) apoptosis, (3) direct consumption and breakdown within protozoa, and (4) non-programmed cell death and breakdown via extracellular enzymes to release soluble products which can be re-assimilated by neighbouring organisms.

Due to the multiple, complex interactions which occur simultaneously, it is difficult to decipher exactly how C is cycled within the soil or even what the dominant pathways are. Metabolic models generally assume one of two structures (Wolfe and Chinkes, 2005; Cobelli et al., 2000; Manzoni et al., 2012); a catenary structure where independent compartments are aligned sequentially, with each compartment only connected to its neighbour, or a mamillary structure whereby a central compartment is surrounded by subsidiary pools of which none are connected to each other. We devised a conceptual model postulating how C is cycled based on our experimental data (Fig. 6). In our model, we suggest a mamillary model may best describe C allocation within rhizopshere soil (Fig. 6), with the central compartment being a labile DOC pool from which microbial communities access substrates. Once the substrate has been consumed

by the microbial communities a new model structure may then be assumed with the microbial biomass acting as the central pool, which will in turn feed back to the labile DOC pool as the cycle continues.

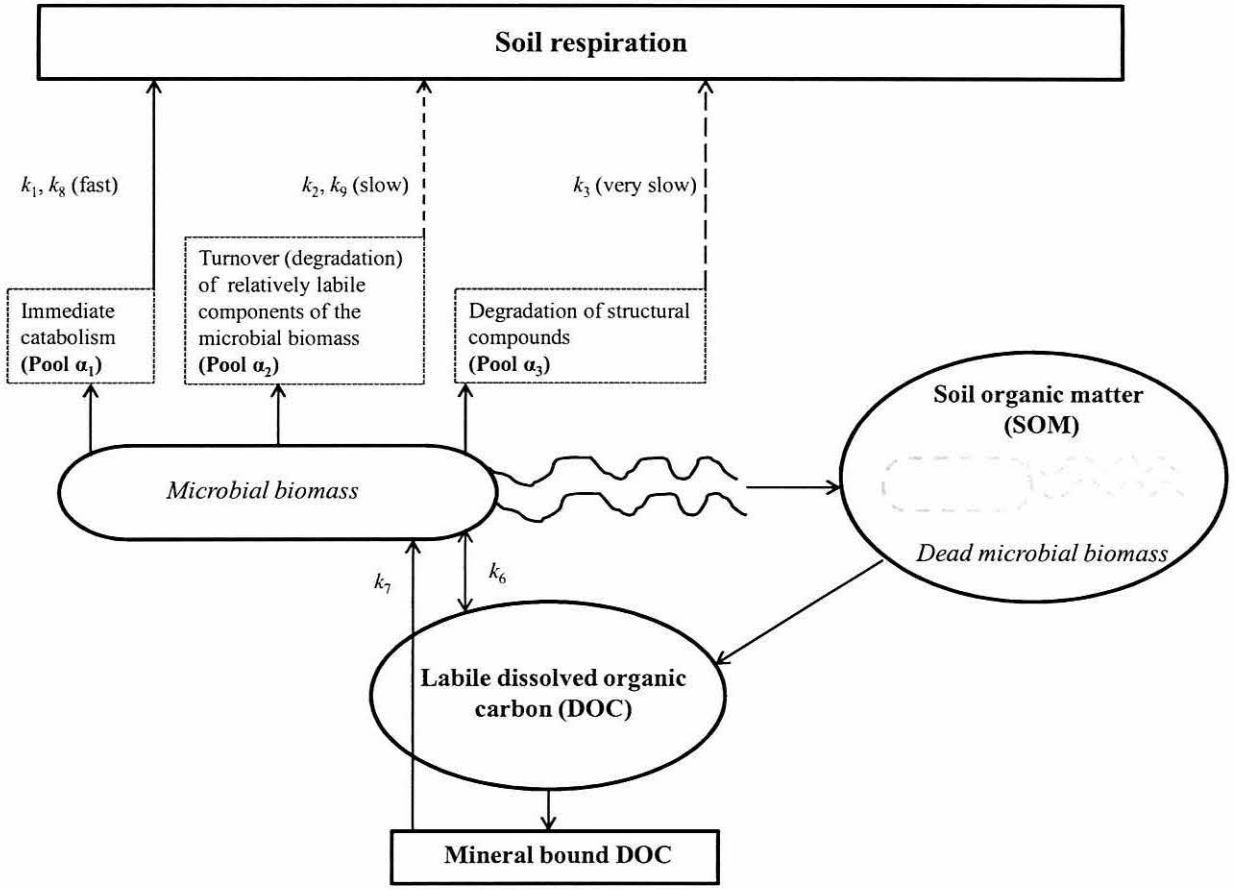

Fig. 6. Conceptual model of soil C pools. The C pools are provisionally attributed after performing extractions on soils spiked with ^{14}C-labelled glucose and alanine. The rate constants (*k* values) are those obtained after fitting exponential decay models to the data and have been assigned to exchanges (arrows) which we hypothesize best reflect their associated pool. For a full description of model parameters see Materials and Methods.

5. Conclusions

We have demonstrated that the mineralization of low molecular weight C substrates corresponds better to a three pool model as opposed to the well documented biphasic model. As expected, however, this is dependent upon the duration of the experiment, and number of time points used to fit the model. Critically, a three pool model is unsuitable based on analysis of dependency values, for short experiments (< 2 d), with limited data points (< 8 time points), despite having high model fit values (r^2). With increasing length of the experiment and increasing number of time points; the biphasic pattern does not provide the best fit to the data and a triphasic pattern succeeds it, without being too complex. The use of $^{14}CO_2$ evolution as a proxy for substrate mineralization is robust, providing the correct model is assigned to the data. Assigning mathematical models to $^{14}CO_2$ respiration data must represent biological functional pools and currently we can only infer how these pools are assigned. Our data comparing kinetic parameters from different chemical extractions with the $^{14}CO_2$ mineralization data did not provide conclusive evidence to define these pools and further research is required. In addition, the use of exponential decay models do not account for interactions between the ascribed pools. It is inevitable that each pool is likely to interact with neighbouring pools and including these interactions in future mathematical models will provide a more robust and reliable model for C cycling dynamics. Although our results identify appropriate models to describe C turnover in soil they have done little to improve our mechanistic understanding of C cycling within microbial metabolic pools. Maybe it is too much to assume that the microbial biomass, consisting of thousands of species existing in different metabolic states, can be described so simplistically. If we accept the latter, then significant advances will need to be made in soil metabolomics before our understanding is significantly improved.

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Appendix V

Supplementary material to accompany Chapter V.

Supplementary material

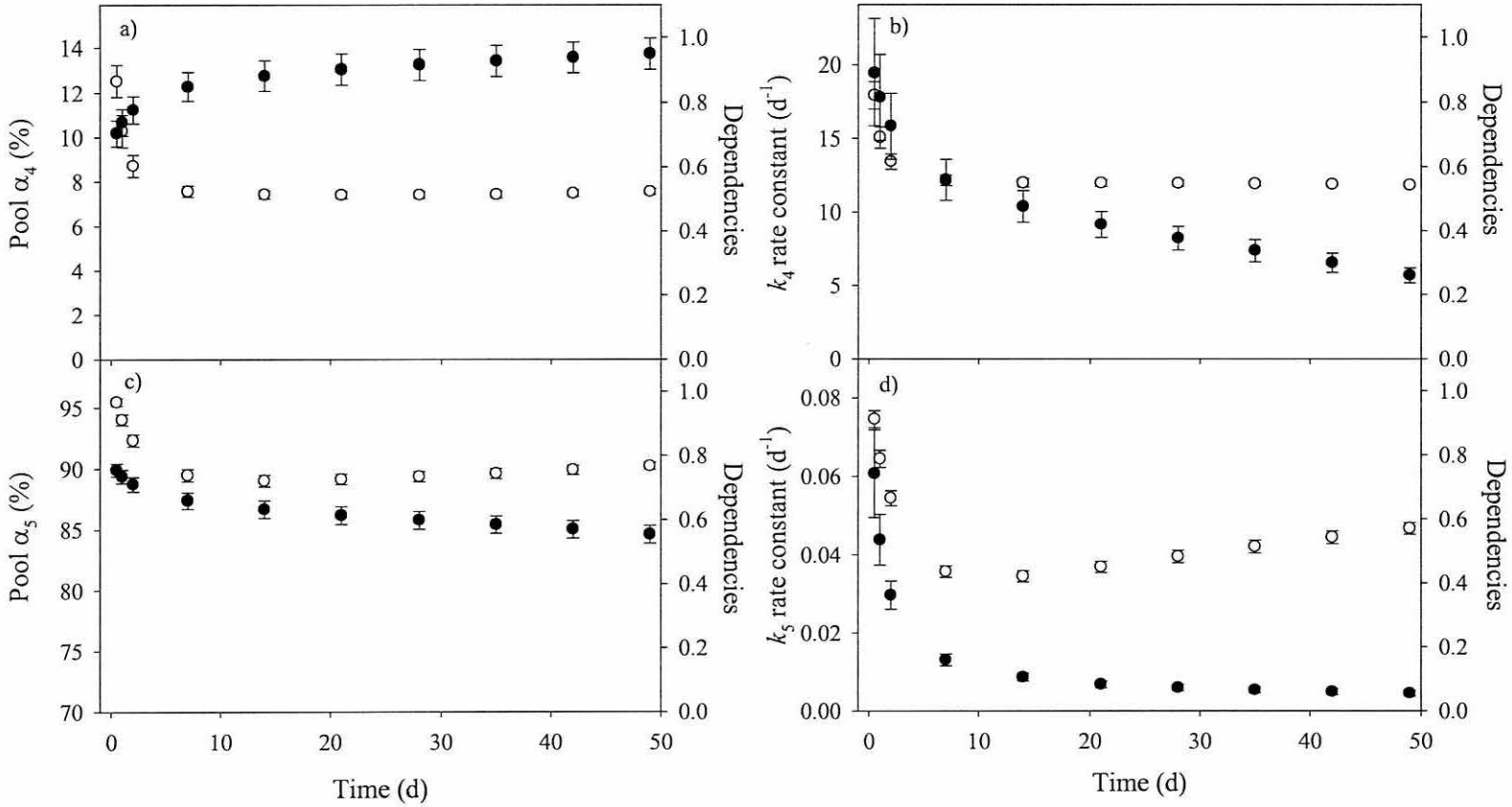

Fig. S1. Influence of the number of measurement points on model parameter estimates describing the turnover of glucose with a first-order double exponential decay model. Solid circles represent parameter values at each time point. Open circles represent dependency values associated with each parameter. Points represent means ± SEM (n = 3). For a full description of model parameters see Materials and Methods (Eqn. 2).

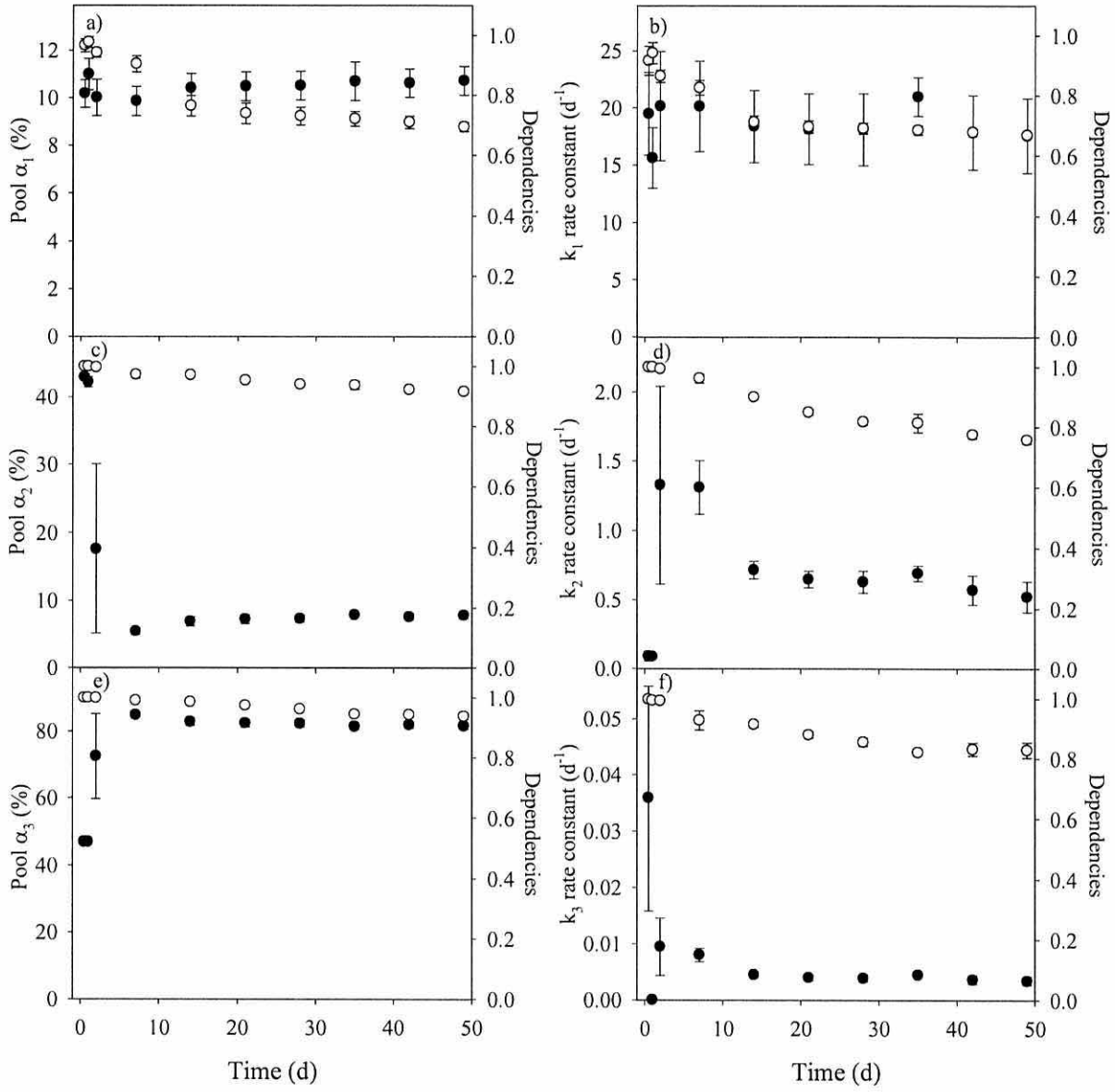

Fig. S2. Influence of the number of measurement points on model parameter estimates for glucose with a first-order triple exponential decay model. Solid circles represent parameter values at each time point. Open circles represent dependency values associated with each parameter. Points represent means ± SEM (n = 3). For a full description of model parameters see Materials and Methods (Eqn. 1).

3. Discussion

This final chapter aims to summarise all the data presented in this thesis and relate the findings to the 8 objectives identified during Section 2, with a broad overall aim to:

- **Identify mechanisms influencing the mineralization rates of individual DOC components and how these contribute to overall soil respiration.**

Soil respiration is the second major pathway of C efflux from terrestrial ecosystems, behind photosynthesis (Kuzyakov, 2006); the magnitude of this flux is dependent upon a variety of factors. These include soil properties (e.g. soil temperature, soil water content, pH, salinity, and nutrient availability), climate (e.g. precipitation, temperature, seasonal and yearly changes), and anthropogenic influences (e.g. land-use change, pollution) (Fang and Moncrieff, 1999; Raich and Tufekcioglu, 2000). This multitude of factors, combined with their interactive effects, often confound our understanding of the mechanisms involved in regulating C cycling. The terrestrial biosphere contains almost twice as much stored C compared to that stored within the atmosphere and there is much debate about how natural (e.g. climate change) and anthropogenic changes (e.g. land-use changes) could potentially affect this terrestrial C pool (Houghton, 2004; 2005; Conant et al., 2001; Bellamy et al., 2005). Improving our understanding of the regulating mechanisms involved in C cycling is particularly important for helping validate Global Climate Models GCMs), and informing climate policy makers on effective mitigation/adaptation strategies.

This thesis investigated how four key aspects can potentially influence soil respiration;

1. Choice of method employed to measure CO_2 efflux.
2. Changing climatic variables.
3. Physical soil properties.

4. Mathematical modelling of CO_2 flux.

These factors can all influence our understanding of the biogeochemical cycling of the macro-nutrient, C, and consequently affect our quantification of total soil respiration. These divisions are not mutually exclusive as there is much crossover between them, particularly for climatological and pedological influences on soil respiration which are strongly interlinked.

3.1. Methodological influences on soil respiration

Soil respiration is difficult to measure *in-situ*, not only due to confounding environmental and physical soil properties influencing measurements, but also because there is currently no internationally recognised or standard protocol for measuring soil respiration (Widén and Lindroth, 2003; Pumpanen et al., 2004; Norman et al., 1997). Laboratory studies investigating controls on C cycling are often conducted in preference to in-situ experiments due to time and logistical constraints. Laboratory results are thus deemed to be analogous to *in-situ* results; however, few studies have directly compared how the kinetics of soil respiration may differ between *in-situ* and *ex-situ* experiments. This thesis had specific objectives designed to help ascertain how methodological approaches may influence soil respiration

3.1.1. To investigate whether using different commercially available, in-situ, CO_2 gas analyzers can reliably be compared.

In chapter I, we directly compared two different non-steady state/closed IRGA-based CO_2 analyzers; the LI-COR 8100 (LI-COR Biosciences, Lincoln, NE, USA) and the PP Systems EGM-4 with an associated SRC-1 chamber (PP Systems, Hitchin, Herts, UK).

This was conducted *in-situ*, across three contrasting ecosystems; lowland grassland, upland grassland and upland heathland. Different CO_2 analyzers have slight variations in how the CO_2 flux is calculated, which could potentially influence measurements (Table 1). However, despite these differences, the main aim of the analyzers is to accurately calculate CO_2 flux, so the various systems, in theory, should be relatively comparable to each other.

Table 1. Summary of some of the main differences between the two non-steady state/closed systems used in Chapter I.

PP Systems EGM-4 with SRC-1 chamber	LI-COR 8100 with 102 chamber
• Manual chamber placement	• Automated chamber placement
• Fan used to mix air inside chamber	• No fan as chamber geometry designed to mix the air.
• No pressure relief vent – fan mixes air which minimises pressure differences	• Pressure relief vent design - pressure inside the chamber tracks ambient pressure under calm and windy conditions.
• Manual selection of data fit before measurement begins – either linear or quadratic	• Automated selection of data fit during measurement – either linear or quadratic
• Flow rate ~ 350 ml min^{-1}	• Flow rate ~ 2000 ml min^{-1}

Overall, we found good similarity in respiration estimates across the three different ecosystems for the two different IRGA systems (Fig. 2; Chapter I). This suggests that soil respiration measurements taken from different ecosystems can be reliably compared, irrespective of the two different systems being used to calculate flux. It is important to note that this data only applies to the two IRGAs used within this study and so care should be taken when comparing other CO_2 analysers and other approaches (e.g. static chambers). Further studies could include switching the chambers and the IRGAs i.e. fitting the SRC-1 chamber with the LI-COR 8100, and LI-COR-102 chamber with the EGM-4 IRGA, this would enable a direct comparison of both chambers and both IRGAs. Also, comparison

with other techniques such as eddy flux and static NaOH traps in order to see how comparable respiration measurements are across different systems.

3.1.2. To examine whether the inclusion/absence of collars on CO_2 gas analyzers in-situ, adversely affect respiration measurements.

In addition to directly comparing two IRGA systems, we also investigated the absence/inclusion of collars on soil respiration measurements. Results from our studies (Chapter I), for both IRGAs, showed great variability in respiration results when collars were included, across all three ecosystems. Across all sites, results showed a 25 ± 11 and 20 ± 6 % decrease in CO_2 efflux for the LI-COR 8100 and EGM-4 systems respectively, when collars were included compared to no collar treatments (Fig. 1; Chapter I). Our results showed that there is a systematic bias when collars are used during respiration measurements. This bias was present irrespective of the type of IRGA used, and as such, suggests there is some physical effect of using a collar. This could be due to a poor seal between the collar and the chamber base thereby resulting in CO_2 loss. However, intrinsic, site specific properties could also affect respiration measurements, such as areas with high organic matter content and with high soil water content where decomposition and mineralization are already limited (e.g. peaty areas). Our data showed greatest variation in the upland grassland site which had the highest organic matter content (71.2 ± 3.89 %) and also the highest soil water content (76.8 ± 1.05 %). This suggests that the use of collars in these systems will likely have a greater influence on soil respiration measurements and so caution is needed on emplacement of collars prior to any measurements being taken irrespective of the type of CO_2 analyzer being used. This corroborates with results from other studies where collars were also seen to influence respiration measurements (Rochette

et al., 1997; Davidson et al., 2002). However, the magnitude of this effect and perhaps more importantly, the duration of this effect is poorly understood and requires further study. In addition, is this effect important when compared to temporal and spatial variability of soil respiration? Further investigation should be undertaken to assess whether using collars or not provide the best estimation of soil CO_2 flux. This could be assessed using *ex-situ* experiments using known CO_2 effluxes and comparing the effects of including/excluding collars.

3.1.3. To investigate how the mineralization rates of individual DOC components compare under field (in-situ) and laboratory (ex-situ) conditions.

Results from laboratory studies are assumed to be analogous to field results and are often scaled-up accordingly and are used for modelling large-scale ecosystem dynamics (Carlyle et al. 1998; Oburger and Jones, 2009). It is therefore imperative to assess whether laboratory results realistically represent results from the natural environment. We directly compared the mineralization rates of 31 different ^{14}C-labelled substrates under field and laboratory conditions (Chapter II), with a particular focus on whether C partitioning into individual metabolic pools is affected. Our results showed the mineralization of the 31 ^{14}C-labelled substrates was highly reproducible between laboratory and field conditions. This suggests that estimates of C dynamics from the laboratory are indeed representative of results obtained from the field. Model parameters obtained from the mineralization data showed the partitioning of substrate C into two microbial pools; the first being a labile pool which is associated with immediate use for catabolic processes (e.g. respiration), the second, slower pool is thought to represent use for anabolic processes (e.g. formation of new biomass, growth and repair) and then eventual turnover of the microbial community

(Jones, 1999; Jones and Hodge, 1999). The use of mathematical models can also help describe the kinetics of substrate mineralization by providing rate constants which can be used to calculate substrate half-lives. Our studies showed that the amount of C partitioned into each respective pool did not differ between laboratory and field conditions. However, 3 substrates showed differences in rate constants which in turn influenced the overall substrate half-life. This could be as a result of changing microbial activity in the different experimental conditions, whilst the size and composition of the microbial community remains unaffected by the different conditions. This could be due to the microbial populations requiring different substrates as conditions change. Further research is needed to identify if different metabolic pathways are active under the different experimental conditions.

This research has important implications for further research investigating the breakdown of C compounds by microbial communities because it provides evidence supporting the notion that laboratory results are analogous to field results. However, it is important to note that results obtained in Chapter II depict conditions at a single point in time and despite our results showing good correlation between the laboratory and field, this could change if field conditions alter e.g. with seasonality. The research in Chapter II was conducted during the autumn and soil conditions e.g. temperature, were relatively comparable between the laboratory and the field, and thus disturbance to the intrinsic soil conditions was minimal. However, if this study was conducted during the winter, soil conditions, in particular temperature would be considerably different between the field and laboratory. Therefore, taking soil from *in-situ* and transporting it to the laboratory during the winter could influence mineralization rates and as such results may not be representative of those found in the field. This has implications for ecosystem modellers

who are interested in C dynamics and require accurate measurements to improve not only our understanding of current C cycling dynamics but also how changing climatic variables may influence C cycling in the future. This promotes the need for further research investigating how mineralization rates are likely to alter seasonally and whether these results are equally comparable in the laboratory.

3.2. Climatological influences on soil respiration

Understanding how the terrestrial biosphere is likely to be influenced by climate change is inherently difficult because of a lack of a mechanistic understanding on how C dynamics are likely to be affected by key environmental factors (e.g. temperature, soil water content, and increased CO_2 levels). Global climate models currently project rises in temperature and atmospheric CO_2 levels and also increased variability in precipitation patterns (Houghton, 2005).

However, uncertainty between climate models exists regarding seasonal and spatial precipitation patterns. This is particular pertinent to high latitude areas which are expected to experience higher than average rates of warming and also experience more varied precipitation patterns (Anisimov et al., 2007). This thesis outlined specific objectives in order to investigate the influences of climate variables on C cycling.

3.2.1. To investigate the effect of temporal variability on mineralization rates of individual DOC components in-situ.

In-situ environmental conditions vary not only between seasons, but also between years, with fluctuations in precipitation patterns and temperature regimes. *In-situ*

experiments often represent only a snapshot of environmental conditions at a single time point in the year. Results are then often incorporated into ecosystem models to investigate potential changes on a yearly scale. However, it is important to examine how these potential measurements may change from year-to-year. We assessed the inter-annual field variability of the mineralization rates of the same 31 ^{14}C-labelled substrates mentioned above (Section 3.1.3.), across two separate years (Chapter II). Our results again showed good correlation in the modelled rate parameters between the two years, showing good reproducibility of data (Fig. 3; Chapter II). However, there were notable differences in specific rate parameters between the two years, which in turn affected the overall half-life for individual substrates (Fig. 5; Chapter II). As such, 13 out of the 31 substrates showed significant differences between the two years. This emphasizes the importance of including inter-annual measurements in models looking at ecosystem contribution to a particular flux, in this case CO_2. We speculate that the differences between years could be as a result of short-term localised climatic variations. Results from 2010 results showed shorter half-lives compared with 2009 which could be linked to a period of low rainfall early in the year, potentially delaying the onset of the growing season which could influence root exudation patterns. Understanding the specific role each substrate plays in metabolic processes, using advanced techniques such as the use of proteomics and metabolomics will be invaluable in helping to explain why certain substrates differ between years. Again, it is important to note that measurements taken at one point in time only represent conditions at that point of sampling and if sampled the following week, results may differ. Therefore, more comprehensive studies looking at substrate mineralization and turnover rates are required in order to provide more reliable and accurate estimates of diurnal/seasonal/yearly influences, this will also account for periods when microbes become starved of root exudates. This will

vastly improve current and future ecosystem models looking at nutrient cycles and how these might be affected by future climate and/or land-use change.

3.2.2. To investigate the response of vegetation and below-ground communities to soil thaw along a natural snow melt gradient, in the high Arctic tundra, to assess the potential feedbacks to climate change.

Climate change is projected to have a great impact in high latitude areas with temperature and precipitation regimes both likely to be affected. However, the effect of these changes on ecosystem functioning is difficult to predict because of our poor understanding of fundamental soil-microbial-plant processes in polar environments. The high Arctic tundra is characterised by a short growing season (Grogan and Chapin, 1999; Williams et al., 2009; Wookey et al., 2009) and as such soil microbial communities and vegetation are hypothesised to respond rapidly to snow melt. Monitoring how Arctic ecosystems respond to natural events *in-situ*, such as snow melt, can help improve our understanding of how ecosystems may be influenced by future climate change. Combining *in-situ* observations with detailed laboratory techniques can provide a more comprehensive picture of ecosystem responses to climatic events. We investigated the response of vegetation (e.g. vegetation emergence and leaf expansion) and below-ground soil communities (soil respiration and 16S rRNA) to snow melt and attempted to identify what are the key drivers in mediating the response (Chapter III).

Soil parameters measured along the transect identified soil temperature and nutrient availability as the main influential drivers regulating changes during snow melt, as soil water content was not a limiting factor along the transect. Our results showed both

vegetation (emergence data and leaf expansion) and below-ground microbial communities (soil respiration) changed rapidly in response to snow melt. However, genetic analysis which looked at quantifying microbial biomass in terms of gene copy numbers, did not find a similar rapid response. In contrast immediately post melt, gene copy number decreased and then remained relatively constant with increasing distance away from the snow melt front. However, the type of genetic analysis used (16S rRNA) includes both active and non-active cells (Coci et al., 2011) and so results could comprise previously un-decomposed microbial populations remaining from the previous season. Temperature may be the main driver initiating plants and microbial communities to respond after snow melt, however, if higher temperatures are sustained as currently projected, then this could have major implications for Arctic ecosystem functioning (Lal, 2004; Anisimov et al., 2007). If prolonged warmer temperatures dominate in the Arctic, then the growing season will likely be extended due to an increased length of time free from snow cover, due to increased plant productivity. If plant productivity increases then there will be a greater supply of nutrients from root exudation which could potentially fuel a complete change in vegetation communities with vascularised plants dominating, unless nutrient availability becomes limiting (Mack et al., 2004; Grogan and Jonasson, 2005). Other studies suggest bryophyte colonization may increase along with an increase in "immigrant" angiosperm species (Robinson et al., 1998; Madan et al., 2007). Our study emphasizes the importance of combining *in-situ* measurements and advanced laboratory techniques in order to try and elucidate mechanisms controlling Arctic ecosystems functioning. It is important to try and understand the processes currently acting before we can try to postulate potential impacts of a changing climate.

3.3. Pedological influences on soil respiration

Soil provides the foundation for life in terrestrial ecosystems. Soil provides an essential habitat for vegetation and microbial communities to live in and many fundamental processes between the biosphere and the atmosphere are controlled by the soil, such as, nutrient cycling, storage of gases and water flow. Soil temperature and soil water content are two important and influential soil properties which can affect processes occurring within the soil. These two variables are most likely to be affected by climate change. However, there are other soil properties which are also important, such as pH. This thesis outlined specific objectives to focus on how soil properties are can affect the mineralization of specific C components.

3.3.1. To examine the sensitivity of the decomposition of different C compounds to changing soil properties, in particular, soil temperature and soil water content, in the high Arctic tundra.

Soil organic matter contains both recalcitrant, older, high MW C which is often difficult to metabolise; it also contains more labile, low MW organic C which can be easily decomposed and metabolised by microbial communities. Soil temperature and soil water content are two factors which can potentially influence the breakdown rates of different C compounds, both of which are likely to be affected by climate change, especially in high latitude areas. The Arctic tundra provides a relatively pristine environment in which to make direct studies of climate change impacts on soil C dynamics. The Arctic is currently experiencing dramatic alterations to its climate, with temperatures warming at twice the global average rate over the last 100 years, resulting in reduced snow cover and thawing of

the soil. Currently, C stored in the Arctic is mostly protected from decomposition whilst it remains frozen and under snow cover (Post et al., 1982; Grogan and Jonasson, 2005). However, with climate change altering temperature and precipitation patterns, this C store is likely to be exposed, making it vulnerable to decomposition. We conducted a series of manipulation studies investigating the influence of the two key environmental variables; soil temperatures and soil water content, on the breakdown of both low and high MW C compounds in Arctic soils (Chapter III). Our results showed both environmental variables influenced mineralization rates (Fig. 6; Chapter III) and microbial pool partitioning of C (Tables 4 and 5; Chapter III) of both low and high MW compounds. However, we identified that soil water content had the greater influence on the mineralization rates of the high MW compounds. Microbial decomposition of organic matter may therefore be more sensitive to changes in soil water content than in temperature changes. Field observations along the snow melt front identified temperature as being the main regulator influencing vegetation changes immediately post snow melt, initiating the start of the growing season, However, data from our manipulation studies suggests that soil water content may become more important in regulating C cycling as the growing season progresses. This work has helped improve our understanding of the fundamental soil-microbial-plant interactions in polar environments and how these processes are likely to be influenced by future climate change.

3.3.2. To examine if changing soil properties, such as pH, can influence mineralization rates of low MW DOC components and to investigate if this correlates to a change in microbial community composition.

Soil pH is very important for regulating chemical reactions as many enzymes are pH dependent, and is deemed to be as important as C and N concentrations for influencing soil microbial biomass and microbial community composition. There is much focus on how the activity of microbial communities is likely to be influenced by changing soil and/or environmental variables. However, it is important to also investigate whether any change in activity is due to a change in community composition structure or whether it is a direct effect on metabolic processes. We investigated how the mineralization rates of different low MW ^{14}C-labelled compounds were affected by soil pH and examined whether this correlated with any change in the microbial community composition (Chapter IV) as identified using phospholipid fatty acid analysis (PLFA). Soils for this experiment were taken from a natural pH gradient so we were not altering the intrinsic soil properties in order to manipulate pH changes. Our results showed no changes in mineralization patterns irrespective of pH change. However, microbial community composition showed distinct differences across the pH gradient, with a significant increase in bacterial growth as pH increased. Results from this study suggest that microbial community composition does not impact on the mineralization rates of low MW compounds. Therefore, in terms of C cycle models, it is perhaps more important to focus on the controls of microbial C cycling and whether individual metabolic processes are affected by changing soil properties. New advances in metabolomics, proteomics and transcriptomics techniques can provide more detail about specific changes to the metabolic activity of microbial populations, and can identify if specific genes involved in particular metabolic pathways are activated/de-

activated as soil conditions change. This will provide a more detailed understanding as to the mechanisms controlled soil C cycling.

3.4. Mathematical modelling of soil respiration results

Soil C dynamics are currently not well understood because of the complexity of soil-microbial-plant interactions and the numerous confounding factors (methodological, climatological and pedological) which can influence C cycling. Improving our understanding of these processes and attempting to disseminate mechanistic controls on C cycling is important for being able to simulate future changes to soil C storage across different ecosystems. The application of mathematical models can provide quantification on how specific C components pass through the soil microbial biomass. One objective of this thesis was to investigate the application of different mathematical models to soil respiration data and examine whether subsequent interpretation of model outputs can be biologically justified. In addition, we have also investigated how results obtained from modelling can be used to estimate total ecosystem CO_2 fluxes.

3.4.1. To examine methods and mathematical models currently used to determine C dynamics.

Throughout this thesis we employed the method of adding isotopically ^{14}C-labelled substrates to soil to enable CO_2 evolution to be monitored over time and thus used as a proxy for estimating substrate turnover rates. The kinetics associated with substrate turnover can then be estimated by applying mathematical models, providing a more mechanistic understanding of substrate flow through the soil microbial biomass. The

mineralization of low MW C compounds follows an exponential decay pattern (e.g. double or triple first-order exponential decay models), and the number of exponential components used can infer how C is partitioned inside the microbial community once assimilated. It is imperative that the correct model is applied to the experimental data, not only to avoid over-fitting of the data but also to provide the most accurate quantification of fluxes (Wolfe and Chinkes, 2005). We investigated the validity of both double and triple exponential decay models by examining relationships between model parameters obtained from the mineralization data of two low MW C compounds (Chapter V). Our results indicate that the type of exponential decay model selected is dependent upon the number of time points used to fit the model as well as the duration of the experiment. Relationships between model parameters (dependency values) showed triple exponential decay models are unsuitable for short experiments (< 2 d) and when limited measurement points are used (< 8 time points), regardless of the model appearing to show a good fit to the data (r^2 values). When longer duration experiments with more measurement points are included then likewise the double exponential decay does not provide the best fit to the data and there is a danger of under-estimating the complexity of substrate turnover. There is a wealth of literature suggesting that the mineralization of low MW C compounds corresponds better to a double exponential model (Chotte et al., 1998; Saggar et al., 1999; van Hees et al., 2005; Boddy et al., 2007). However, we suggest that this kinetic modelling approach is not always valid and more attention should be placed on looking at relationships between model parameters rather than just assuming a double exponential model will always provide the best fit to the data. Results obtained from the exponential decay models are then used to infer substrate pool partitioning and flow rates through the microbial biomass. We attempted to define these biologically functional pools using a series of different chemical extractions (Chapter

V) and comparing results with the model parameters obtained from the mineralization data, but we were unable to provide conclusive evidence supporting the postulated biological functional pools.

This research highlights the importance of identifying the appropriate mathematical model to apply to C substrate turnover, however, the lack of correlation to known biological pools implies a lot more further research is required before we can improve our mechanistic understanding of how C is cycled within microbial metabolic pools. Mathematical modelling is useful for inclusion in ecosystem models as it provides some quantification as to how substrates are cycled by the microbial communities which is invaluable for ascertaining overall ecosystem fluxes. New advances in metabolomics techniques may help disseminate the complex mechanisms controlling C substrates cycling within the microbial biomass. Soil C fluxes will only contribute a small fraction to GCMs which endeavour to encompass numerous terrestrial-atmosphere interactive processes. It is important that the values obtained from the biosphere are as accurate as possible and so often the hugely complex soil-microbial-plant processes are over-simplified for inclusion in GCMs. Accurately accounting for soil C fluxes is essential for quantifying GHG fluxes and predicting how these fluxes are postulated to be influenced by global changes.

3.4.2. Up-scaling of experimental data and wider implications

Mathematical models not only help provide a more mechanistic understanding of complex processes occurring at a fine-scale resolution, but are also used as a tool for projecting large-scale regional/ecosystem/global changes (Blagodatsky and Smith, 2012; Standing et al., 2007). This is important for policy makers who implement

adaptation/mitigation strategies in response to how ecosystems/regions may be affected by climate or land-use changes. Ascertaining values for large-scale changes often come from the up-scaling of fine-scale experimental data as most scientific experimental research is conducted on a much smaller scale to what policy makers require. It is therefore imperative that the data incorporated into large-scale models is as robust and accurate as possible.

Soil respiration is the second major pathway of C efflux from terrestrial ecosystems after photosynthesis and is postulated to account for 60-90% of total ecosystem respiration (Kuzyakov, 2006). Soil respiration is divided into two functional sources; one derived from autotrophic (plant) respiration and the other derived from heterotrophic (microbial) respiration (van Hees et al., 2005). Currently, there is much discrepancy surrounding the relative contributions to soil respiration from root and microbial sources, with root/rhizosphere respiration estimated to contribute as little as 10 % or up to 90 % to total soil respiration (Hanson et al., 2000). It is difficult to experimentally separate root and microbial contributions to respiration due to their close interactions (Hanson et al., 2000; van Hees et al., 2005). However, in order to better understand current C cycling and potential implications to global C storage from future climate change, then it is important to try and calculate the contribution from each group (Hanson et al., 2000).

We attempted to calculate microbially derived ecosystem soil C and CO_2 fluxes using the modelled field data reported in Chapter II combined with new analysis quantifying concentrations of each substrate found in soil solution. This research aims to provide a unique and comprehensive analysis estimating relative substrate contribution to overall soil respiration via the microbial biomass. Some substrates have been omitted from further analysis due to poor limits of detection. Urea was omitted because concentrations are highly variable in a grazed environment and inclusion in an up-scaled model could

result in a major over-estimation of contribution to overall CO_2 efflux. Glucosamine and adenosine are difficult to quantify due to their low concentrations in soil solution. The amino acid mix was also omitted because it combines equal amounts of the individual amino acids already quantified. Model parameters (pool sizes a_1 and a_2, and rate constants k_1 and k_2) obtained from double exponential decay models (taken from Chapter II) were then incorporated into the calculation in order to work out relative substrate contribution to overall flux. Values were then scaled up accordingly into either tonnes of C or CO_2 per hectare, accounting for soil conditions at the time of sampling (e.g. soil water content, bulk density), then fluxes were estimated as a proportion of soil respiration (field based IRGA measurements) and soil organic C content (total C) respectively, to give percentage organic matter C loss y^{-1} and percentage of substrate C contribution to respiration (Fig. 5a and b respectively). It is important to note that these fluxes represent only a single time point and so soil parameters are likely to change throughout the year. Our results showed good reproducibility between the two years (Fig. 5) which suggests that data obtained from the mineralization studies are a reliable proxy for soil respiration. Our data showed glucose and peptides dominate respiration fluxes, which given that plants are mainly comprised of cells and proteins is not surprising. However, initial concentrations of these compounds found in soil solution are 54 μM and 125 μM respectively, which are substantially higher than the other compounds measured, improved quantification of these values is required to verify their respective concentrations. Our results indicate that in autumn 2009, microbial communities contributed 18.6 ± 0.07 % to total soil respiration; this decreased to 17.6 ± 0.08 % in autumn 2010. This implies the remaining percentage is attributed to root respiration, or from other substrates not investigated in this study. Up-scaling this flux to represent CO_2 flux per year should be treated with caution because soil properties, such as

soil water content and temperature are likely to affect mineralization rates and model parameters. In order to validate a yearly flux, monthly experiments are required in order to observe any intrinsic soil and/or climatological variability. As mentioned previously, the values obtained within this study represent one field site at one point in time and as such, there is no account of seasonal or spatial variability. This study utilised the top 15 cm of soil to calculate fluxes as this is assumed to represent the biologically active mineral horizon. However, the depth of this horizon can change depending upon soil type and profile depth and amount of organic matter accumulation will also change along with bulk density. It is important to take into account site-specific functions when applying a similar method to a different soil type. The developments of new molecular techniques, such as metabolomics, proteomics and transcriptomics are helping provide the links between microbial diversity and ecosystem functioning (Standing et al., 2007). Encompassing both biological and physical processes into dynamic mathematical models is vital if we are to provide the most accurate determination of ecosystem functioning (Blagodatsky and Smith, 2012). In addition, experiments looking at the mineralization rates of other compound groups, such as lipids and phenolics (derived from the breakdown of lignin) would also be important to investigate in terms of their relative contribution to overall soil respiration. This is turn can help implement future policy regarding mitigating the potential feedbacks of climate change in different terrestrial ecosystems.

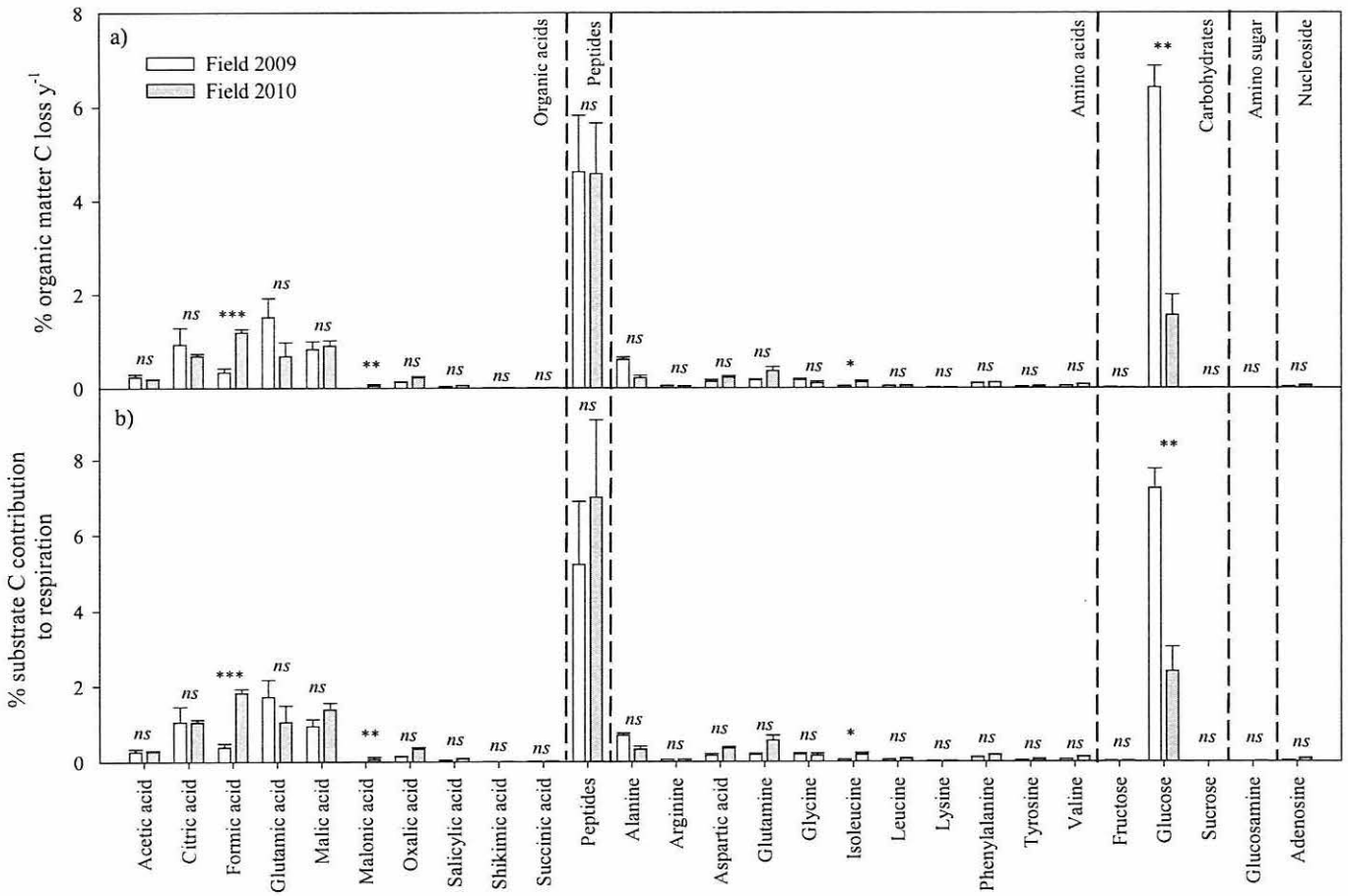

Fig. 5. Modelled estimates showing percentage of organic matter C loss per year (panel a) and substrate contribution to total soil respiration (panel b). Values represent means ± standard error of the mean (SEM), n = 3. *ns*, *, **, ***, represent values of $P > 0.05$, $P < 0.05$, $P < 0.01$ and $P < 0.005$ respectively.

4. Conclusions and suggestions for future work

The research conducted within this thesis has helped to further our understanding of the complex, and fundamental soil-microbial-plant processes involved in the cycling of C in terrestrial ecosystems. We have attempted to improve our current knowledge of the main controls on soil CO_2 flux across two contrasting ecosystems; an agricultural grassland and Arctic tundra. In particular, we investigated the relative contribution from soil microbial communities on total soil CO_2 flux and examined the different factors controlling these fluxes in the two different ecosystems. This has important implications for predicting how terrestrial C dynamics are likely to respond to future changes from both natural (e.g. climate change) and anthropogenic (e.g. land-use change) sources.

We looked at 4 key influences on soil respiration measurements and investigated how they can potentially affect how we interpret C fluxes. The conclusions of each are summarized below:

4.1. Methodological influences on soil CO_2 measurements

We show that the type of IRGA-based CO_2 analyzer used *in-situ* does not significantly impact upon soil respiration measurements and results therefore from the two different IRGAs can be reliably compared. This is important for when soil respiration is being investigated across a broad range of habitats and where often multiple CO_2 analyzers have been used and as such it is valuable to know that results can be reliably compared. In terms of validating CO_2 fluxes, there needs to be an internationally recognised standard for measuring soil respiration, which the IPCC could help provide. However, this is made difficult *in-situ* because of uncertainty

surrounding known CO_2 efflux from soils and a lack of absolute calibration of methods. Studies comparing different respiration methods with known CO_2 effluxes *ex-situ*, are useful for identifying systematic errors associated with each system used for measuring soil respiration. However, once these systems are used *in-situ* a whole suite of disturbance caveats occur which can influence results. Therefore, it is important to continue to test how reliably comparable CO_2 efflux values are when using different IRGA-based methods in-situ.

In addition, we found that the inclusion of a collar onto the soil respiration chamber, to extend the chamber volume, had an adverse effect on soil CO_2 efflux due to the amount of disturbance created, irrespective of the type of IRGA used. This is particularly noticeable in areas with high organic matter content and high soil water content. However, further studies are required to examine the duration and magnitude of this effect again across multiple ecosystem types.

Methodological influences apply not only to the type of equipment being used to measure fluxes but also whether the experiment is being conducted *in-situ* or *ex-situ*. Often *ex-situ* experiments are preferentially used over in-situ experiments because they offer limited logistical constraints and results are often assumed to accurately represent those obtained *in-situ*. Our results show that *ex-situ* studies are highly reproducible between parallel studies conducted *in-situ*. We therefore can conclude that for low MW C compounds, *ex-situ* experiments looking at their relative contribution to soil respiration do accurately represent *in-situ* processes. This is only true for *in-situ* conditions at the time soil was collected for *ex-situ* experiments. Further research is required to investigate how this may change seasonally throughout the year when conditions are likely to differ between *in-situ* and *ex-situ* conditions i.e. during winter. In addition quantifying C fluxes based on initial concentrations of substrates present in soil solution will help accurately determine terrestrial C fluxes, which in turn can help

validate future climate models. It would also be interesting to look at the influence of cropping and/or management systems to see what impact these may have on the mineralization rates of individual DOC components. Investigating how mineralization rates compare between top and subsoil depths would also be interesting especially with regards to the C storage potential of subsoils. Studies suggest that topsoils store 615 Gt C within the top 0.2 m, whereas the subsoil (up to 3 m depths) can store 2344 Gt C (Fontaine et al., 2007). How this vast subsoil C store may respond to global change is not yet well understood but has the potential for having a large impact on terrestrial CO_2 efflux. In addition, we have looked directly at the mineralization of low MW DOC components, but it would be interesting investigate the link with upstream processes by looking at the breakdown of high MW C components into low MW fractions e.g. enzymatic breakdown of polymers such as cellulose into sugars

4.2. Climatological influences on soil CO_2 measurements

Validation of global climate models and the formulation of effective mitigation/adaptation strategies are hampered by a lack of clear understanding about how terrestrial carbon dynamics influence global climates. Predicting ecosystem responses to climate change remains difficult partly because of poor understanding of fundamental soil-microbial-plant processes in terrestrial environments.

We show from an agricultural soil, that that the contribution to soil CO_2 efflux from individual low MW DOC components are variable between different years when measured *in-situ*. This emphasizes the need for more detailed studies to be conducted looking at how the mineralization rates of DOC components are likely to alter from year to year. In order to predict the consequences of climate change on C dynamics and any potential feedbacks, there needs to be an improved understanding on how mineralization

rates fluctuate on a diurnal, seasonal and yearly scale *in-situ*, across different ecosystems. Further research also needs to be conducted looking at how the individual concentrations of DOC components change throughout the year and mineralization rates quantified accordingly to account for this. It is important to attempt to link mineralization results back to exudation rates and how the mix of different compounds exuded may differ during the year. Identifying the source of the low MW compounds would also be useful as to whether the compounds originate from root or litter turnover or directly through root exudation. This will also be dependent upon the different plant species present in the system being studies. This would help to examine the contribution of the soil microbial community to overall soil respiration.

In-situ measurements are valuable for directly observing natural responses to changing climatic variables. Results can then be extrapolated and used for attempting to predict how these systems could respond to future projected changes. The high Arctic tundra provided a relatively pristine environment in which to directly monitor the response of vegetation and soil microbial communities to snow melt and what is the main driver of this response. We show temperature and nutrient availability to be the controlling drivers regulating the vegetation and soil microbial response during snow melt. However, we postulate that C dynamics may initially be controlled by temperature changes but then this is likely to be replaced by soil water content being the more influential driver as the growing season progresses. Future studies, incorporating advanced genetic analyses such as metabolomics, proteomics and transcriptomics would provide a more detailed study looking at how the activity of the microbial populations is affected during snow melt, such as those studies conducted by Mackelprang et al., (2011) who conducted a metagenomic analysis of permafrost microbial populations and found a rapid response to thaw. The genetic technique we utilised (16S rRNA) included both living and dead cells and so future work could attempt to separate living and dead

cells using more detailed RNA techniques and including a greater number of species to quantify. Investigating the role of mycorrhizae in C turnover would also be interesting to look at because they provide a direct link between vegetation and soil microbial communities. Detailed studies looking into the effects of extending the growing season would be useful to examine if priming effects, via the prolonged release of root exudates in the soil, potentially increasing decomposition rates, or if there was the potential for sequestration of C. The use of these advanced techniques would provide a more comprehensive understanding of how microbial populations use particular substrates for metabolism and whether the activity of microbial communities could be influenced by changing climatic variables. In addition longer term in-situ studies would be useful to integrate the response of plant and microbial communities over several seasons.

4.3. Pedological influences on soil CO_2 measurements

Climate change strongly influences soil properties which in turn can affect soil respiration measurements. Two important variables are soil temperature and soil water content. Results from the *in-situ* measurements of the vegetation and soil microbial response to snow melt showed temperature to be one of the main influential drivers. Our laboratory results also show that C cycling is likely to be regulated by soil temperature at the start of the growing season. This is particularly relevant for the cycling of low MW DOC components which are likely to be released as the soil thaws releasing a flush of nutrients potentially remaining from the previous season. Soil temperature also affected high MW C components, but showed a greater sensitivity to changing soil water content. We postulate that decomposition of organic matter may be more sensitive to soil water changes compared to temperature. As the growing season commences, soil

temperature appears to be the most important regulator of responses as seen from *in-situ* observations and the mineralization results of low MW C compounds. However, over time, temperature no longer drives the response and soil water content becomes more important in regulating C dynamics. Future research should look at the interactive effects of these two important variables by conducting a simultaneous study combining soils held at different soil water content whilst under different temperatures. This could also be combined with metabolomic work looking at whether there is a shift in the metabolic activity of the microbial populations under the different conditions. The use of 454 pyro-sequencing would help give an insight into community shifts of microbial communities and provide more detailed results than PLFA analysis. In addition, looking at shifts in microbial community structure and activity between aerobic and anaerobic metabolism would also be interesting to investigate.

Soil pH was another soil property we investigated. Our results showed that the mineralization rates of different low MW DOC compounds were not affected by changing soil pH. However, the composition of the soil microbial community did alter with pH as detailed from PLFA analysis. Therefore, looking at community composition alone is of limited use when looking at predicting C dynamics. As already mentioned, the incorporation of more detailed genetic approaches (e.g. metagenomics, proteomics and transcriptomics) involved specifically in looking at how microbial communities metabolise and utilize specific C compounds will be a more useful approach for developing our understanding of how C is cycled. Improving our understanding of C dynamics by quantifying a metabolic response to direct stimuli, such as changing climatic or pedological variables will be invaluable in helping to validate future models looking at how C fluxes may be influenced by future ecosystem changes, either from natural or anthropogenic sources.

4.4. Mathematical modelling of soil CO_2 measurements

The application of exponential decay curves to soil respiration data helps to provide a more quantitative and mechanistic understanding of the processes involved in soil C cycling, and enables predictions to be made regarding how ecosystem functioning is likely to be affected by climate change (Ostle et al., 2009). We show that results from soil CO_2 evolution data serves well as a proxy for substrate mineralization. However, to aid our understanding, the model outputs must represent biologically functional pools and currently we can only infer how these pools are assigned. Our results show for short-term experimental data (< 2 d), with limited data points (< 8 time points), then a simpler model (first-order double exponential decay model) best fits the data and this would relate to 2 biological pools. However, for longer term experiments, with more data points, we suggest a more complex model (first-order triple exponential decay model) is required and provides the best fit to the data. This more complex model describes in more detail how some substrates are compartmentalized during their metabolism. Our data did not provide conclusive evidence supporting biological justification of the inferred pools and as such further research is required. This again would come from the use of more advanced molecular and genetic techniques which attempt to identify not only the exact composition of the microbial community but also identify active metabolic pathways helping us understand how substrates are preferentially utilised within the microbial biomass. In addition, the use of first-order exponential models do not account for interactions between different pools and so these models may provide a snapshot as to how substrates are metabolised, however, more detailed models are required to more realistically represent how microbial communities cycle C.

5. Summary

Within this thesis we aimed to investigate how the microbial metabolism of specific components of DOC contributes to overall soil respiration. We show that results from the mineralization of individual DOC components can be used to extrapolate larger-scale CO_2 fluxes from an agricultural system. We combined this data with known concentrations of the specific compounds measured to account for the relative proportions of each substrate contained with soil solution. Model parameters from the exponential models were incorporated with soil properties in order to attempt to measure the microbial contribution to overall soil CO_2 efflux. Our estimates show that the microbial contribution to respiration is approximately 18%, suggesting the remaining contribution is from root respiration, or from substrates not including within this thesis. Further work investigating this flux is required to corroborate our measurements. Accurate measurements of individual DOC components are essential in order to ascertain the most reliable estimate of total C flux.

This thesis helps improves our understanding of the complex soil-microbial-plant processes involved in C cycling. We have focussed on investigating how microbial populations cycle macro-nutrients such as C and looked at how changes to specific soil properties can influence the C dynamics. This thesis provides the platform for further studies utilising advanced molecular techniques to help decipher how substrates are metabolised within the microbial biomass. We suggest that combining experimental biological data with detailed mathematical models describing the kinetics (including pool interactions) of substrate metabolism will enhance our understanding of the complex interactions that exist within terrestrial systems. This improved understanding of what happens on the micro-scale is essential for validating larger-scale climate models used for predicting how regions/ecosystems may respond to future

climate and/or land-use change scenarios. This also has important implications for helping inform climate policy makers on formulating effective mitigation/adaptation strategies. This could include selecting specific plants which favour soil C sequestration, e.g. grassland forbs and legumes. These plants increase the quality of plant litter therefore promoting habitats for macrofauna (i.e. earthworms and ants) which aid in improving soil structure, increase the amount C bound within soil aggregates and enhancing nutrient mineralization (De Deyn et al., 2008). In addition, root exudates can also help form soil aggregates via interactions with soil minerals, especially clay particles. Other plant traits can also help promote this interaction between soil minerals and root exudates compounds, such as deep-rooting, root density and branching capabilities (De Deyn et al., 2008) can all increase exudation all of which are important factors influencing soil C sequestration.

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