DOCTOR OF PHILOSOPHY

Impact of climate change on belowground carbon storage in the Arctic and UK

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Award date:
2008

Awarding institution:
Bangor University

Impact of Climate Change on Belowground Carbon Storage in the Arctic and UK

A thesis submitted to the University of Wales by Elizabeth Boddy in candidature for the degree of Philosophiae Doctor

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March 2008

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Summary

Despite the large quantity of research on the sensitivity of soil organic matter (SOM) degradation to temperature change, there is still no consensus as to whether soil efflux of CO_2 will increase with elevated temperatures. Understanding the mechanisms that control CO_2 efflux from the soil is therefore critical for predicting the response of ecosystems to climate change, particularly in polar regions where warming is occurring at rates 2-3 times greater than in temperate regions. This thesis considered two methodologies for evaluating whether soil efflux of CO_2 is driven by recent inputs from plants and, secondly, the degree of temperature sensitivity. Firstly, Part I (Chapter 2) considered basal CO_2 and nutrient limitations in these soils. Secondly, Part II (Chapters 3-6) utilized ^{14}C-labelled glucose and amino acids to assess the mineralization of low molecular weight (MW) C in soils from the UK and Arctic and the temperature sensitivity of mineralization.

UK and Svalbard soils had similar respiration rates per gram of O horizon and metabolism of low MW C with an exponential decay coefficient k_1 averaging < 1.5 h in the laboratory. However, cycling of C through the microbial biomass was significantly slower in Svalbard soils than in UK grassland soils, though when Svalbard soils were incubated at 20 ^{o}C, turnover was similar to UK field rates. Respiration measurements undertaken on an area basis were also significantly different, with the UK having much greater respiration rates, highlighting the importance of the sampling methodology when considering results. Both soils were substrate limited, indicating that respiration rates were dominated by recent contributions of labile C from plants.

Temperature changes did cause an increased respiratory loss of SOM, but not to the magnitude expected with the Q_{10} value being < 2. Temperature dependence of SOM degradation was shown to be C pool dependent; with mineralization of labile substrate-C insensitive to temperature, but microbial biomass-C turnover and mineralization of higher MW SOM fractions increased with elevated temperatures. Partitioning of ^{14}C changed with elevated temperatures, with more C utilized for respiration rather than growth, indicating a possible decrease in efficiency of growth at higher temperatures.

Further research is needed in the temperature-dependence of SOM degradation, particularly turnover of high MW SOM, in the laboratory and field.

Acknowledgements

I would like to acknowledge the following people -

My supervisors Professor Davey Jones and Professor John Farrar for their continual support and guidance throughout my research and for facilitating the two most amazing field trips to Svalbard. Without their help and time I would not have been able to complete this thesis and all their input is gratefully acknowledged and appreciated.

NERC for funding the Ph.D. project and COST 631 for enabling the research visit to Lund University, Sweden.

I am grateful for all the encouragement, support and advice that Dr Paul Hill has given me over the Ph.D., for always finding something positive in my data when I couldn't see the wood for the trees!

Dr Paula Roberts, Jonathan Roberts, Gareth Williams and John Evans for all their technical help and advice.

I am exceedingly grateful for all the help, support and encouragement that my family has given me over the Ph.D., for always being there to support me when I needed some extra encouragement and helping with all life's problems along the way enabling me to focus on my studies.

My friends and colleagues for their interest, support and encouragement; and lastly, Glenn for introducing me to surfing and keeping me calm in the last few months of writing.

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Abbreviations

C – Carbon

ca. - Approximately

CH_4 – Methane

CO_2 – Carbon dioxide

d – Day

DOC – Dissolved organic carbon

DON – Dissolved organic nitrogen

IPCC – Intergovernmental panel on climate change

IRGA – Infra-red gas analyser

g – Gram

h – Hour

ha – Hectare

K_2SO_4 – Potassium sulphate

m – Metre

M – Molar

MRT – Mean residence time

MW – Molecular weight

N – Nitrogen

$NaHCO_3$ – Sodium bicarbonate

NaOH – Sodium hydroxide

NH_4^+ – Ammonium

NO_2 – Nitrous oxide

NO_3^- – Nitrate

OC – Organic carbon

OM – Organic matter

P – Phosphorous

PCA – Principal component analysis

PLFA – Phospholipid fatty acid

ppm – Parts per million

SEM – Standard error of the mean

SIR – Substrate induced respiration

SOM – Soil organic matter

t – Ton

t – Time

y – Year

Chapter 1

Introduction and Literature Review

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1.1. Introduction

1.1.1. General introduction and need for research

The Intergovernmental Panel on Climate Change (IPCC) defined climatic change as a "change in climate over time whether due to natural variability or as a result of human activity" (Houghton et al., 2001). Anthropogenic activities have caused CO_2 concentrations to increase from 260 ppm approximately 150 years ago to 382 ppm by February 2006, which is possibly the highest CO_2 levels have been for several million years. At the accelerating rate of increase in CO_2 concentrations, global CO_2 is expected to reach 700 ppm by the end of the 21st century, and this concentration is likely to have a significant effect on global ecosystems (Nösberger and Long, 2006).

Agriculture is the most common land use. Globally 5×10^{12} ha are under agricultural management and approximately 13×10^{6} ha are converted annually to agricultural use. At present, 69% of agroecosystems consist of pasture (20-40% of the world's land surface; Nösberger and Long, 2006). Thus evaluating the impact of climate change on these ecosystems is of great importance. Whilst polar ecosystems are not significant in terms of provision of food, wood, fibre and renewable energy crops, they are important when considering climate change both because they have large stored C reserves and because they may be especially sensitive to climate change.

Global warming is predicted to be 2-5 ^{o}C by the end of the 21st Century. Direct ecological impacts are to be expected from such a rapid warming, as the coldest period of the last ice age and the interglacial period were between 5 and 6 ^{o}C cooler than current temperatures (Houghton, 1997). Feedback effects are also expected from ecological systems responding to change and generating

additional climatic impacts by modifying carbon cycling, moisture contents and trace greenhouse gases at the earth's surface (Harte et al., 1995). Polar ecosystems are experiencing some of the fastest rates of warming, and are predicted to warm by 0.6 ± 0.2 °C above the global mean. Indeed there is accentuated warming in the Antarctic Peninsula, the Bellingshausen Sea and the Siberian Plateau, and generally across the Arctic region (Vaughan et al., 2003). There is evidence of pole-ward movement of vegetation types during the last glacial period over paleoclimatic scales, but there is no evidence that climate linked soil characteristics or species composition will remain valid over time scales of decades that characterise anthropogenically mediated global warming (Harte et al., 1995). Understanding the mechanisms that control CO_2 efflux from the soil is therefore critical for predicting the response of ecosystems to climate change.

1.1.2. Plan of thesis

The thesis is divided into two parts. Part I focuses on the factors regulating CO_2 efflux from the soil surface, measuring the basal respiration rates of UK and Arctic soils in the field and laboratory and subsequent manipulations to estimate the turnover times of C in UK soils and the C and N limitations of Arctic soils. Part II investigates the fate of low molecular weight (MW) C additions to the soil, their turnover rates and the temperature dependence of turnover.

Each chapter in Part I and II, whilst standing alone, forms an essential part to the thesis continuity with Part I providing an overview into the basal soil respiration in the UK grassland and Arctic tundra soils and the nutrient

limitations of these soils, establishing estimates of C turnover in UK soils. Part II is composed of four chapters written for publication in the journal Soil Biology & Biochemistry and as such there is some unavoidable repetition in the Materials and Methods sections. Part II moves the thesis from the overview of CO_2 efflux to a more analytical approach, considering the turnover of low MW C, in particular the turnover ^{14}C-labelled glucose and amino acids and the fate of the labelled ^{14}C once it has been metabolized by the microbial community. Part II also considers the impact of elevated temperatures on the turnover of ^{14}C in the dissolved organic carbon (DOC) pool and within the microbial community. Thus, though consisting of separate stand-alone chapters the thesis has an overall aim to provide turnover estimates as indicated on Fig. 1.1.

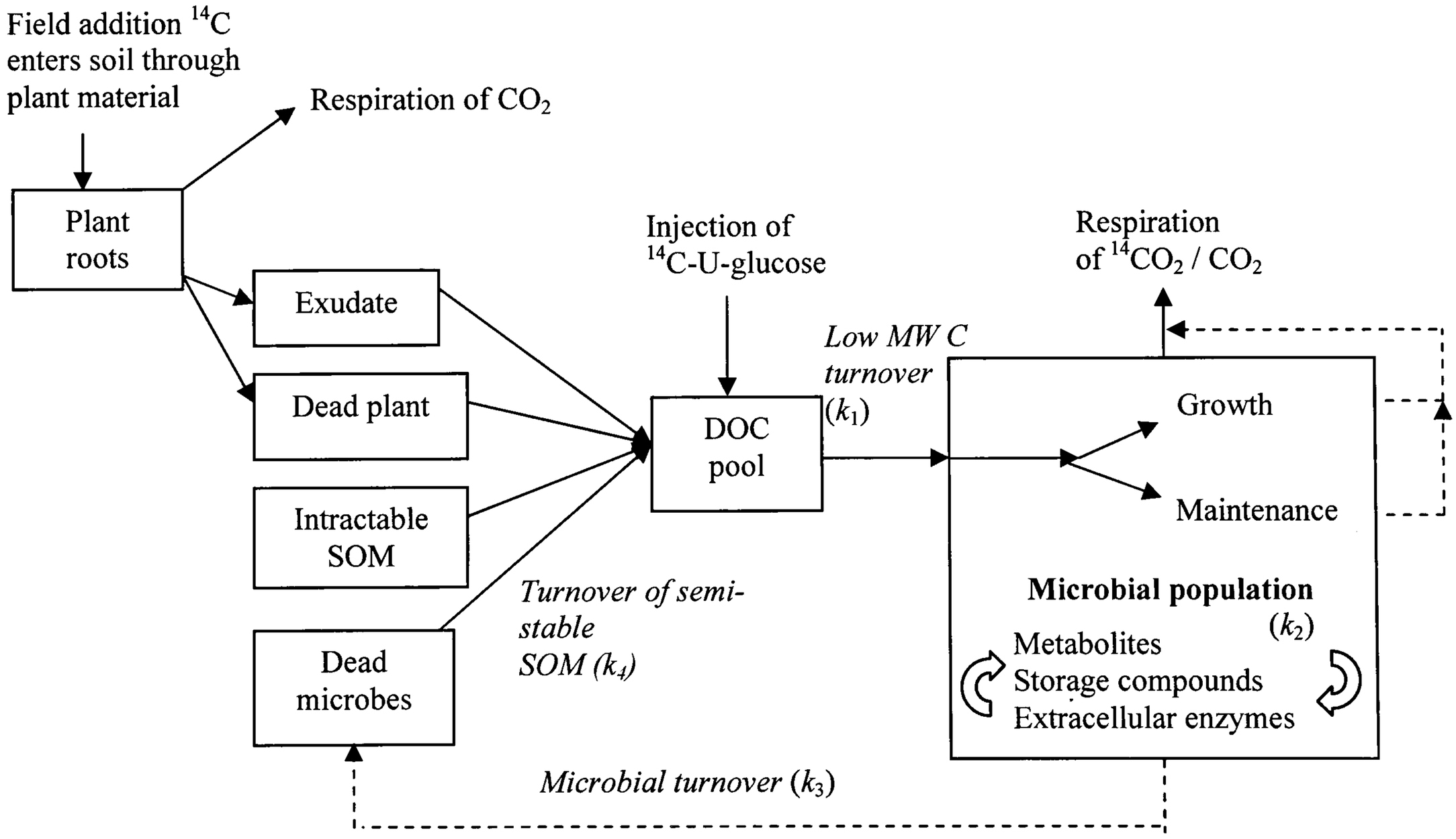

Figure 1.1. Schematic of the controls on CO_2 efflux from the soil surface, with CO_2 measured in the field and laboratory from plant material and the microbial population, identifying the contribution of each to total measured CO_2 efflux (Part I). The fate of added ^{14}C substrate is identified, with substrate added either

to the soluble DOC pool or through labelling of plant material. Also indicated are the suggested location of four temporal soil organic matter (SOM) pools described by the exponential coefficients k_1, k_2, k_3, and k_4, the values of which are calculated in Part II. A fifth, less important pool, is the turnover of recalcitrant SOM taking many thousand years (identified in Part I).

1.1.3. Aims and objectives

Climate change is expected to have a profound effect on the way in which global ecosystems behave. To understand how fast they will respond to our changing climate, however, requires that we understand plant and soil responses to increased temperature and CO_2 across a broad range of ecosystems. The aim of this PhD studentship was to investigate the factors that regulate CO_2 production in soils from the UK and the Arctic evaluating the hypotheses that soil respiration is governed by recent inputs from plants, and that rising temperatures will result in increased respiratory loss of soil organic carbon (OC). To achieve this overall aim the thesis has 8 specific aims, an overview of which is given in this introduction and are discussed in greater detail in each subsequent chapter:

1) to quantify rates of basal CO_2 efflux in the UK and Arctic field sites (Chapter 2).
2) to establish whether there are different pools contributing to the efflux of CO_2 from the soil (Chapter 2).
3) to investigate substrate limitation on UK and Arctic field sites (Chapter 2).
4) to determine the rate *in situ* and in the laboratory of low MW DOC mineralization in UK grassland soils and in the laboratory for Arctic soils (Chapter 3 and 4).

5) to assess the response in metabolism of low MW C to short-term temperature change (Chapter 4).
6) to consider whether short-term temperature change affects gene expression in the microbial community and soil solution chemistry, thus affecting the functionality of the soil community (Chapter 5).
7) to assess the decomposition of SOM during long-term temperature change (Chapter 6).
8) to evaluate whether priming will cause an increase in the decomposition of high MW C at elevated temperatures (Chapter 6).

1.2. Carbon in soil and respiration

Tundra and boreal forest ecosystems contain 27% of the Earth's soil C pool, and Arctic ecosystems store 11% of terrestrial SOM (Grogan and Jonasson, 2005; Schimel and Mikan, 2005). The world's soils contain about 1500 Gt C in the top 1 m and 2000-2400 Gt C to a depth of 2 m (Kirschbaum, 2004). Consequently, even a small change in the release of stored OC in soil could constitute a large positive feedback effect with the extra carbon adding to the CO_2 present in the atmosphere (Fig. 1.2; Schimel et al., 1990).

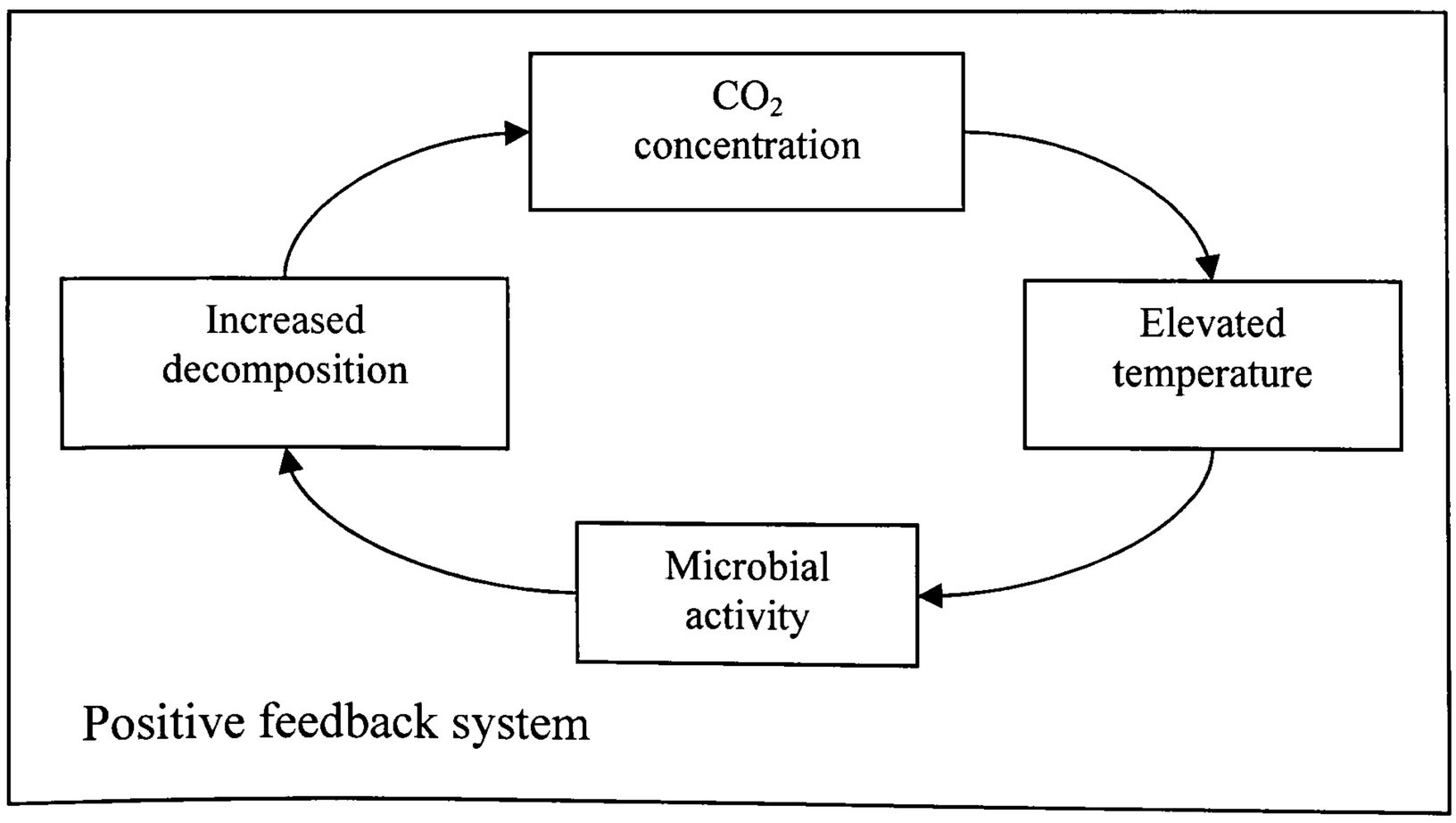

Figure 1.2. Schematic of a positive feedback system for stored OC in soil affected by elevated temperatures enhancing microbial degradation.

Global C storage has steadily increased since the last glacial maximum 18,000 years ago (Kirschbaum, 2000). C storage across climatic gradients suggests a strong effect of temperature on soil OC and N. Moisture limitation is also a key factor in the response of soil OC to temperature change. Organic N availability decreases with decreasing temperatures. Soil OC demonstrates a far greater dependence on temperature than does net primary productivity. The decomposition of SOM is a function of both physical and chemical environmental variables and the composition of the SOM (Subke et al., 2003). Although most newly added OM can be readily decomposed over a few years the remainder becomes part of the inert C pool, which can accumulate over many years. This C forms a sink within the biosphere around which much of the uncertainties over climate change are focused.

Soil C turnover can be calculated from soil respiration rates giving the mean residence time (MRT) of the soil C pool (Table 1.1; Raich and Schlesinger, 1992). Table 1.1 averages all C pools. In most soils the pool of OC consists of a small pool near the surface of DOC and labile C (low MW C) with a rapid turnover, and larger heterogeneous pools of medium, high MW and recalcitrant C (Fig. 1.1) that are dispersed throughout the lower profile. Turnover times generally increase with depth, reflecting the abundance of the different forms of detritus. In a New Zealand soil a study indicated that most of the SOM was <100 years old, but the lower profile contained 16% which was at least 5700 years old (Raich and Schlesinger, 1992).

Table 1.1. Estimated turnover time of soil carbon based on mean pools and mean soil respiration (SR) rates, using an average of 30% for the contribution of roots to total CO_2 efflux (Raich and Schlesigner, 1992).

Vegetation type	**Soil C ($kg\ m^{-2}$)**	**SR ($g\ C\ m^{-2}\ y^{-1}$)**	**Turnover (y)**
Tundra	*20.4*	*60*	*490*
Boreal forests	20.6	322	91
Temperate grasslands	*18.9*	*442*	*61*
Temperate forests	13.4	662	29
Woodlands	6.9	713	14
Cultivated lands	7.9	544	21
Desert scrub	5.8	224	37
Tropical grasslands	4.2	629	10
Tropical lowland forests	28.7	1092	38
Swamps and marshes	72.3	200	520
Global total	**1515 Pg C in soil**	**68 Pg C y^{-1}**	**32**

Gainey first made measurements of soil respiration in 1919 (Schlesinger and Andrews, 2000). Since then there has been an exponential rise in measurements of soil greenhouse gas fluxes. Soil respiration contributes a major component of the terrestrial global C cycle. Most of the C bound within the biosphere is found in the soil and there is a general trend of increasing C storage with decreasing annual average temperatures (Subke et al., 2003). CO_2 is produced in soils by autotrophic root respiration and from heterotrophic microbial respiration (discussed in greater detail in Chapter 2) and, to a small extent, by the chemical oxidation of carbon-containing materials (Raich and Schlesinger, 1992). CO_2 is released from the soil in the process known variably as soil respiration, soil-CO_2 evolution, or soil-CO_2 efflux. The contribution of roots to net respiration measured at the soil surface is generally around 50% (Raich and Tufekcioglu, 2000; Lee et al., 2003). The rate of this efflux is controlled by the rate of production, the strength of the concentration gradient between soil and atmosphere, and soil properties – pore size, air temperature and

wind speed. Changing climate may affect soil structure particularly in northern temperate, tundra and boreal ecosystems where a warmer and in northern temperate a wetter climate may increase the loss of OC from the soil potentially leading to a loss in aggregate stability (Young et al., 1998).

Warming is likely to result in an increase in respiratory losses of C, which may be greater than the gains of C into the system from the increase in net primary production (Saleska et al., 1999). Soil OC averages from 50 to 150 t ha^{-1}, but this varies greatly. In polar regions there can be as much as 80 t ha^{-1}, compared to arid climates where there can be as little as 30 t ha^{-1} (Lal, 2004). The critical factors influencing soil respiration are primarily temperature and moisture content, followed by vegetation and substrate quality, net ecosystem productivity, relative allocation of net primary productivity to above and below ground vegetation, population and community dynamics of above and below ground flora and fauna (Table 1.2; discussed in more detail in Chapter 2; Rustad et al., 2000).

Table 1.2. The controls upon carbon sequestration in soils (Ward and Strain, 1999).

Molecular and Physiological Controls on Carbon Sequestration	Ecological Controls on Carbon Sequestration
A. Molecular responses	**A. Primary organism interaction**
1. Gene transcription	1. Plant-plant
	2. Plant-animal
B. Primary physiological responses	3. Plant-microbes (disease, decomposition, symbiosis)
1. Photosynthesis	
2. Photorespiration	
3. Dark respiration	**B. Secondary organism interaction**
4. Stomatal regulation	1. Evolutionary responses and genetic differentiation
C. Secondary physiological responses	
1. Photosynthate concentration	**C. Tertiary ecosystem responses**
2. Photosynthate translocation	1. Integration of all effects through time
3. Plant water status	
D. Tertiary whole plant responses	
1. Growth rate	
2. Growth form	
3. Reproduction	
4. Phenology (development rate)	

1.3. Carbon pools and cycling

The IPCC shows soils to be net sequestering of C (Fig. 1.3). The total global emission of CO_2 from soils is now recognised as one of the largest fluxes in the C cycle, with small changes in the magnitude of soil respiration potentially having large effects upon the total concentration of CO_2 in the atmosphere. It is estimated that there are 1500×10^{15} g C contained within soils, of which 75×10^{15} g C is released annually; an order of magnitude higher than emissions from anthropogenic activity (Winkler et al., 1996; Rustad et al., 2000; Sjögersten and Wookey, 2002). The flux of CO_2 is closely associated with plant growth, which provides organic residues for microorganisms. CO_2 flux from soils indicates an average residence time (mass/flux) of 32 years for C in SOM. This differs widely

between regions, the greatest rates are in the tropics (Schlesinger and Andrews, 2000).

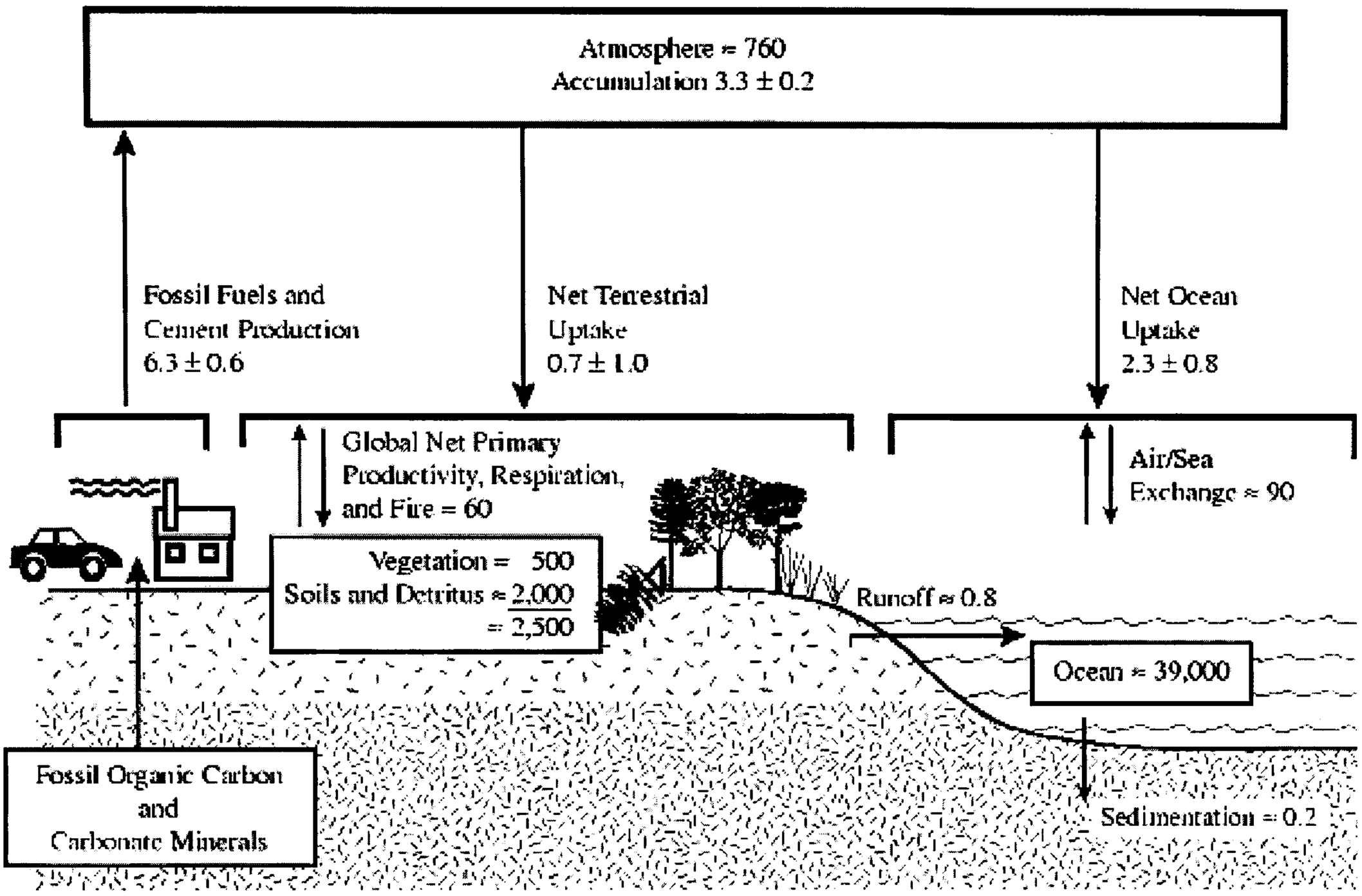

Figure 1.3. Diagram of the carbon cycle, indicating that second only to the oceans the majority of C is stored within terrestrial ecosystems (Gt C). Terrestrial ecosystems are currently sequestering C (Houghton et al., 2001).

The flow of C through soil is controlled by many physical, chemical and biological factors (Table 1.2 and Fig. 1.4). Temperature affects plant growth and so the inputs of C into soil. Changes in root turnover are likely to influence the sequestration of below ground C (Paterson et al., 1997); altering plant inputs (addition of leaf matter, exudation and secretion from roots, sloughing and root turnover of structural compounds) through climate change affects the SOM and the microbial communities. Soil OC displays a large degree of heterogeneity in the turnover of its different pools, with turnover times estimated to range from

0.5 h to 10,000 y (Schlesinger and Andrews, 2000; Knorr et al., 2005; Boddy et al., 2007). The dynamics of soil OC is often modelled as a system of just two pools, a fast and slow pool (Kirschbaum, 2004). In Fig. 1.4 the slow (or high MW C) pool would be the solid C pool and the fast (or labile) pool the soluble C pool.

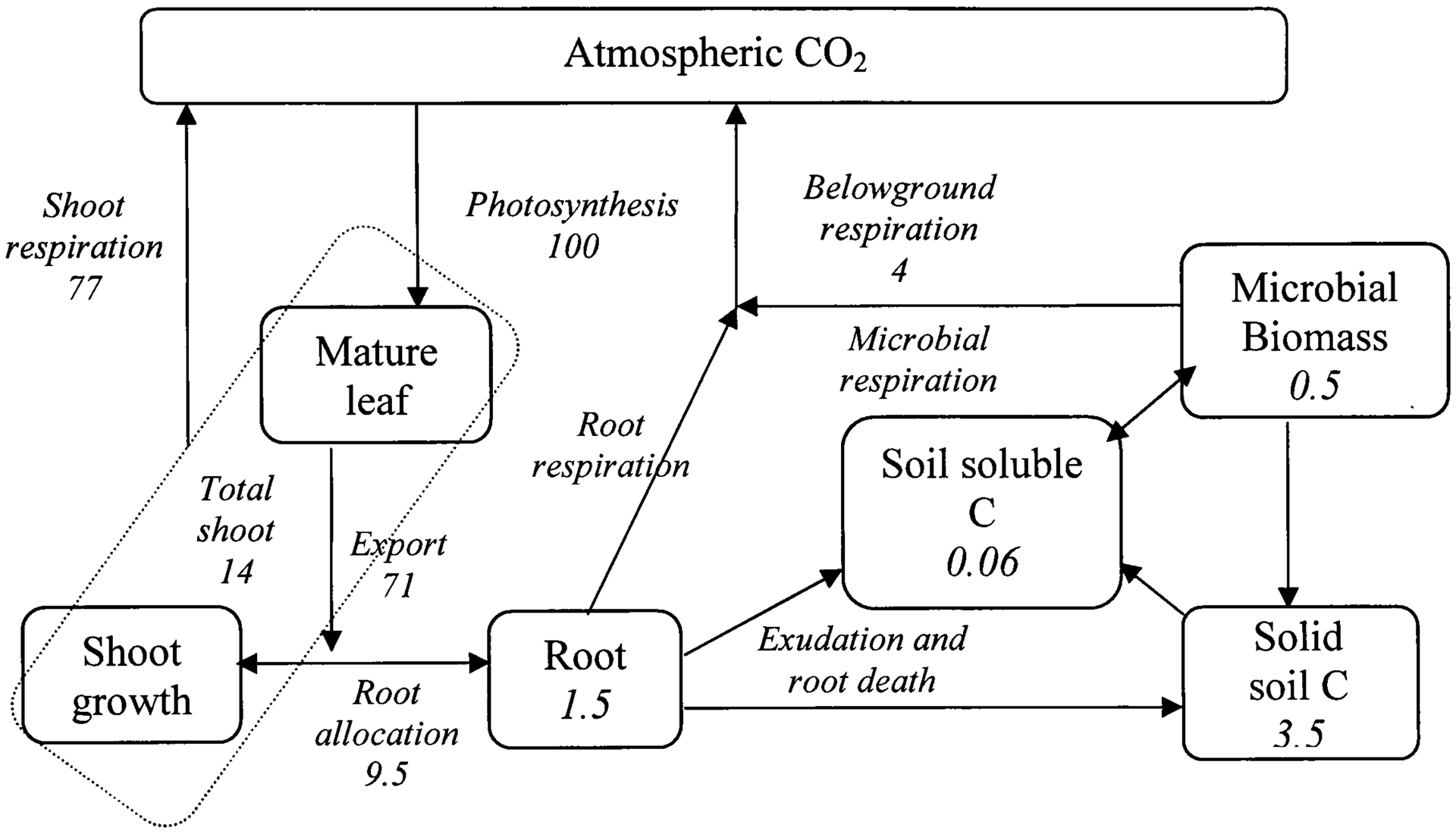

Figure 1.4. The carbon cycle following the fate of net photosynthetically fixed C (100% of available C) in *Lolium perenne* grassland. The diagram illustrates that the soil soluble C pool is a vital step in the degradation of stored OC and in the movement of labile C through soil in the form of root exudates and microbial turnover (Hill et al., 2008).

80-90% of the processes in soil are mediated by microbes (Nannipieri et al., 2003). Soil is a structured, heterogeneous and discontinuous system, generally nutrient poor and energy limited, with microorganisms living in discrete microhabitats. Generally less than 5% of the total available space is occupied by microorganisms. Despite this, the microbial biomass is large in numbers and extremely diverse, being estimated to be 1-2 t ha^{-1} bacteria and 2-5 t ha^{-1} fungi, with microbial C contributing only 1-5% of total soil OC (Hu et al.,

1995; Nannipieri et al., 2003). Rhizosphere communities (the microorganisms in habitats around roots) provide a vital role in nutrient cycling and perturbations in their activity and composition are likely to affect ecosystem function. Roots release exudates into the soil, which can affect rhizosphere processes in several ways, notably they can modify the solubility, sorption, and transport of mineral elements to the roots through changing pH, redox potential and organic complexation (Kuzyakov et al., 2003). Root exudates provide the main source of labile substrate for the microbial biomass, dominated by sugars and amino acids (Table 1.3; discussed in further detail in Chapters 2-6; Farrar et al., 2003; Pelz et al., 2005).

Table 1.3. The composition of root exudation; sugars and amino acids dominate root exudation (Jones, 1993).

Compound	**Rate of C loss µg C g $^{-1}$ root d^{-1}**	**Plant species**
Mucilage**	34000	Bean
Lactic acid*	19458	Maize
Sugars	*7880*	*Maize*
Amino acids	*123*	*Maize*
Organic acids	104	Maize
Phenolics	86	Wheat
Sterols	12	Oil seed rape
Vitamins	6	Maize
Flavonoids	3	Bean
Growth hormones	0.07	Peanut

*anaerobic conditions
**root tip only

Microorganisms form a dynamic and vital pool of SOM, responsible for the cycling of nutrients from stored OC through the DOC pool (Fig. 1.4; Nguyen and Henry, 2002). Although the DOC pool is very small the flux through the pool is extremely rapid (van Hees et al., 2005; discussed in greater detail in

Chapters 2-6). The low MW DOC pool provides the link between the breakdown of high MW microbial and plant necromass and recalcitrant SOM in the soil. Indeed the rate at which the microbial community can cycle higher MW C compounds limits the productivity of the system and thus controls respiration (Toal et al., 2000; Nguyen and Henry, 2002). In general, the availability of soluble C and N, nutrients, water and temperature governs the activity of soil microbes (discussed in greater detail in Chapter 2; Blagodatsky et al., 1998; Galicia and Garcia-Oliva, 2004; Pennanen et al., 2004).

1.4. Temperature dependence of carbon turnover in soil

As discussed in detail in Chapters 4, 5 and 6, global warming has wide-ranging implications for soil microorganisms and stored C. The impact of temperature on soil and respiration is not a new concept; indeed Deherain and Demoussey studied the impacts of temperature in 1896 (in: Bunt and Rovira, 1955). Under cold climates the soil's rates of chemical and biological reactions are slow; limiting the rate at which nutrients are made available. Adsorption and transport of nutrient ions by higher plants is also inhibited under lower temperatures (Brady and Weil, 1999). Decomposition of SOM by microbes is strongly limited by temperature, which has implications for nutrient cycling, affecting the release of N, and thereby reducing productivity of boreal systems. Paleoecological evidence suggests that the tree line in Northern Sweden extended 300-400 m above its current position, which corresponded to a 1.5-2 ^{o}C warmer summer temperature (Sjögersten and Wookey, 2002).

Trends in global soil respiration show that mean air temperature was the single best predictor of soil respiration rate when net primary production is

compared to soil respiration (Raich and Schlesinger, 1992). The majority of research focuses on the temperate latitudes (between 30 and 60° latitude) with boreal, arid and tropical regions providing little, variable, data (Raich and Schlesinger, 1992). Winkler et al. (1996) also correlated soil respiration with temperature, moisture content and nutrient status. The dependence of respiration upon temperature can be simply defined by first order rate kinetics, where the change in OM over time is linked to a rate constant specific to each soil type (Eqn. 1.1). This equation over-simplifies the system as SOM is not homogenous and the different fractions are degraded at different rates,

$$dx/dt = -kx \qquad \text{(Eqn. 1.1)}$$

where k is the rate constant, x is the SOM content, and t is time.

Increases in soil temperature will result in an increase in microbially mediated reaction rates, provided adequate water is available (Mosier, 1998). Temperature effects upon soil respiration are often described using Q_{10}, Arrhenius equations, which is the difference in respiration over a 10 °C interval. There are a variety of calculations that can be used to ascertain Q_{10}. However, Fang and Moncrieff (2001) define Q_{10} by the simple calculation (Eqn. 1.2),

$$Q_{10} = \frac{R_{T+10}}{R_T} \qquad \text{(Eqn. 1.2)}$$

where R_T is the respiration rate at each temperature.

The Q_{10} of soils is generally assumed to be about 2 (Nicolardot et al., 1994; Bekku et al., 2003). However, the value of Q_{10} needs to be used with care in soil with its large number of microorganisms and an array of different

compounds, each with their own enzymatic reactions and activation energies (Davidson and Janssens, 2006; see Chapters 4-6 for a detailed discussion).

The temperature sensitivity of decomposition of SOM is complex. Conceptual models describing microbial decomposition of SOM range from one pool to 200 pool models (Giardina and Ryan, 2000; Blagodatsky and Richter, 1998). The diverse range of soil OC compounds has different temperature sensitivities. Temperature-dependent labile and recalcitrant components of SOM have been reported in soil (Nicolardot et al., 1994; Barrett et al., 2006; see Chapters 4-6 for discussion). Thus it is important that the different soil pools and compounds are considered in isolation, as averaging temperature dependencies by methods such as the Q_{10} can mask the temperature response of individual pools (Davidson and Janssens, 2006).

Temperature is highly variable over short time periods; the greatest fluctuations are at the soil surface, with damping effects and lag periods increasing with depth (Subke et al., 2003). The organic layer is exposed to the largest variation and contains the most significant pool of easily decomposable OM. Temperature at the surface is subject to both diffuse and direct radiation, with the direct radiation causing the most extreme surface peaks in temperature. Smith et al. (2003) indicated that below 10 cm, diurnal fluctuations are 20% less than at the surface. Organic soils are far more effective than mineral soils at damping the diurnal effects due to thermal conductivities and diffusivities between ¼ and ⅛ of a mineral soil.

The Arctic was long thought to be a strong sink for C because of the low temperatures and poor drainage limiting the rates of decomposition, however, the current sink or source status of the Arctic is unknown (Welker et al., 2000;

Mikan et al., 2002). The failure to include winter respiration has also led to incomplete, and great uncertainty in, C budgets. Respiration can continue in frozen soils, and although the rates are low the accumulated winter CO_2 flux can contribute significantly to the total annual C budget (Welker et al., 2000; Mikan et al., 2002). Decomposition of older more recalcitrant SOM may be more temperature dependent that that of labile OM (Mikan et al., 2002). Mikan et al. (2002) established two different gradients for respiration response to temperature change above and below 0 °C (with a Q_{10} of 4.6-9.4 above freezing and 63-237 in frozen soils). These gradients are associated with changes in activation energy. The Q_{10} is inappropriate for describing the temperature dependence of respiration under these cold climates. High Q_{10} values can be attributed to the withdrawal of available water – in that water participates directly in numerous metabolic reactions.

The duration of the effects of elevated temperature are a matter of discussion. Acclimation has been observed in warming studies lasting several years after an initial increase in soil SOM decomposition (Knorr et al., 2005; Davidson and Janssens, 2006). Respiration rates in plants have also acclimated to changes in temperature over periods of days to months (Wythers et al., 2005). Thus there is a need to investigate the response of soil to temperature change over a range of time scales (see Chapters 5 and 6 for a detailed discussion). The temporal pattern could be due to the depletion of labile substrate at higher temperatures, due to the slow turnover of higher MW C (Kirschbaum, 2004).

1.5. Summary

It is clear that SOM is a complex and dynamic system; the temperature dependency of its degradation has been described variably from one pool to 200 pool models (Giardina and Ryan, 2000; Blagodatsky and Richter, 1998). Understanding the mechanisms controlling the production of CO_2 from soils both in the UK and the Arctic, evaluating experimentally viable numbers of SOM pools, and investigating the impacts of temperature change on these pools is critical for predicting the response of ecosystems to climate change.

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Part I

CO_2 efflux from UK and High Arctic soils

This thesis section focuses on the total CO_2 efflux from soil, and considers the C and N limitations of microorganisms in the laboratory. The aim of Part I is to establish the background characteristics of the soils studied in later chapters and to gain insight into the underlying mechanisms controlling soil respiration (Fig. I.1).

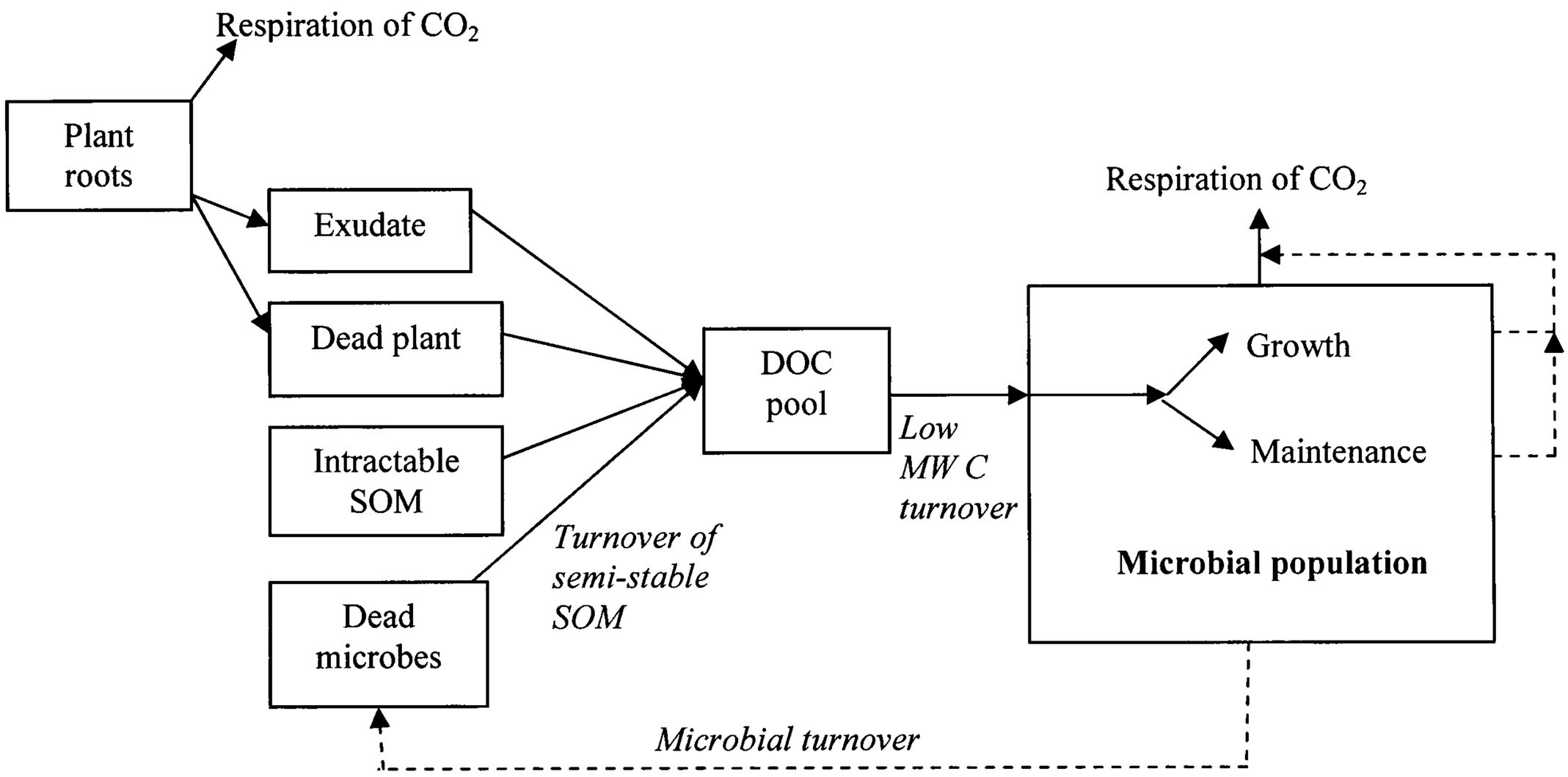

Figure I.1. Schematic of the major controls on CO_2 efflux from the soil surface, indicating the three main modes of C turnover, namely that of low molecular weight (MW) C turnover, microbial biomass turnover and the turnover of the semi-stable soil organic matter (SOM) pool; a fourth pool of lesser importance for short term C fluxes is the turnover of intractable SOM.

Chapter 2

Basal CO_2 efflux from UK grassland and High Arctic soils: Contribution of different organic matter pools and C and N limitation

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2.0. Abstract

Understanding the mechanisms that control CO_2 efflux from soil is critical for predicting the response of ecosystems to climate change. Polar ecosystems are experiencing some of the fastest rates of warming and so it is vital to understand the factors regulating C turnover. The primary aim of this study was to establish the basal CO_2 efflux from UK grassland and Arctic tundra soils, secondly, to establish whether respired CO_2 depends on a number of different C pools, and thirdly to investigate C and N substrate limitations in Arctic soils. To address this, respiration measurements were undertaken in the field and laboratory for soils from two Arctic sites (Zeppelinfjellet and Stuphallet) and compared to a UK grassland Eutric Cambisol. Following on from this, three UK grassland soils (cambisol, gleysol and podzol) were sampled and intact cores, sieved soil, excised shoots and root respiration monitored for >1 y at 10 ± 1 °C. Lastly to investigate C and N limitation, glucose, glutamate and inorganic N were added to topsoil, subsoil and lichen-modified soil from the two Arctic field sites and the impact on CO_2 efflux measured. Arctic basal respiration measured in the field (0.3 ± 0.1 g CO_2 m^{-2} h^{-1}) was much smaller than UK grassland soil (1.1 ± 0.3 g CO_2 m^{-2} h^{-1}), however, respiration measured in the laboratory at 10 °C was similar in soil from both locations (ca. 11 ± 1 μg CO_2 g^{-1} h^{-1}). Measuring the decline in CO_2 efflux over time from the components of grassland soils identified three different C pools, firstly, that of the metabolites and storage compounds, with a mean residence time (MRT) < 50 d; secondly, the microbial biomass pool, with a MRT of < 1.5 y; and lastly the stable soil OM pool with a turnover in excess of 5,000 y. The addition of both organic C, and inorganic N increased CO_2 efflux, though the greatest response was observed

after the addition of low MW C, indicating that the Arctic microbial community is predominantly C-limited.

Keywords: Carbon cycling; Dissolved organic carbon; Methodology; Mineralization; Soil CO_2 efflux; Soil organic matter

2.1. Introduction

2.1.1. Soil respiration – its nature and properties

Soil CO_2 fluxes originate from both autotrophic root respiration and from heterotrophic microbial respiration occurring in the rhizosphere and the bulk soil. Total belowground respiration is therefore ultimately dependent on aboveground plant productivity, and more immediately upon the respiration of roots and the soil microbes, which indirectly obtain substrate via rhizodeposition and from the turnover of soil organic matter (SOM) pools (Fig. 2.1; Ward and Strain, 1999). Soil autotrophic and heterotrophic respiration may react differently to changes in environmental conditions, such as global warming. Consequently, if we are to accurately predict ecosystem responses to environmental perturbation such as climate change it is crucial that we gain more insight into both components of soil respiration (Buchmann, 2000). As a proportion of total respiration, root respiration typically contributes approximately 50%, though this is highly biome dependent, with roots contributing up to 93% in Arctic tundra (Raich and Tufekcioglu, 2000; Lee et al., 2003). The flow of C through soil depends on many physical, chemical and biological factors. Plant inputs to the soil are varied, including inputs of leaf litter, exudation from roots, sloughing of cells and root turnover (Paterson et al., 1997). Root exudates can modify the soil by

altering solubility, sorption and transport of nutrients to the roots via changing pH, redox potential and organic complexation (Kuzyakov et al., 2003).

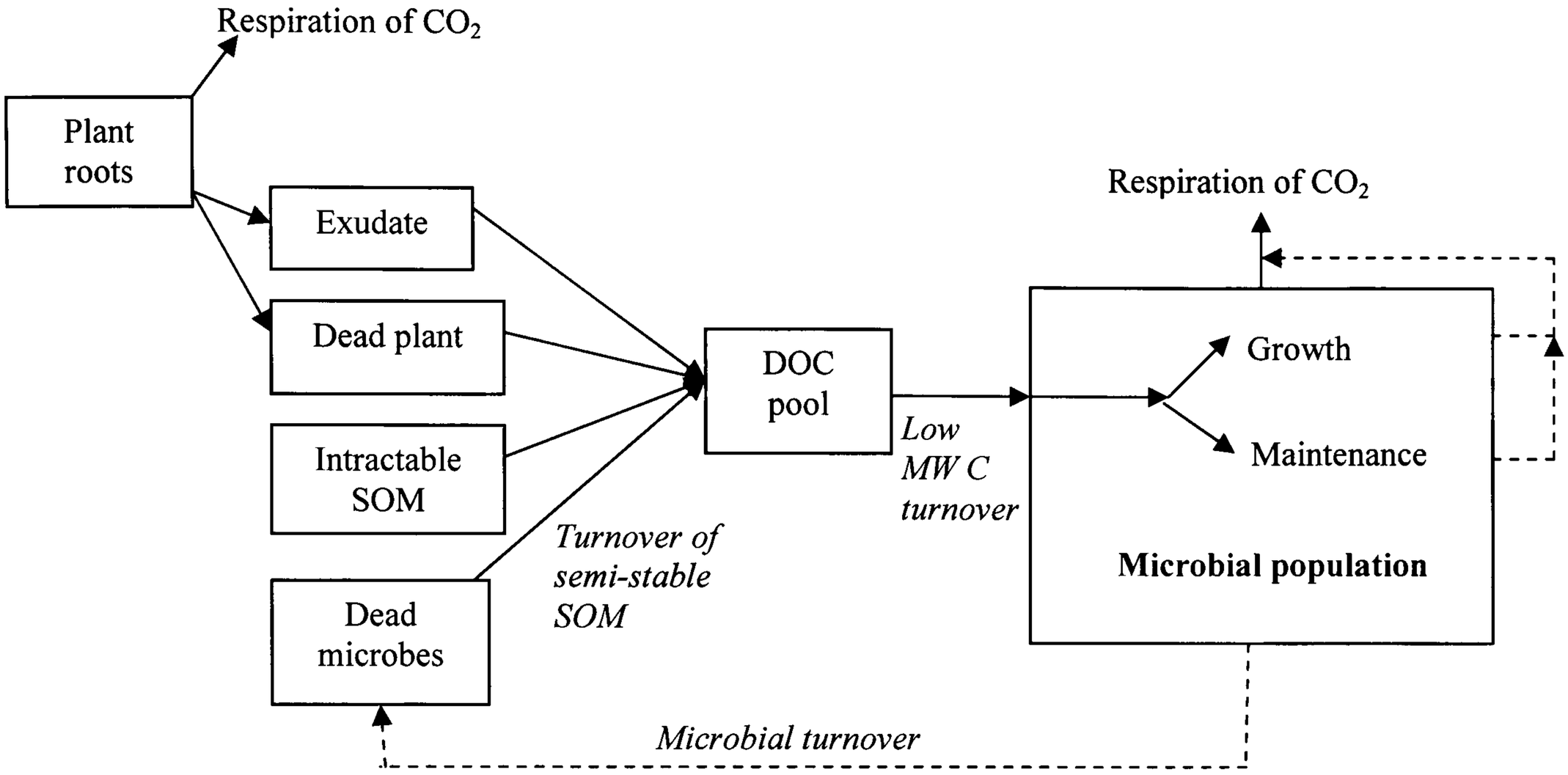


Figure 2.1. Schematic of the major controls on CO_2 efflux from the soil surface, indicating the three main modes of C turnover, namely that of low MW C turnover, microbial biomass turnover and the turnover of the semi-stable SOM pool; a fourth pool of lesser importance for short term C fluxes is the turnover of intractable SOM.

Kutsch and Kappen (1997) proposed that soil respiration can be modelled by three dominant processes: 1) root and rhizosphere respiration, 2) respiration of fast or low molecular weight (MW) organic matter (OM) fractions, 3) respiration of slow or high MW OM fractions. Separating root and microbial respiration of low MW OM and high MW OM has been estimated using an array of different methodologies, which include: (1) following the isotopic C signature of the different components, (2) root cuvettes in the field versus root excision in the laboratory, (3) inhibition with herbicides, and (4) substrate induced respiration (Buchmann, 2000). There is still a large degree of uncertainty attached to estimates of the contribution of root respiration to total soil CO_2 efflux, with

root-to-microbial respiration varying from 1:9 to 9:1 (Buchmann, 2000), though generally root contribution is taken to be 50% (Lee et al., 2003).

Low MW compounds generally enter the soil solution through rhizodeposition (root exudation and turnover; Jones and Murphy, 2007). Root exudation is one of the main sources of C for soil microorganisms and may be the source of the most dynamic components of the soil dissolved organic carbon (DOC) pool (Pelz et al., 2005). Root exudates are largely composed of low MW compounds (e.g. sugars, organic acids, amino acids), and although the size of the DOC pool is very small in comparison to the C in a solid form, its turnover can be extremely rapid (van Hees et al., 2005; Boddy et al., 2007a). Much of the C contained in the solid OM pools is probably not of recent root origin and remains relatively recalcitrant to microbial attack (Kalbitz et al., 2000). The low MW components of DOC are vital as they provide the link between the breakdown of high MW microbial and plant necromass and recalcitrant SOM (Toal et al., 2000).

2.1.2. C and N limitations on the microbial biomass

Soil microorganisms mediate key belowground processes such as nutrient and C cycling, and form a crucial and dynamic pool of soil OM (Nguyen and Henry, 2002). The majority of the microbial community (e.g. bacteria, fungi, actinomycetes etc) can only access soluble C (protozoa can take up insoluble SOM), and consequently it is the rate of supply of C substrates to the soil solution that primarily drives respiration (van Hees et al., 2005). Microbial activity is often limited by C availability especially in Arctic ecosystems (Ruess et al., 2001) and the release of low MW C compounds from roots by

rhizodeposition may be key to controlling soil respiration (Nguyen and Henry, 2002). Low MW compounds, such as sugars, organic acids and amino acids, typically dominate root exudation (Farrar et al., 2003), and these substrates play a central role in microbial metabolism. The microbial community exhibits a high degree of functional redundancy for utilising these compounds in soil (Gonod et al., 2006).

The microbial biomass, largely composed of bacteria and fungi, is the living fraction of SOM. Microbial C generally represents only 1-5% of total soil organic carbon (OC; Hu et al., 1995). Microbial respiration represents the primary mechanism for the decomposition of C fixed by plants (Allan and Schlesinger, 2004). The control of the decomposition process by the microbial community regulates nutrient availability, determines C storage and contributes to atmospheric CO_2 via respiration efflux from the soils surface (Cheng and Virginia, 1993; Wang et al., 2003). In general, the availability of soluble C and N, nutrients, water and temperature governs the activity of soil microbes (Blagodatsky et al., 1998; Galicia and Garcia-Oliva, 2004; Pennanen et al., 2004). Soil microbes' high demand for low MW C and N, induces a rapid turnover of both monosaccharides and organic acids in soils (Jones, 1999). Amino acids represent one of the most labile and dynamic soil organic N pools; approximately 30-45% of the N recovered in agricultural soils is in the form of amino acids after organic matter acid hydrolysis; all microorganisms appear to have the capacity to assimilate amino acids (Jones, 1999; Jones and Shannon, 1999; Vinolas et al., 2001a).

Though most soils are limited by substrate availability (C and N), boreal and tundra soils are also limited by temperature thereby inhibiting decomposition

and causing OM to accumulate (Schlesinger and Andrews, 2000). Soil respiration in boreal systems can be highly variable during the growing season, due to variable moisture and temperature content. For example, frozen soils can accumulate NH_4^+ in a readily available form over the winter months, which then becomes gradually depleted during the growing season (Vanhala, 2002). Where temperature, moisture content and pH remain quasi-constant, variation in soil respiration has been attributed to N availability and soil organic C content (Zak et al., 2000; Vanhala, 2002). The impact of N additions also appears to be soil dependent. For example, N addition increased root growth in acidic soils, but decreased root growth in calcareous sands and decreased the decomposition of soil OM in boreal soils (Hagerdorn et al., 2003).

Arctic soils are often thought to be more N-limited than C-limited (Jones and Kielland, 2002; Madan et al., 2007) due to the high levels of soil OC immobilizing the available soil N (Kirschbaum, 2000). The freeze-thaw cycle in Arctic soils results in a pulse release of inorganic N at the start of the growing-season, coinciding with enhanced microbial activity, though the same is true for labile C (Vanhala, 2002). Decomposition of soil OM in low-N soils may be limited by the inadequate supply of N for microbial activity, leading to N immobilization and reduced system productivity (Vance and Chapin, 2001).

Wheatley et al. (2001) believed that there was an upper limit to the effect of increased N concentrations, as the total microbial biomass is a stable parameter of a particular soil. However, it is more likely that the soil is subject to many co-limiting factors and enhancing the concentration of any one component has a limited effect on total soil respiration. The degree to which environmental limitation affects the soil microbial community is less clearly understood than

with plants (Vance and Chapin, 2000). C substrate supply is often viewed as a major limiting factor for microbial activity, however, most soils contain more dead OC than live microbial C indicating that other factors must provide additional controls over microbial activity.

2.1.3. Aims of the study

Polar ecosystems are currently experiencing some of the fastest rates of warming (Grogan and Jonasson, 2005), a rate 2-3 times faster than that forecast for temperate regions (1.5 ^{o}C by 2050, Mikan et al., 2002). Arctic ecosystems have been estimated to store a large proportion (11%) of the total global soil OM (Grogan and Jonasson, 2005; Schimel and Mikan, 2005). Soil respiration (total CO_2 efflux) of tundra soils is much slower than in temperate soils (60 compared to 660 g C m^{-2} y^{-1} respectively) and turnover time is also much longer in tundra soils (490 y compared to 60 y; Raich and Schlesinger, 1992). Soil respiration provides a sensitive indicator of belowground changes in microbial activity and system C losses. Consequently, understanding the mechanisms regulating soil organic C dynamics in temperate and polar regions is vital for future climate predictions. The primary aim of this study was to establish the baseline CO_2 efflux from temperate grassland and tundra soils, and in so doing establish whether different C pools contribute to the efflux of CO_2 from the soil, and to establish the impact of sampling disturbance and root exclusion on total soil CO_2 efflux. The secondary aim of the study was to investigate C and N limitations on the Arctic tundra soils, and to assess the impact of removing a soil from new substrate inputs for >1 y on a UK grassland soil to estimate the substrate limitation of these soils.

2.2. Materials and methods

2.2.1. UK grassland and Svalbard tundra sampling sites

The UK grassland soils were obtained from three temperate oceanic agricultural grasslands located at Abergwyngregyn, Gwynedd, North Wales (53°14'N, 4°01'W; Table 2.1). The mean annual soil temperature at 10 cm depth is 11 °C and the annual rainfall is 1250 mm. Soil was sampled from three sites (Eutric Cambisol, Dystric Gleysol, and Haplic Podzol) located along a glacial parent material catena sequence. The Eutric Cambisol was formed from mixed origin glacial till, the Dystric Gleysol from lacustrine deposits and the Haplic Podzol from Ordovician mudstones and shales. The Eutric Cambisol field site contained three delineated areas of improved pasture for high-density sheep grazing and three areas that had been covered with membrane to prevent light reaching the soil surface for 2 y but yet still enabled moisture and gaseous exchange. Soil was sampled in April 2005; at this time the soil temperature averaged 12.3 ± 0.7 °C across the sampling sites. For soil characterization, intact soil cores were taken to a depth of 5 cm using a 4.2 cm diameter soil corer. For respiration analysis, soil was collected from each of the field sites to a depth of 5 cm and sieved through 7 mm to remove roots.

Table 2.1. Characterisation of selected properties of the UK grassland soils used in the study. Values represent means ± SEM (n = 3). The symbols *, NS (not significant) indicate the significant differences at the $P < 0.05$ level between the grassland field sites.

Field site	Moisture content ($g\ kg^{-1}$)	pH	Total C ($g\ kg^{-1}$)	Total N ($g\ kg^{-1}$)	C-to-N ratio	Soil respiration ($mg\ CO_2\ kg^{-1}\ h^{-1}$)
Gleysol	35.3 ± 0.7	5.7 ± 0.7	43 ± 2	*4.5 ± 0.3	*9.6 ± 0.2	3.3 ± 0.9
Podzol	28.4 ± 1.0	4.4 ± 0.2	*104 ± 11	*9.1 ± 0.8	11.3 ± 0.3	2.0 ± 0.4
Cambisol grazed	32.6 ± 0.8	5.4 ± 0.6	31 ± 2	2.6 ± 0.3	*12.2 ± 1.0	2.9 ± 0.6
Cambisol no vegetation	*44.5 ± 0.2	4.8 ± 0.6	26 ± 1	2.4 ± 0.2	10.9 ± 0.6	2.6 ± 0.4
	*	*NS*	*	*	*	*NS*

Two sites in the High Arctic were selected in June 2005 near Ny-Ålesund, Svalbard, Spitsbergen on the strandflat, the region of lowland plain adjacent to the coast, formed due to the combined actions of frost weathering and abrasion by the sea during interglacial periods. The first site was a polar meadow at Zeppelinfjellet (78°56'N, 11°58'E; Table 2.2), where the plant community is classified as Kantlyngsonen (*Cassiope tetragona*) vegetation. The second site was a polar heath at Stuphallet (78°56'N, 11°42'E), with a plant community classified as Reinrosesonen (*Dryas octopetala*) zone. The parent material at both sites consists largely of calcareous deposits of marine origin. At each site, three experimental areas dominated by either *Carex misandra* R. Br. (short leaf sedge) *Dryas octopetala*, *Saxifraga oppositifolia* L. vegetation types or crustose lichens (e.g. *Rhizocarpon* spp, *Lecanora* spp) were located at least 2 m apart.

At both Arctic field sites, the soil consisted of a distinct dark humose O horizon (ca. 5 cm in depth), which overlaid a largely structureless mineral B horizon (ca. 30 cm in depth). Most roots were present in the O horizon. Intact soil blocks with vegetation attached were collected during the mid-growing

season from each site alongside samples of the subsoil (mineral B horizon). Vegetation percentage cover at each field site was determined using ten replicate 1 m^2 quadrats and the grid intersection method (n = 100 quadrat^{-1}). The surface soil temperature at the time of sampling was 10 ± 1°C. The intact samples were placed in gas permeable polyethylene bags, and flown back to the UK at 4 °C for analysis within 48 h.

Background soil characteristics for the UK and Arctic soils were analysed as per Boddy et al. (2007a) and Boddy et al (2007b). Briefly, soil moisture content was assessed by oven drying soil at 80 °C. Dry, root-free soil was analysed for C and N content in a Leco CHN 2000 analyser (Leco Corp., St Joseph, MI, USA). Soil pH was measured with a BDH Gelpas electrode (VWR International, Lutterworth, UK) in a 1:1 (w/v) mixture with deionised water. Respiration measurements were made within 72 h of sampling using an infra red gas analyser in the UK (PP-Systems Ltd., Hitchin, UK) or *in situ* using an infra red gas analyser (EGM-4, PP-Systems Ltd.).

2.2.2. Background soil characteristics for temperate and Arctic field sites

UK grassland soil solution was extracted according to Giesler and Lundström (1993). Briefly, intact soil cores were centrifuged (4000 *g*, 15 min, 20 °C) to obtain soil solution and the collected solutions passed through a Whatman 42 filter paper before freezing at -20 °C to await chemical analysis (Table 2.3). Soil solution samples were analysed for total DOC and total dissolved N (TDN) using a Shimadzu TOC-V-TN analyser (Shimadzu Corp., Kyoto, Japan). NO_3^- was determined colorimetrically by the Cu-Zn-hydrazine reduction method of Downes (1978) and NH_4^+ by the salicylate-hypochlorite procedure of Mulvaney

Table 2.2. Selected properties of the vegetation cover and topsoil properties (0-5 cm) at the Zeppelinfjellet (polar meadow) and Stuphallet (polar heath) field sites. Values represent means ± SEM (n = 3). Other vegetation includes ground cover of *Silene acaulis*, moss, *Polygonum vivparum*, *Salix polaris*, *Equisetum arvense*, *Luzula arctica*, and *Cassiope tetragona* species present at each site *NS* indicates no significant difference between the two sites ($P > 0.05$) while * indicates a significant differences at the $P < 0.05$ level.

Field site	Vegetation ground coverage (dominant vegetation types)						Moisture content ($g\ kg^{-1}$)	pH	Total C ($g\ kg^{-1}$)	Total N ($g\ kg^{-1}$)	C-to-N ratio	Soil respiration ($mg\ CO_2\ kg^{-1}\ h^{-1}$)
	Dryas (%)	*Saxifrage* (%)	Lichen (%)	*Carex* (%)	Bare soil (%)	Other (%)						
Zeppelinfjellet	4.3 ± 2.0	3.1 ± 0.9	51.0 ± 1.9	3.4 ± 1.3	5.8 ± 1.3	31.8 ± 2.3	35.6 ± 0.9	6.3 ± 0.3	115 ± 20	6.3 ± 0.7	18 ± 1	4.36 ± 0.48
Stuphallet	4.5 ± 4.5	5.9 ± 1.7	36.3 ± 3.8	3.5 ± 1.3	7.3 ± 1.9	42.8 ± 6.8	23.5 ± 5.1	6.2 ± 0.2	108 ± 17	6.8 ± 0.6	16 ± 1	4.06 ± 0.16
	NS	*NS*	*	*NS*	*NS*	*NS*	*	*NS*	*NS*	*NS*	*NS*	*NS*

Table 2.3. Characterization of selected properties of the soil solutions across a catena sequence of UK grassland soils. Free amino acids assumes an average C content of 62.4 g mol^{-1} and an average N content of 19.6 g mol^{-1}. Values represent means ± SEM (n = 3). The symbols * and *NS* (not significant) indicate the significant differences at the $P < 0.05$ level between the grassland soil solutions.

Sample site	DOC	DON	Glucose	Total free amino acids		Total phenols	NO_3^-	NH_4^+
	(mg C l^{-1})	(mg N l^{-1})	(mg C l^{-1})	(mg C l^{-1})	(mg N l^{-1})	(mg C l^{-1})	(mg N l^{-1})	(mg N l^{-1})
Gleysol	176 ± 37	27 ± 4	14.4 ± 1.7	3.7 ± 0.1	1.2 ± 0.1	9.1 ± 1.8	1.1 ± 0.1	3.0 ± 0.5
Podzol	* 573 ± 73	66 ± 12	16.9 ± 0.5	4.3 ± 0.3	1.3 ± 0.1	15.6 ± 1.2	0.8 ± 0.0	4.3 ± 0.9
Cambisol grazed	131 ± 22	40 ± 12	17.3 ± 2.7	6.7 ± 4.2	2.1 ± 0.8	20.2 ± 3.6	1.0 ± 0.0	3.5 ± 0.4
Cambisol no vegetation	176 ± 16	82 ± 50	25.9 ± 7.3	1.8 ± 0.1	0.6 ± 0.1	7.8 ± 2.3	1.9 ± 0.3	3.6 ± 0.3
	*	*NS*	*NS*	*NS*	*NS*	*NS*	*NS*	*NS*

Table 2.4. Selected properties of the soil solution from the Zeppelinfjellet and Stuphallet field sites (0-5 cm depth). Free amino acids assumes an average C content of 62.4 g mol^{-1} and an average N content of 19.6 g mol^{-1}. Values represent means ± SEM (n = 3). *NS* indicates no significant difference between the two sites ($P > 0.05$) while * indicates a significant difference at the $P < 0.05$ level.

Sample site	DOC	DON	Glucose	Total free amino acids		Total phenols	NO_3^-	NH_4^+
	(mg C l^{-1})	(mg N l^{-1})	(mg C l^{-1})	(mg C l^{-1})	(mg N l^{-1})	(mg C l^{-1})	(mg N l^{-1})	(mg N l^{-1})
Zeppelinfjellet	51 ± 6	3.3 ± 0.6	1.8 ± 0.1	1.5 ± 0.3	0.48 ± 0.09	4.4 ± 0.6	0.46 ± 0.06	1.1 ± 0.2
Stuphallet	83 ± 12	6.5 ± 1.6	1.8 ± 0.1	1.8 ± 0.4	0.58 ± 0.11	3.6 ± 0.4	0.46 ± 0.07	1.2 ± 0.1
	*	*NS*	*NS*	*NS*	*NS*	*NS*	*NS*	*NS*

(1996). Phenolic concentrations were assessed using the Folin-Ciocalteau reagent, calibrated with a phenol standard, according to Swain and Hillis (1959). Free amino acids were measured fluorimetrically according to Jones et al. (2003). Glucose was determined spectrophotometrically with a Glucose (GO) Assay® kit (Sigma-Aldirch, Misouri, USA).

In Svalbard, soil solution was extracted from three samples (collected 2 m apart) by gently shaking 10 cm^3 of soil with 10 ml of deionised water for 30 s and then recovering the soil solution using a 5 cm long Rhizon soil moisture sampler (Rhizosphere Research Products, Wageningen, Netherlands) and sterile Venoject® vacuum tubes (Terumo Corp., Tokyo, Japan). The solutions were then frozen at -20 °C and flown to the UK for analysis (Table 2.4). Samples were analysed as for the UK grassland soils.

2.2.3. Measurement of basal respiration in the field and laboratory

Respiration rates of soil from Svalbard and North Wales were compared in the field and in the laboratory. Measurements at Zeppelinfjellet, Stuphallet and UK Cambisol field sites used a portable infrared gas analyser (PP-Systems Ltd., EGM-4 with one sample chamber). Measurements were taken in a random pattern across the dominant vegetation groups (*Dryas*, *Carex*, *Saxifraga*, Lichen and subsoil at Svalbard and from the *Lolium perenne* dominated grass sward in the UK; $n = 3$). Measurements at Svalbard were undertaken in June 2005 and UK respiration measurements were undertaken in April 2005 when soil temperature was comparable (10 ± 1 °C).

Laboratory measurements used a 12-chambered infrared gas analyser incubated at 10 ± 1 °C to be comparable to field temperature measurements (PP

Systems Ltd.; n = 3). 5 g samples of soil were prepared – with either plant material remaining or excised at the surface and respiration was measured over an 8 h period. Soils were collected from under *Dryas*, *Carex*, *Saxifrage*, lichen and subsoil at Svalbard and in the UK from grazed Eutric cambisol and non-vegetated Eutric cambisol soil.

2.2.4. Influence of disturbance and temporal scale of sampling on respiration

Intact soil cores were collected from three sites (grazed Eutric Cambisol, Dystric Gleysol, and Haplic Podzol) along the catena sequence in the UK. Extracted cores were stored at 4 °C for up to one week before preparation. Components were separated by removal of shoots and roots from the soil. Roots were removed by sieving through a 7 mm mesh. Shoots were excised at the soil surface. Individual treatments included:

1) intact cores + shoots

2) intact cores minus shoots

3) sieved soil minus roots

4) sieved soil + 1 g of roots

5) sieved soil + 3 g of roots

6) sieved soil + 5 g of roots.

Respiration rate was measured in each of the treatments over a 1.15 y sampling period using an infrared gas analyser (PP-Systems Ltd.). Respiration was monitored at 0, 0.06, 0.1, 0.19, 0.6, 1.15 y at 10 ± 1 °C. Cores were maintained in the dark from the start of the experiment so that the respiration rates could be measured over time with no new inputs of carbon. In all cases, three replicates

were analysed. The decline in respiration over time was described by fitted a double exponential model to the experimental results where

$$S = [a_1 \times \exp(-k_1 t)] + [a_2 \times \exp(-k_2 t)] \quad \text{(Eqn. 2.1)}$$

where S is the total remaining C in the soil, k_1 is the exponential coefficient describing the respiration and mineralization of readily available substrate by the microbial biomass, k_2 is the exponential coefficient describing the secondary, slower phase of respiration and mineralization of less available C, a_1 and a_2 describe the proportion of C associated with pools with exponential coefficients k_1 and k_2, and t is time.

Mean residence time (MRT) of the C in the soil is calculated from k_1 and k_2 using:

$$\text{MRT} = \ln(2)/k_{CO_2} \quad \text{(Eqn. 2.2)}$$

2.2.5. Analysis of C and N limitation on respiration of Arctic tundra soils in the laboratory

In the laboratory, substrate additions were analysed for their impact on soil microbial respiration in soil samples from both Zeppelinfjellet and Stuphallet. At each site, subsoil, topsoil and lichen-modified soil were analysed. Topsoil consisted of three separate pooled samples from under *Dryas*, *Carex* and *Saxifraga* modified soils, sieved to 7 mm with roots and shoots removed. Respiration rates were measured from 2 g of soil for 3 h to achieve a stable respiration level. At this point additions of substrate consisting of either organic C (50 mM glucose; Jones and Murphy, 2007), organic N (50 mM glutamate; Jones and Murphy, 2007), or inorganic N (20 mM ammonium and nitrate; Jones and Kielland, 2002) were added to the soil. Additions of 200 µl substrate were

made to 2 g of sterile sand, and this subsequently thoroughly mixed with the soil sample. Sand was used to ensure a thorough distribution of the substrate and to prevent loss of soil structure in the subsoil. Respiration measurements were then monitored for 5 h to see the effect of the added substrate. Maximum respiration rates were measured 0.5 – 2.5 h following substrate addition (Wang et al., 2003). Three controls were used; soil shaken at 3 h with no sand, soil with sand alone added and soil with sand and 200 µl of de-ionised water added at 3 h. To estimate the proportion of the microbial community that was affected by the added substrates listed above, additions of a potent microbial toxin (20 mM $HgCl_2$) was added to the soil as described above and the decline in respiration measured over 5 h. The proportional decline in respiration was assumed to be a measure of the proportion of the community that was reached by the substrate additions.

2.2.6. Statistical and data analysis

Field respiration measurements were compared by site using one-way ANOVA. Laboratory respiration measurements were also compared for UK and Svalbard field sites using one-way ANOVA. The data from UK agricultural catena sequence soils was analysed using Univariate GLM ANOVA, to compare the three soils over time by each component. Substrate limitation of respiration was assessed similarly, comparing soil type (topsoil, subsoil, lichen at Zeppelinfjellet and Stuphallet), against time and substrate. Statistical procedures were carried out with the software package SPSS 12.0 for Windows (SPSS Inc., Chicago, IL) with $P < 0.05$ used as the upper limit for statistical confidence, $n = 3$.

2.3. Results

2.3.1. UK grassland and Arctic tundra basal respiration

Basal respiration rates measured at the two Arctic sites (Stuphallet and Zeppelinfjellet) did not differ significantly from each other (ANOVA, $P > 0.05$; Fig. 2.2), however, these were significantly lower than the respiration rates observed in the UK grassland soils (UK Cambisol: 1.09 ± 0.25, Svalbard: 0.34 ± 0.08 g CO_2 m^{-2} h^{-1}; $P < 0.05$). In addition, there were no statistical differences observed between the respiration rates from the soils sampled from the different types of vegetation at the two Arctic field sites (*Dryas*, *Carex*, *Saxifrage*, lichen and subsoil; $P > 0.05$). UK grassland that had been covered for two years to prevent vegetation growth and C inputs had a much lower rate of respiration than in the vegetated UK plots but a similar rate of CO_2 efflux as that observed at the Svalbard field sites (covered UK grassland = 0.21 ± 0.02 g CO_2 m^{-2} h^{-1}).

Basal respiration rates per gram of O horizon in the laboratory were generally greater at Zeppelinfjellet than at Stuphallet (Fig. 2.3). When respiration from the polar soils in the laboratory was compared to the UK grassland, no statistical difference was observed (UK: 11. 9 ± 1.6, Zeppelinfjellet: 12.9 ± 2.1, Stuphallet: 9.0 ± 1.8 μg CO_2 g^{-1} h^{-1}; $P > 0.05$). Respiration samples from different vegetation modified soils (*Carex*, *Saxifrage*, *Dryas* and lichen) also showed no statistical difference by vegetation type (21.7 ± 2.1 Zeppelinfjellet, 16.6 ± 2.2 μg CO_2 g^{-1} h^{-1}; $P > 0.05$). Overall, respiration rates were much lower where plant material (roots and shoots) had been removed from the soil (plant, no plant: average ± SEM; 21.7 ± 2.1, 5.2 ± 0.7 at Zeppelinfjellet, 16.6 ± 2.2, 3.1 ± 0.4 at Stuphallet, and 13.5 ± 1.8, 6.5 ± 0.4 at the UK grassland μg CO_2 g^{-1} h^{-1}; $P < 0.05$). Respiration rates from Zeppelinfjellet and Stuphallet subsoils were much

smaller than in the topsoils, but statistically similar to topsoils with plant material removed (0.35 ± 0.10 Zeppelinfjellet, 1.22 ± 0.30 Stuphallet μg CO_2 g^{-1} h^{-1}; $P >$ 0.05).

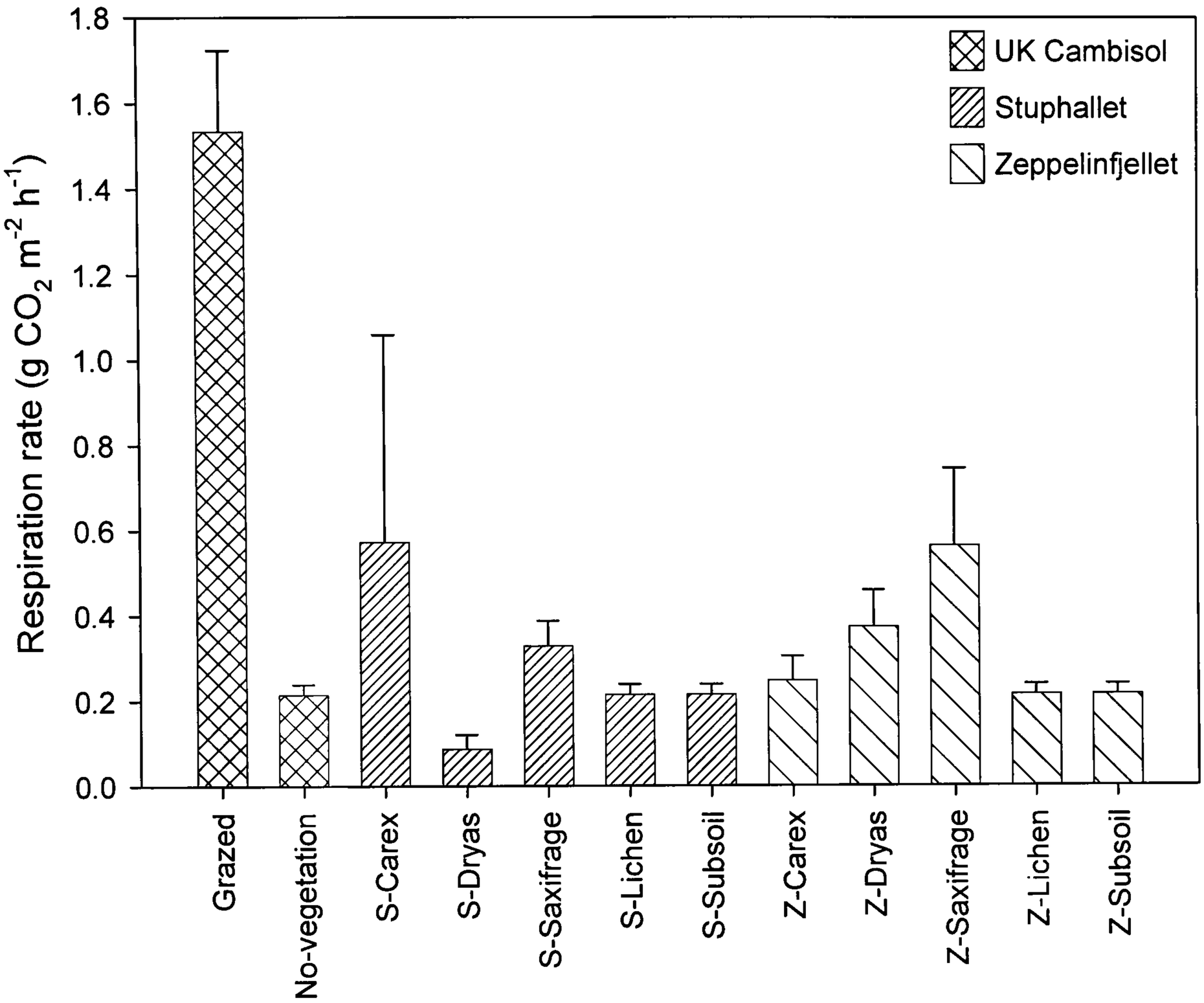


Figure 2.2. Field respiration rates using an EGM-4 from the UK (grazed and no-vegetation) and from two polar field sites [Zeppelinfjellet (Z) and Stuphallet (S)] with different vegetation cover. Values represent mean ± SEM, $n = 3$.

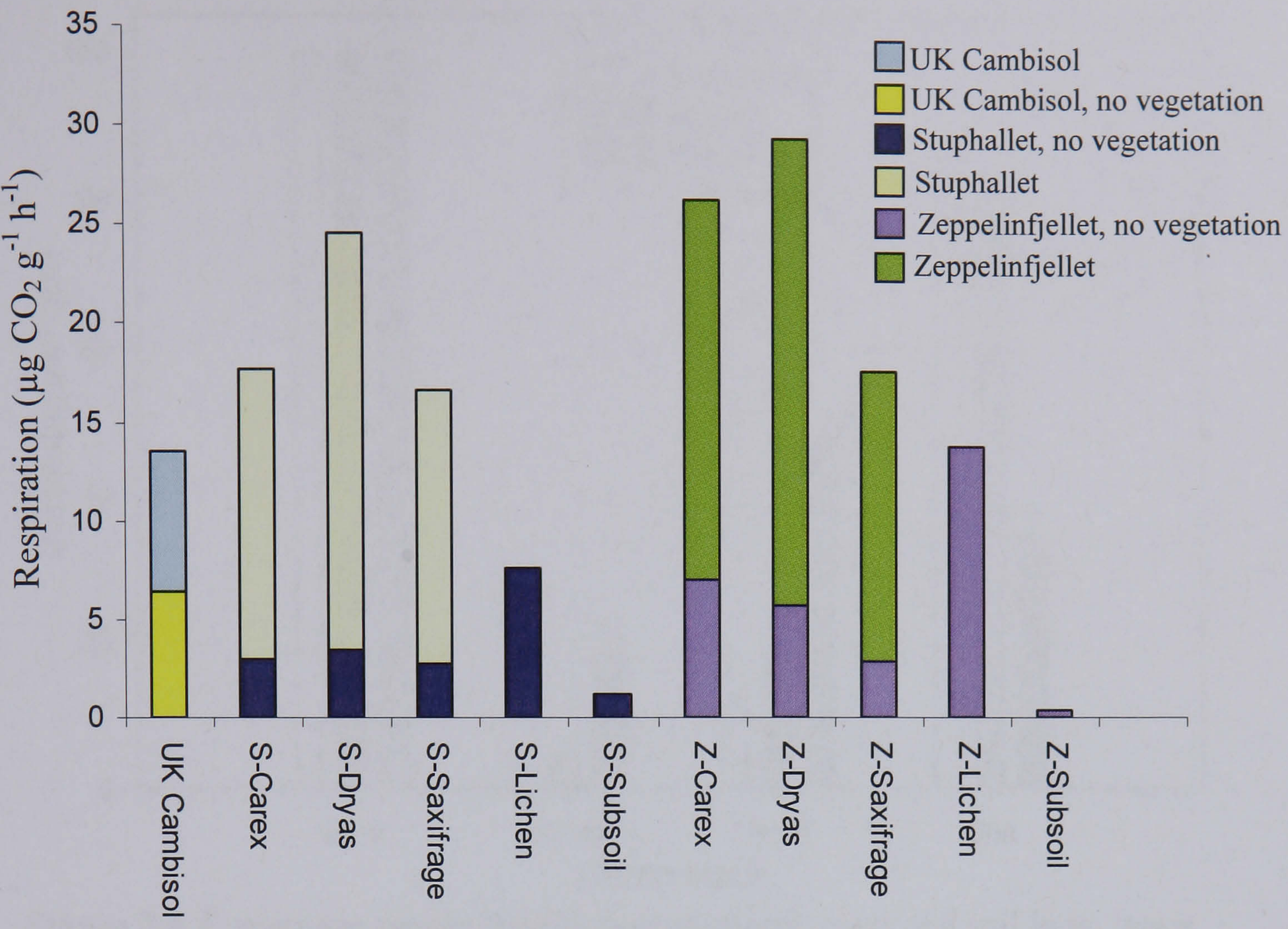


Figure 2.3. Laboratory respiration rates for soils from UK grassland (Cambisol), and obtained from different vegetation types at either Zeppelinfjellet (Z) or Stuphallet (S), measured at 7 h after placing in a PP-Systems 12-chambered respirometer. Respiration rates per gram of soil material, with either root and shoot material included or excised and sieved through 7 mm to remove the roots. Values represent mean ± SEM, $n = 3$.

2.3.2. Impact of disturbance and temporal scale on respiration in three UK soils

There were no significant differences between the individual components (soil, roots or shoots) in the intact cambisol and gleysol soil cores. In contrast, the podzol cores had proportionally more shoot and root present and subsequently less soil (average ± SEM cambisol and gleysol: podzol; shoot 3.2 ± 0.3: 18.6 ± 2.8%; root 13.1 ± 1.7: 47.1 ± 1.0%; soil 83.7 ± 1.9: 34.4 ± 3.5%; $P <$ 0.001; Fig. 2.4).

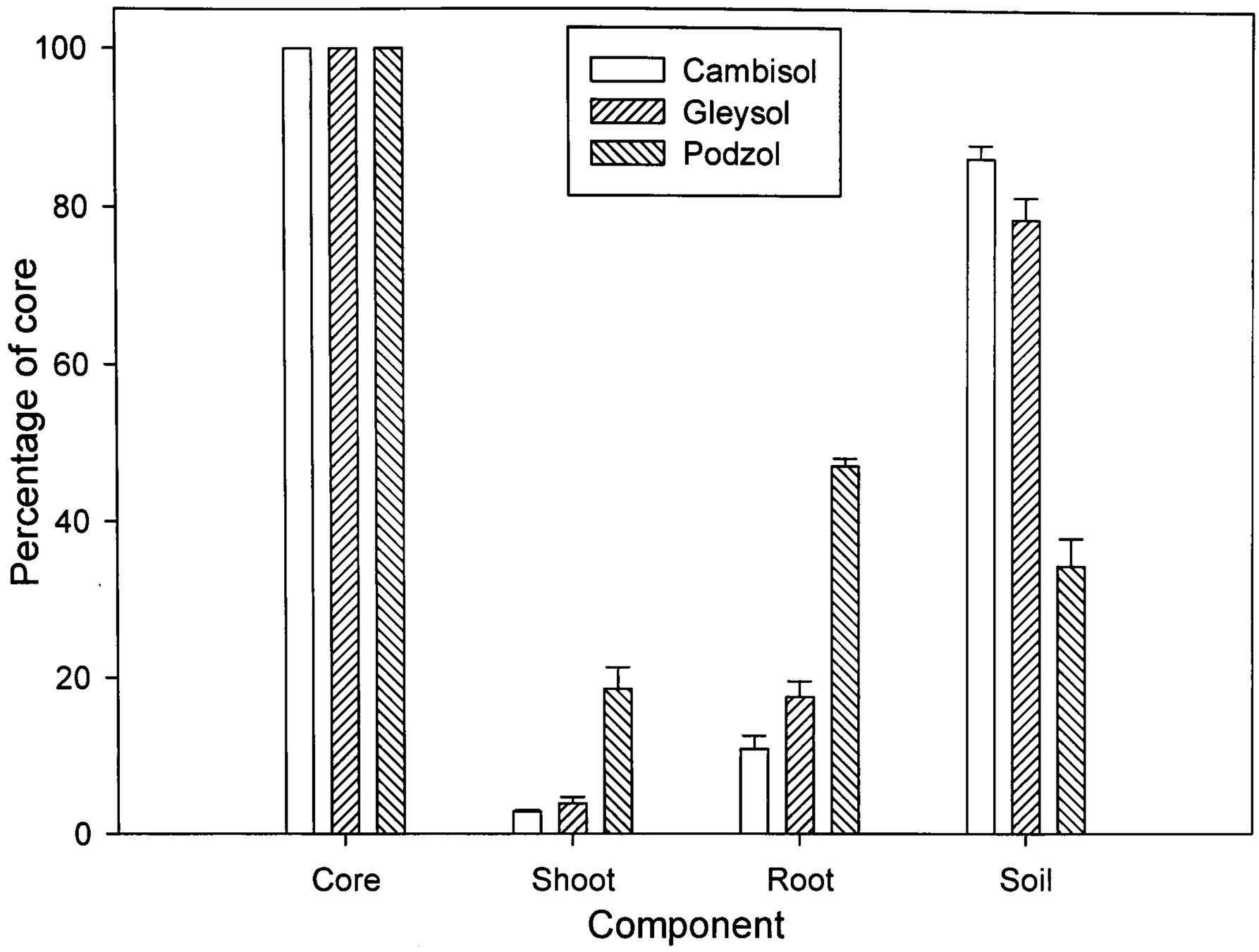


Figure 2.4. Percentage weight distribution of shoots, roots and soil in an intact core sampled from cambisol, gleysol and podzol soils. Bars are SEM, $n = 3$.

There was also no significant difference in respiration rates for the three soils at the initial time period, excepting the efflux of CO_2 from the excised shoots, where shoots from the podzol had lower CO_2 efflux than those from the cambisol and gleysol (52 ± 4 podzol, 90 ± 4 cambisol, 92 ± 7 gleysol μg CO_2 g^{-1} h^{-1}; $P = 0.002$; Fig. 2.5). CO_2 efflux from the intact cores averaged 6.3 ± 0.3 μg CO_2 g^{-1} h^{-1} across the three soil types, while CO_2 efflux from cores with shoots excised approximated 90% of the intact core's respiration rate (5.7 ± 0.5 μg CO_2 g^{-1} h^{-1}), which was expected from the proportion of shoots within an intact core (Fig. 2.4). CO_2 efflux from the sieved soil averaged 3.9 ± 1.9 μg CO_2 g^{-1} h^{-1} across the three soil types, approximately 70% of the respiration of intact cores, though the variability was high (SEM 35%), as expected from Fig. 2.4. The CO_2 efflux from excised shoots and roots was much greater (25 and 10% respectively

of an intact core), with CO_2 efflux from shoots averaging 78 ± 7 μg CO_2 g^{-1} h^{-1} across the three soil types and roots 136 ± 27 μg CO_2 g^{-1} h^{-1}.

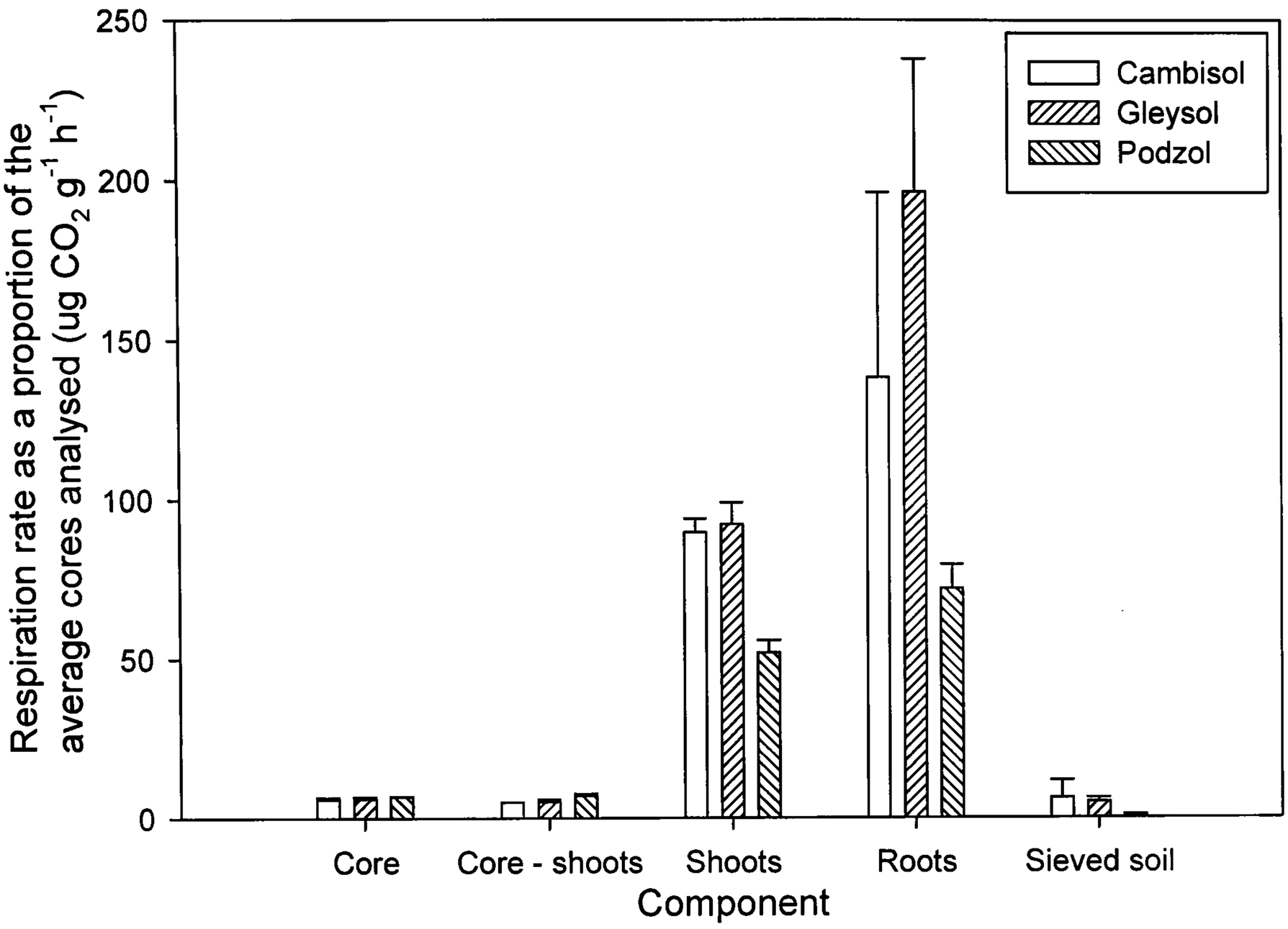


Figure 2.5. Respiration rate of components measured at t = 0, giving the respiration of the core, core minus shoots, excised shoots and roots, and sieved soil scaled to the amount present in an intact core sampled from cambisol, gleysol and podzol soils. Bars are SEM, n = 3.

Initially, CO_2 efflux following the addition of roots back to sieved soil had a linear relationship (r^2 = 0.42 ± 0.23; Fig. 2.6). However, no statistical differences were observed with the different soils or with the root additions (P = 0.169 and 0.210 respectively). This is likely to be a factor of the high variance.

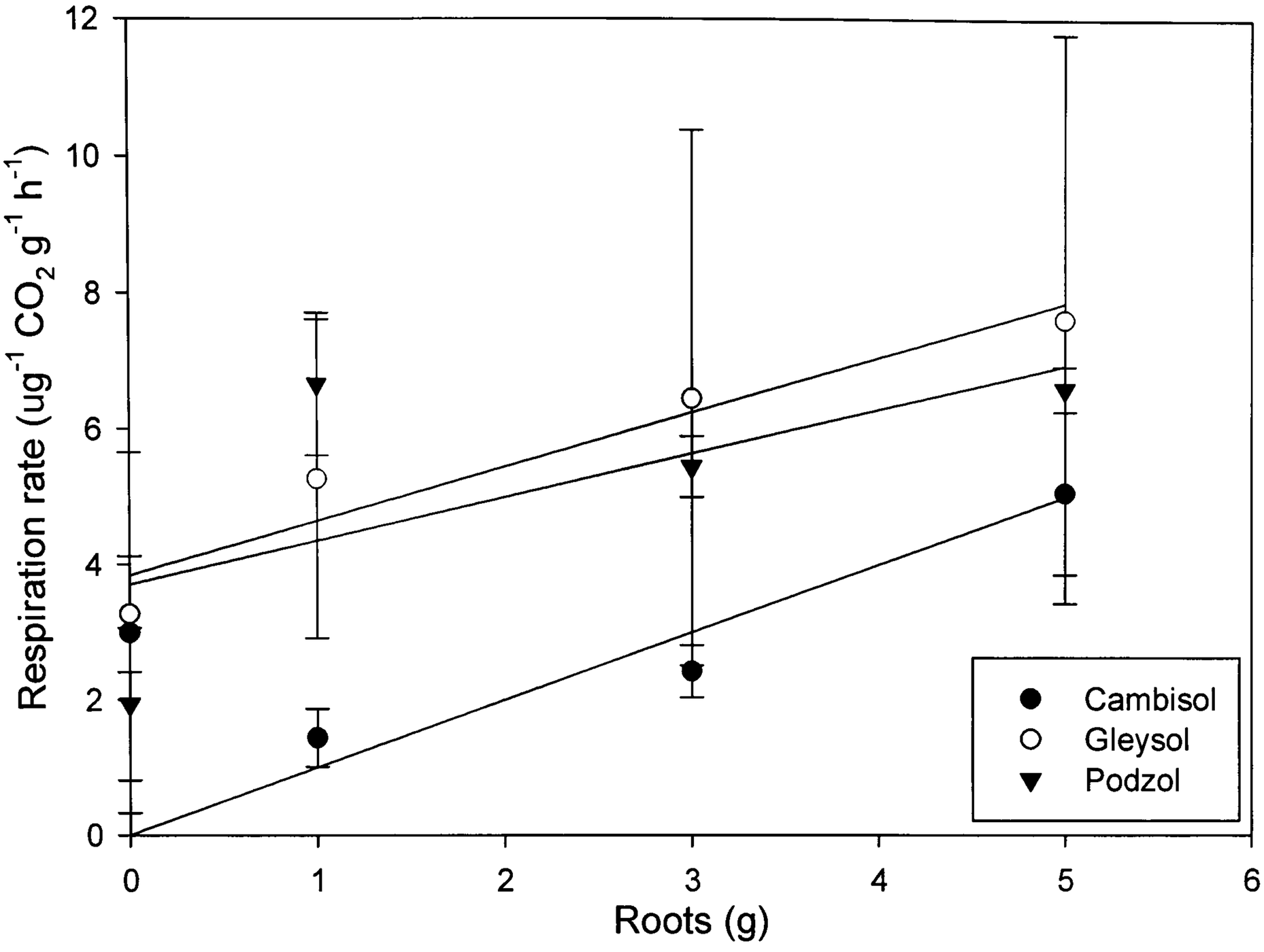


Figure 2.6. The effect on CO_2 efflux of the addition of excised roots (0, 1, 3 and 5 g) to sieved soil (40 g) from cambisol, gleysol and podzol soils at time 0 d. Bars are SEM, $n = 3$.

Subsequently, CO_2 efflux was monitored in the cores at 6 times over a 1.15 y period from the three soils incubated at 10 °C in the dark. Overall, respiration declined in all cases except for the intact core of the gleysol, which is probably due to high variability in the later sampling periods. Respiration rate in the intact cores from the cambisol and podzol soils progressively declined over time (falling from 6.38 ± 0.26 μg CO_2 g^{-1} h^{-1} at $t = 0$ to 0.37 ± 0.08 μg CO_2 g^{-1} h^{-1} at $t = 1.15$ y; $P = 0.011$) and generally, this decline followed a double exponential decay pattern (Eqn. 2.1; Fig. 2.7). In contrast, the gleysol cores did not follow the same pattern of decline. While respiration in the gleysol was originally similar to the other soil types, the respiration rates at the end of the incubation were significantly greater ($P = 0.031$). All cores with the shoots

excised fitted well to a double exponential model (r^2 = 0.92 ± 0.05). The respiration decline in the cambisol and gleysol cores were similar (falling from 4.98 ± 0.45 μg CO_2 g^{-1} h^{-1} at t = 0 to 0.69 ± 0.35 μg CO_2 g^{-1} h^{-1} at t = 1.15 y), but significantly different from the podzol cores with shoots excised (podzol CO_2 efflux falling from 7.05 ± 0.55 μg CO_2 g^{-1} h^{-1} at t = 0 to 0.63 ± 0.08 μg CO_2 g^{-1} h^{-1} at t = 1.15 y; P = 0.003).

Respiration from excised shoots and roots studied in isolation also decreased in CO_2 efflux over time and fitted well to a double exponential decay model (r^2 = 0.55 ± 0.13). CO_2 efflux from the shoots decreased from 78 ± 7 μg CO_2 g^{-1} h^{-1} at t = 0 to 14 ± 8 μg CO_2 g^{-1} h^{-1} at t = 1.15 y across the three soil types (P < 0.001). Shoots from the podzol soil were significantly different from cambisol and gleysol, with initially smaller rates of CO_2 efflux (Fig. 2.7; P = 0.003). CO_2 efflux from the excised roots decreased from 135 ± 27 μg CO_2 g^{-1} h^{-1} at t = 0 to 6 ± 1 μg CO_2 g^{-1} h^{-1} at t = 1.15 y across the three soil types (P < 0.001). There were no clear significant differences between soil type in the efflux of CO_2 from the excised roots (P > 0.05). CO_2 efflux from sieved soil did not decline significantly over time and there was no significant difference by soil type (averaging 1.9 ± 0.4 μg CO_2 g^{-1} h^{-1} across the three soils and sampling intervals; P < 0.05).

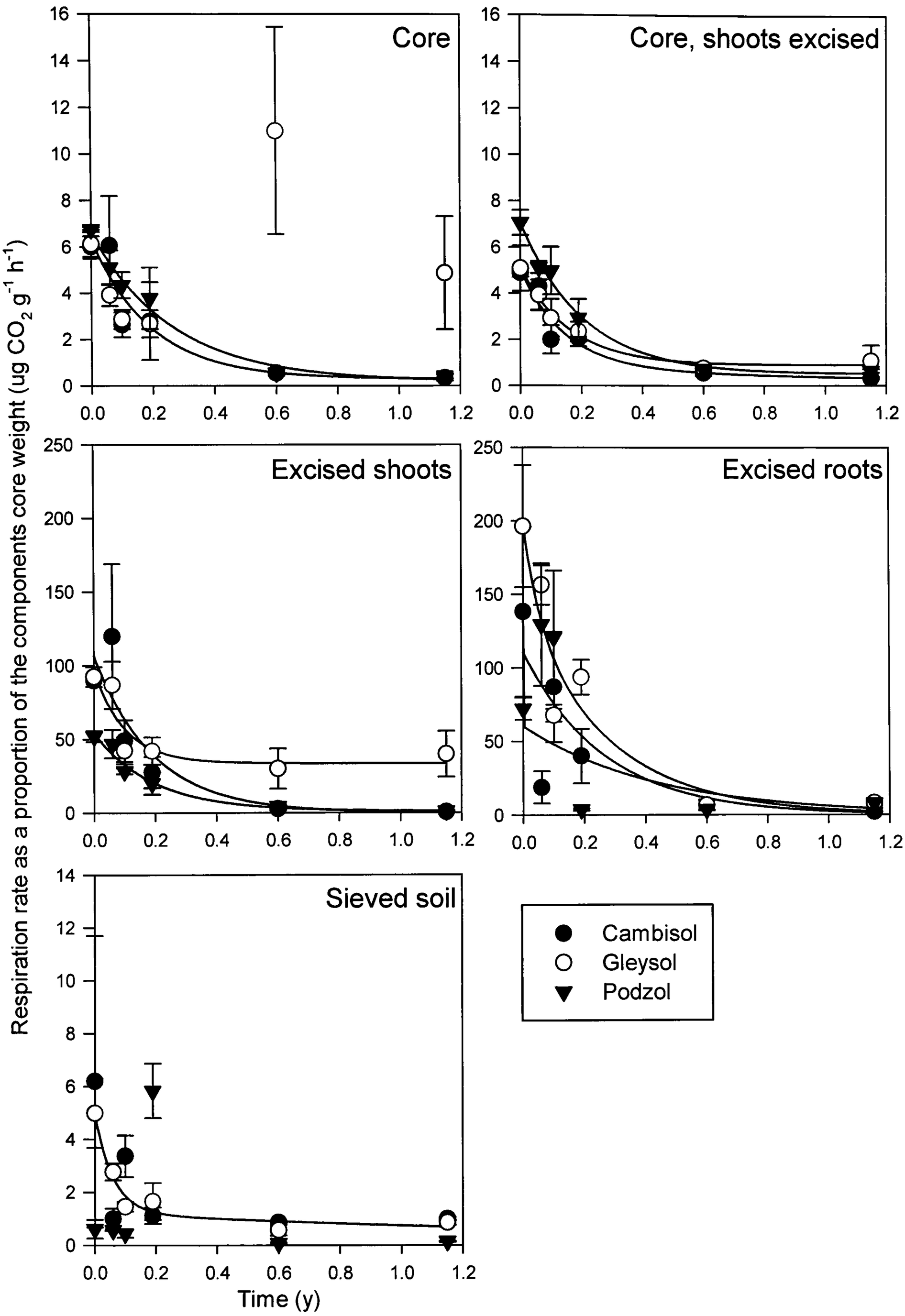


Figure 2.7. Respiration rate of individual components of soil cores measured over a 1.15 y period. Panels show the respiration of the intact core (soil + roots + shoots), core minus shoots, excised shoots and roots alone, and sieved soil as the proportionate weight of the component in an intact core sampled from cambisol,

gleysol and podzol soils over the incubation period at 10 °C. All samples were maintained in the dark. Bars are SEM, $n = 3$.

MRT calculated using Eqn. 2.2 from the double exponential kinetic model for all variables (intact cores, cores with shoots excised, excised shoots and roots, sieved soil) all gave similar residence times for k_1, with C remaining in the soil for 0.15 ± 0.02 y (Table 2.5). The residence time of C in the second phase (k_2) for intact cores and cores with shoots excised were much longer (> 5000 y). The second phase MRT for shoots was longer than k_1 with a turnover of 4.20 ± 2.07 y, while roots had a shorter second phase turnover at 0.20 ± 0.01 y. The MRT for C in the second phase for sieved soil was also longer than first phase turnover averaging 1.13 ± 0.17 y.

Table 2.5. Summary of the mean residence time (MRT) calculated from the double exponential equation, with MRT calculated from k_1 and k_2 using Eqn. 2.2. Values are averages across all three soil types and represent means ± SEM (n = 3).

Parameter	MRT for k_1 (y)	MRT for k_2 (y)
Intact core	0.17 ± 0.02	> 5000
Core with shoots excised	0.12 ± 0.01	> 5000
Excised shoots	0.15 ± 0.06	4.20 ± 2.07
Excised roots	0.15 ± 0.02	0.20 ± 0.01
Sieved soil	0.21 ± 0.10	1.13 ± 0.17

2.3.3. C and N limitations in the laboratory

Additions of either organic C, organic N or inorganic N were incorporated into topsoil, subsoil or lichen modified soil from the two Arctic field sites and CO_2 efflux monitored for 5 h following the addition of substrate. To enable direct comparisons between treatments, the respiration rate at the start

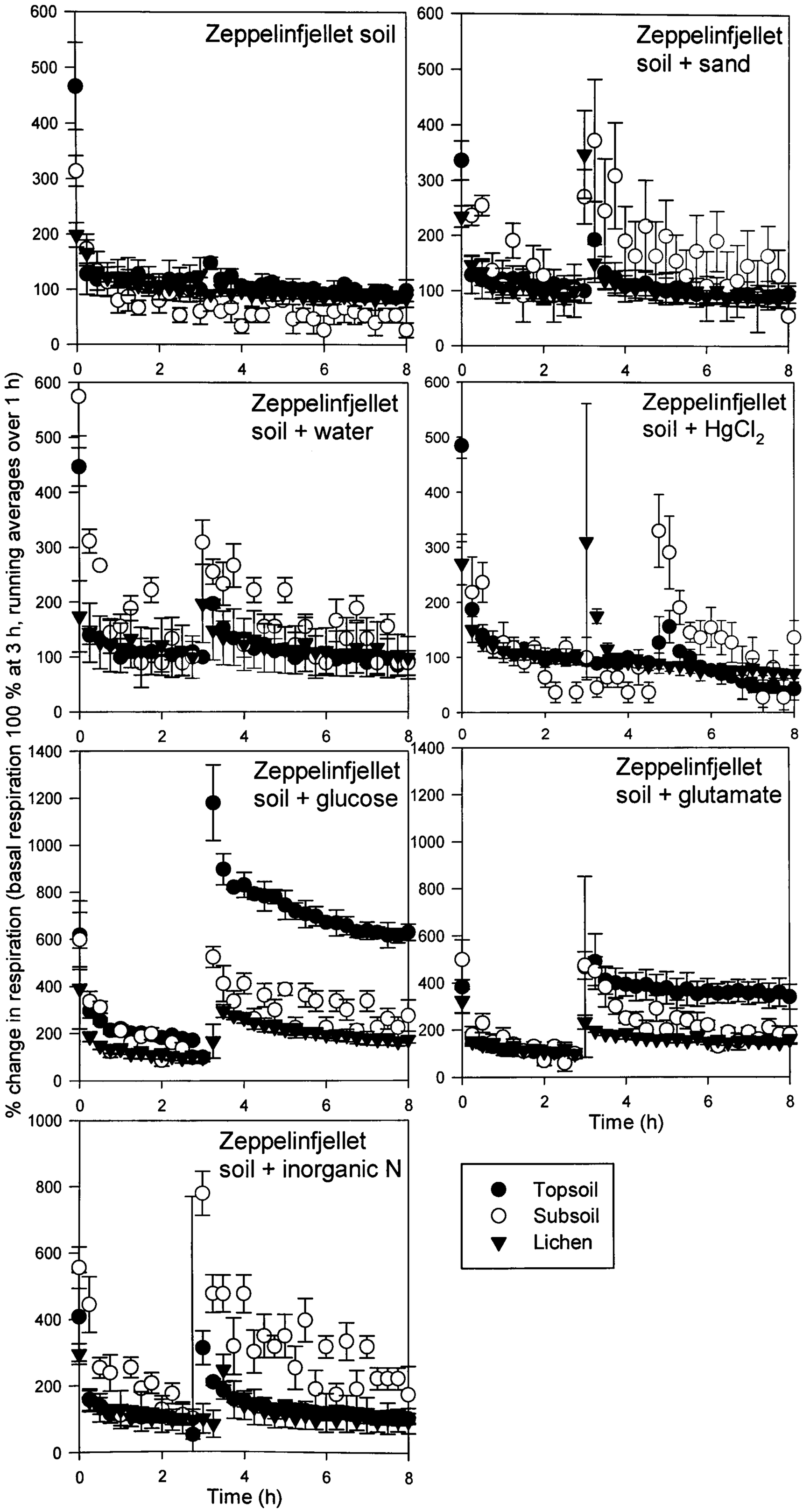
of the additions was established as the baseline respiration rate – i.e. 100% respiration rate and subsequent respiration expressed on a proportional basis (Fig. 2.8). This enabled comparison of the magnitude of the increase or decrease in CO_2 efflux following addition of different substrates to different soils.

Sand and 'sand with water' additions across the two soil sites did not alter CO_2 efflux above that of the basal soil CO_2 efflux throughout the subsequent 5 h monitoring period. The addition of $HgCl_2$ had differing impacts on CO_2 efflux across the 5 h period. At 2 h, $HgCl_2$ had a greater impact on CO_2 efflux than inorganic N (though not statistically different, 182 ± 26 vs. 247 ± 70%); however, by 5 h $HgCl_2$ had the smallest CO_2 efflux (70 ± 10%) though not significantly smaller than basal soil CO_2 efflux, regardless of soil type (81 ± 7%).

Low MW C additions produced the greatest effects on CO_2 efflux. The CO_2 efflux response following glucose substrate addition was maintained at elevated levels across the 5 h period but decreasing from 5.5 times the original respiration to 3.7 times at 5 h (549 ± 90 to 365 ± 64% that of basal respiration). This was the greatest CO_2 efflux response following substrate addition at each of the 5 time periods ($P < 0.001$). Glutamate also increased CO_2 efflux over the 5 h monitoring period, but to a significantly lesser extent than glucose with the response falling from 3.2 times original CO_2 efflux to 2.4 times at 5 h (319 ± 29 to 240 ± 23% of basal respiration). Inorganic N additions affected CO_2 efflux to a lesser extent. Initially, CO_2 efflux increases were seen (2.3 times original efflux, 226 ± 31% of basal respiration), but after 2 h the increases in CO_2 efflux were similar to that seen in the $HgCl_2$, sand and water addition treatments.

Averaging across the 5 h monitoring period showed that there was no statistical difference in the percentage increase in CO_2 efflux following additions

to the two different polar soils (Zeppelinfjellet and Stuphallet; 182 ± 8% and 170 ± 7% of basal respiration respectively). Lichen soil had a statistically lower CO_2 efflux than the plant modified topsoil and subsoil following all additions (lichen: 122 ± 2%, subsoil: 195 ± 9%, topsoil: 210 ± 12% of basal respiration; $P < 0.001$). Across the 5 h monitoring period, glucose (333 ± 23%, statistically greatest response in CO_2 efflux), glutamate (234 ± 15%) and inorganic N (160 ± 7%) additions all showed statistically greater CO_2 efflux than soils where only sand or sand and water had been added (131 ± 9%, 146 ± 10% of basal respiration respectively; $P < 0.001$). There was no significant decline in respiration following the addition of $HgCl_2$ (129 ± 11%, compared to 97 ± 3% for un-amended soil), which was unexpected.


Zeppelinfjellet soil
Zeppelinfjellet soil + sand
Zeppelinfjellet soil + water
Zeppelinfjellet soil + $HgCl_2$
Zeppelinfjellet soil + glucose
Zeppelinfjellet soil + glutamate
Zeppelinfjellet soil + inorganic N
% change in respiration (basal respiration 100 % at 3 h, running averages over 1 h)
Time (h)
Topsoil
Subsoil
Lichen

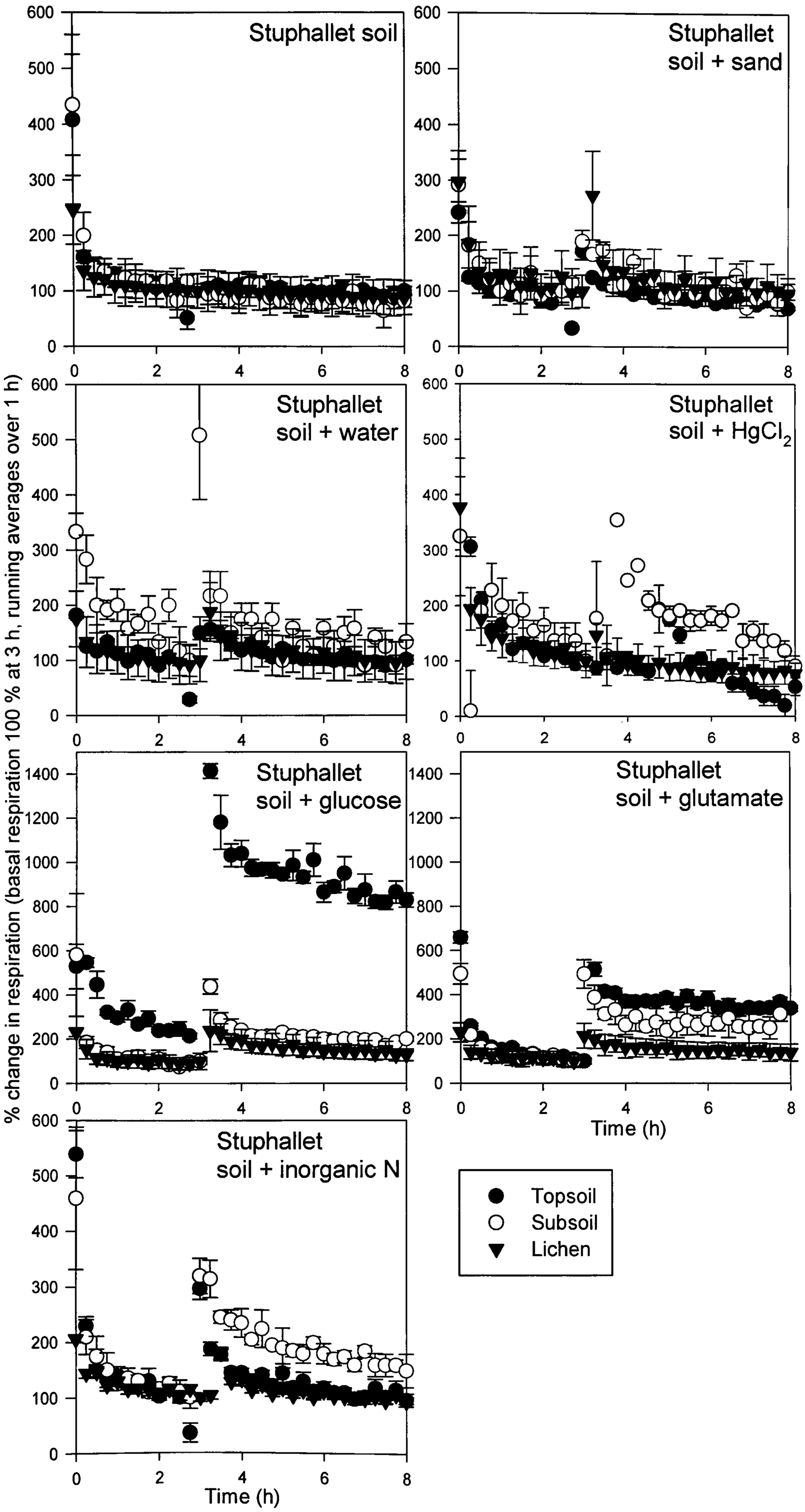

Stuphallet soil
Stuphallet soil + sand
Stuphallet soil + water
Stuphallet soil + $HgCl_2$
Stuphallet soil + glucose
Stuphallet soil + glutamate
Stuphallet soil + inorganic N
% change in respiration (basal respiration 100 % at 3 h, running averages over 1 h)
Time (h)
Topsoil
Subsoil
Lichen

Figure 2.8. Laboratory respiration of Zeppelinfjellet and Stuphallet soils, comparing pooled topsoil samples from under *Dryas*, *Saxifrage* and *Carex* (n = 3) to lichen and subsoil, with their response to additions of C and N. Bars are SEM, $n = 3$.

2.4. Discussion

2.4.1. UK and Svalbard basal respiration rate

Principally, soil respiration is controlled by the inputs of C from plants and root respiration, and by the microbes obtaining substrate from C inputs and soil OM pools (Fig. 2.1; Ward and Strain, 1999). It is important to understand the mechanisms controlling the total CO_2 efflux from the soil in order to be able to quantify the impact of climate change on soil carbon storage. This study focused on establishing the basal CO_2 efflux from High Arctic and UK grassland soils and to establish the effects of substrate limitation on CO_2 efflux following the removal of new substrate for 1.15 y. This study clearly shows that the magnitude of the measured CO_2 flux in different soils depended on how the measurements were made. Measurements of CO_2 efflux per unit of soil surface in the field indicated a much smaller rate of efflux from the High Arctic soils than in the UK soils (1.09 ± 0.25 in UK vs. 0.34 ± 0.08 g CO_2 m^{-2} h^{-1} in Svalbard), which is expected, as measurements of soil respiration by major biome types demonstrate significant differences (Raich and Schlesinger, 1992).

However, measurement per weight of soil where O horizons alone were compared revealed no significant difference between High Arctic and UK grassland soils (CO_2 efflux averaged 11 ± 1 μg CO_2 g^{-1} h^{-1}), indicating that the microbial community is just as active in the O horizon in the two climatic regions. The microbial communities found in the Arctic tundra are also found in

less extreme climates (Robinson and Wookey, 1997), therefore the main difference between tundra and other ecosystems is the rate of decomposition and the delivery of substrate rather than the processes involved. Therefore, when the measurements are compared at the same temperature, moisture and spatial scale it is likely that they would be comparable (Robinson and Wookey, 1997).

The type of vegetation influencing the topsoil had no net effect on the CO_2 efflux from the High Arctic soils. This supports the conclusions of Raich and Tufekcioglu (2000) who found no predictable differences in soil respiration based on vegetation, with temperature, moisture content and substrate being more important factors controlling respiration rates. In contrast, however, Raich and Schlesinger (1992) did find that belowground respiration correlated well with vegetation cover type. CO_2 efflux from the High Arctic soils was significantly lower when plant material (roots and shoots) was removed, reducing to approximately 20% of the CO_2 efflux when plant material was included. Sieved soils from the UK agricultural catena contributed approximately 70% of the respiration of an intact core, though there was high variability between soils. This finding is similar to the respiration rates obtained from forest soils where topsoil had been removed – the mineral soil approximated 60% of the respiration (Buchmann, 2000). We conclude that that microbial respiration can therefore constitute a significant component of the total flux in both grassland and High Arctic soils.

When expressed on a per unit weight basis, the respiration rates of excised shoots and roots from the UK grassland site had much greater respiration rates than the soil. This is probably due to the inherent respiration within plant cells within the first few days (until their internal C supply becomes exhausted)

and then cell lysis and subsequent microbial mineralization of plant derived C (Jones and Darrah, 1994). Root addition to soil caused a linear increase to the CO_2 efflux from sieved soil, which again could be due to a combination of CO_2 derived from the microbial breakdown of root cell lysate as well as actual root respiration.

In most cases, the efflux of CO_2 from UK grassland soils declined exponentially over the 1.15 y experimental period with respiration rates taking up to 7 months to stabilize. In agreement with Jones and Shannon (1999), sieving appeared to have little overall effect on the activity of the microbial population (the main impact being in the removal of root material). In the experiments detailed here the soil cores received no fresh additions of C (due to the removal of photosynthetic inputs); the decline in respiration rates indicates that the soils are C substrate limited as respiration rates begin to decline immediately. The inclusion of shoot and root material did not decrease the rate of decline, indicating that the limiting substrate is not medium to high MW compounds, but moreover low MW substrate, such as root exudates.

Three half-times were identified from this study. Firstly, the calculated MRT for the rapid metabolism of substrate within the soil (approximately 40 d) was similar to the turnover of ^{14}C in the microbial biomass < 25 d (Table 2.5; Boddy et al., 2007a) and 6 to 49 d for the microbial biomass in New Zealand soils (Saggar et al., 1999). Therefore this first phase is likely to be the MRT for the readily decomposable substrates, such as simple metabolites and storage compounds [e.g. organic acids, amino acids, sugars, poly(3-hydroxybutyrate), polyhydroxyalkanoates etc. (Anderson and Wynn, 2001)]. Secondly, the second phase MRT for sieved soil was longer, 1.41 y, which is similar to the MRT

reported for microbial biomass turnover (0.25 to 2.5 y; van Hees et al., 2005; Boddy et al., 2007c). Thirdly, the third phase MRT for intact cores and excised shoots and roots was much longer, in excess of 5,000 y, which is likely to reflect the turnover of recalcitrant C (Boddy et al., 2007c), as 1000-2000 y has been reported for the MRT of semi-stable soil OM (van Hees et al., 2005; Boddy et al., 2007c).

2.4.2. C and N limitations on respiration in the laboratory

Soil microbes have a high demand for low MW C and N (Jones, 1999). In most soils, microbial activity is limited by the availability of organic substrates (Schlesinger and Andrews, 2000). C and N limitation has been shown in microbial respiration at Ny-Alesund, Svalbard, in early successional field sites (Yoshitake et al., 2007). In my experiments, there was a clear order of CO_2 efflux response with glucose having the greatest effect followed by glutamate and lastly inorganic N. Glucose and L-asparagine maintained respiration above that of a water control for 8 h, following a similar pattern to that shown here, with a large initial increase (3 times that of the control) declining over the monitoring period (Pennanen et al., 2004). Amino acid mineralization in soil appears to be highly dependent upon the chemical composition of the individual amino acid (e.g. net charge, location in assimilatory pathway) and its reaction with soil (e.g. sorption and metal complexation processes; Vinolas et al., 2001b). Low microbial assimilation efficiency has been shown for glutamate when compared to glycine and lysine (Vinolas et al., 2001b). Boddy et al. (2007b) also demonstrated higher mineralization rates for an amino acid mixture than glucose in Arctic soils. Indeed, amino acid mineralization is generally poorly diagnosed by measuring

soil respiration, as there are many biotic and abiotic factors controlling the biodegradation rate – such as water content, extracellular enzymes (e.g. deaminases), and the microbial community structure (Jones, 1999).

The response and magnitude of CO_2 efflux following glutamate amendment suggests that the Arctic microbial community assimilation of low MW DON is rapid, however, it is probable that the response is due to C limitation rather than N limitation, which would explain the reduced CO_2 efflux response to added inorganic N (Jones and Murphy, 2007). Additions of inorganic N can enhance, suppress or have no effect on organic matter mineralization (Jones and Shannon, 1999). N addition can cause a suppression of microbial respiration for a number of reasons including: (1) a toxic or osmotic pressure from high addition of N salts, (2) reduced C availability due to the condensing of the added N with soil humus, (3) suppression of fungal ligninolytic activity by added N, or (4) reduced production of enzymes to attack N-containing OM (Vance and Chapin, 2001). In this study, inorganic N had a small, but significant effect on respiration in the short term. Respiration, in the short-term, responded primarily to additions of labile C, but longer-term measurements may have shown an additional response from the addition of N. Vance and Chapin (2001) demonstrated a lag period of 20-40 d before a respiratory response to inorganic N became apparent. Other nutrients, such as phosphorous and sulphur may also limit the soil response to increased N inputs (Allan and Schlesinger, 2004; Madan et al., 2007).

Unexpectedly $HgCl_2$ did not suppress CO_2 efflux below basal respiration until 5 h after additions. Previous studies had demonstrated an immediate effect of adding $HgCl_2$ on respiration, though other microbial inhibitors such as sodium

azide can increase respiration before causing a subsequent reduction in CO_2 efflux (Jones et al., Unpublished data). The results suggest that Hg initially causes an increase in microbial activity possibly due to the activation of stress and defence related pathways in the microbial community that require large amounts of energy and consequently CO_2 production.

2.4.3. Conclusions

From this study, it can be concluded that biome differences in basal respiration rates between UK and Arctic field sites clearly depend on the method utilised to measure the CO_2 efflux. Field measurements showed that High Arctic soils had significantly smaller respiration rates than UK grassland soils, whereas laboratory measurements showed no significant difference per gram of O horizon. This highlights the need to define a standardized methodology for quantifying CO_2 fluxes in laboratory and field studies so that valid and direct comparison can be made between soils. One point that certainly needs to be made is whether measurements should be made on an area or weight basis.

Measuring the decline in CO_2 efflux over time from the components of grassland soils identified three distinct C turnover pools, firstly, that which we ascribe to the pool of low MW metabolites and storage compounds, with a MRT < 50 d; secondly, the microbial biomass pool, with a MRT of < 1.5 y; and lastly the stable soil OM pool with a turnover in excess of 5,000 y. C and organic and inorganic N additions to High Arctic soils all increased CO_2 efflux, though the greatest response was from the addition of low MW C. This indicates that the microbial community is predominantly C limited and that changes in

rhizodeposition and litter in an elevated CO_2 atmosphere could result in additional CO_2 efflux from the soil (Allan and Schlesinger, 2004).

2.5. Acknowledgements

This work was funded by the UK Natural Environment Research Council. We thank Jonathan Roberts for helping with the sample analysis in the UK and Nick Cox for helping with the sample collection near Ny-Ålesund, Svalbard, at the NERC Arctic Station.

2.6. References

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Part II

Turnover of SOM pools in UK and High Arctic soils

Part II (Chapters 3-6) focuses on the turnover of soil organic matter (SOM) pools from UK and Arctic soils, particularly focusing on the turnover of low molecular weight (MW) C in the dissolved organic carbon (DOC) pool, though with extrapolations being made on large MW C pools, and the impact of temperature on the rate of turnover (Fig. II.1). Part II is divided into four papers, two of which are published in Soil Biology & Biochemistry and two are in the process of being submitted. As such there is some unavoidable over-lap in the methodologies of the four papers.

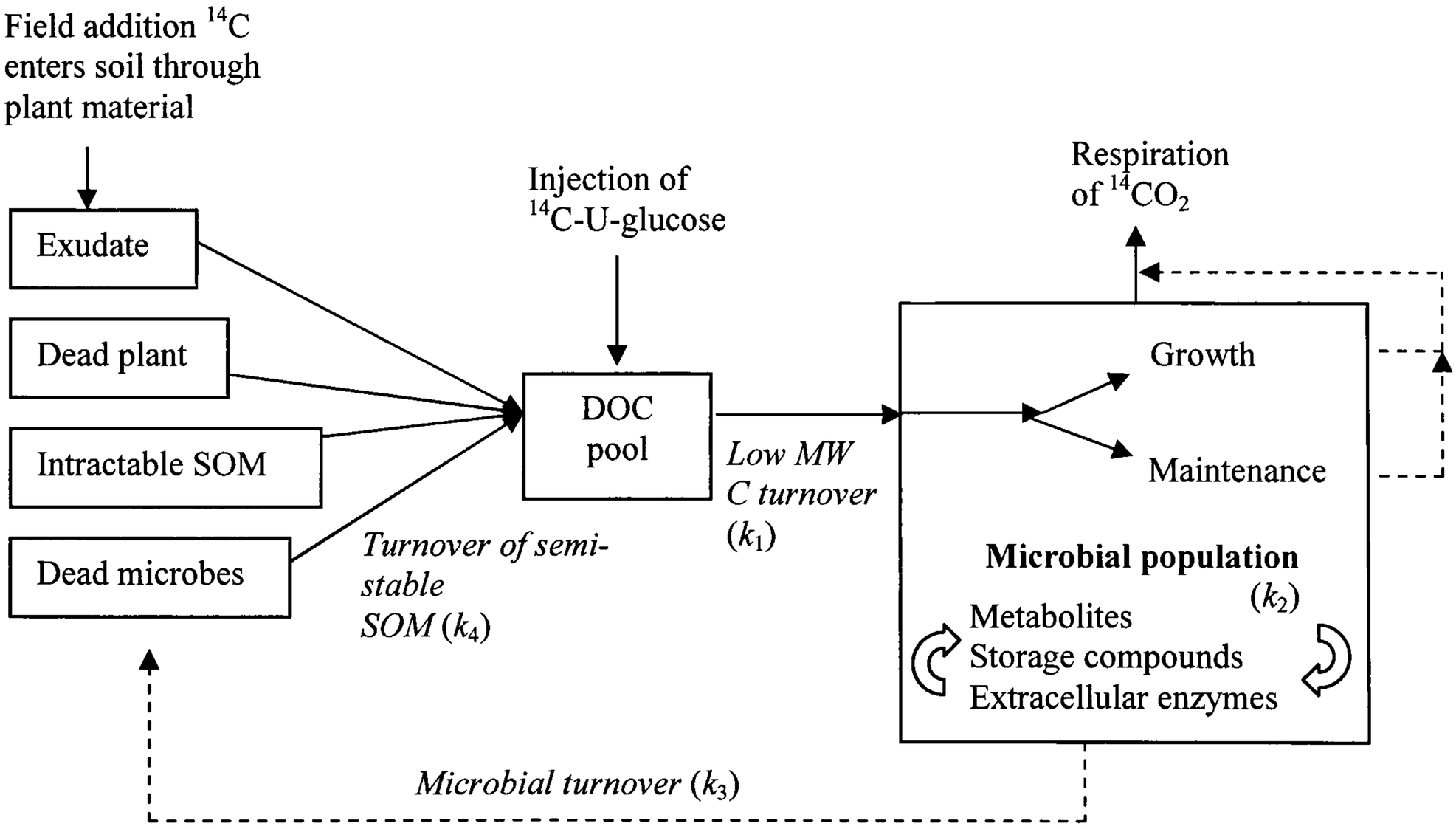

Figure II.1. Schematic of the fate of ^{14}C substrate either added to the soluble DOC pool to plant material. Indicating the suggested location of the temporal SOM pools described by the exponential coefficients k_1, k_2, k_3, and k_4.

Chapter 3

Fast turnover of low molecular weight components of the dissolved organic carbon pool of temperate grassland field soils

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Published in Soil Biology & Biochemistry 39, 2007, 827-835

Nearly all of the experimental and written work in this paper was undertaken by Elizabeth Boddy. Dr Paul Hill assisted in establishing the field plots and I am grateful for his proof-reading. I thank my supervisors for their continued support and guidance.

3.0. Abstract

Large amounts of low molecular weight (MW; <250 Da) carbon (C) are lost from roots into the rhizosphere as a consequence of root turnover and exudation. Their rates of turnover after release into the soil remain poorly understood. We extracted soil solution from a temperate grassland Eutric Cambisol, isotopically labelled the glucose and amino acid components, and then re-injected the solution back into the soil. We followed the subsequent evolution of $^{14}CO_2$ and incorporation of the low MW C into the soil microbial biomass or grasses for 48 h. The experiments were performed both on grazed and un-grazed swards in the field, and in the laboratory. In the field, we showed that glucose and amino acids had short half-lives ($t_{½}$) in soil solution ($t_{½}$ = 20-40 min), but that they persisted in soil microbes for much longer. A first order double exponential model fitted the experimental data well and gave rate constant (k) values of 1.21-2.14 h^{-1} for k_1 and 0.0025-0.0048 h^{-1} for k_2. Only small amounts of the added ^{14}C were recovered in plant biomass (<5% of total added to soil) indicating that plant roots are poor competitors for low MW dissolved organic C (DOC) in comparison to soil microorganisms. The first phase of glucose and amino acid mineralization in the laboratory was slower ($t_{½}$ = 40-60 min) than measured in the field reinforcing the importance of making flux measurements *in situ*. Whilst grazing stimulated below-ground respiration, it exerted only a small influence on the turnover of low MW DOC suggesting that the increase in respiration was due to increased root respiration and not turnover of soil organic matter (SOM). Our results suggest that some components of the low MW DOC pool are turned over extremely rapidly (ca. 4000 times annually).

Keywords: Biodegradation; Carbon cycling; Carbon dioxide; Dissolved organic nitrogen; DON; Mineralization; Rhizodeposition; Soluble carbon; Dissolved organic carbon; DOC.

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Chapter 4

Turnover of low molecular weight DOC and microbial C demonstrate different temperature sensitivities in Arctic tundra soils

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Submitted to the Journal of Soil Biology & Biochemistry – In Press

Nearly all of the experimental and written work in this paper was undertaken by Elizabeth Boddy. Dr Paul Hill and Dr Paula Roberts assisted in the polar field work and I am grateful for Dr Paul Hill's proof-reading. I thank my supervisors for their continued support and guidance.

4.0. Abstract

Polar ecosystems are currently experiencing some of the fastest rates of climate warming. An increase in soil temperature in Arctic regions may stimulate soil permafrost melting and microbial activity, thereby accelerating losses of greenhouse gases. It is therefore important to understand the factors regulating the rates of C turnover in polar soils. We investigated microbial mineralization of labile low molecular weight (MW) dissolved organic C (DOC) in two High Arctic tundra soils. Soil solutions spiked with either ^{14}C-labelled glucose or amino acids were added to the top- and sub-soil from two ecosystem types (lichen and *Carex* dominated tundra), maintained at three temperatures (4-20 °C), and their microbial mineralization kinetics monitored. The soils' concentration-dependent glucose and amino acid mineralization kinetics (0-10 mM) were also investigated. $^{14}CO_2$ evolution from the tundra soils in response to ^{14}C-glucose and -amino acid addition could best be described by a double first order exponential kinetic equation with rate constants k_1 and k_2. Both forms of DOC had a short half-life ($t_{½}$) in the pool of microbial respiratory substrate ($t_{½}$ = 1.07 ± 0.10 h for glucose and 1.63 ± 0.14 h for amino acids; exponential coefficient k_1 = 0.93 ± 0.07 and 0.64 ± 0.06 h^{-1} respectively) whilst the second phase of mineralization assumed to be C that had entered the microbial biomass, was much slower (average k_2 = 1.30 × 10^{-3} ± 0.49 × 10^{-4} h^{-1}). Temperature had little effect on the rate of mineralization of ^{14}C used directly as respiratory substrate. In contrast, the turnover rate of the ^{14}C immobilized in the microbial biomass prior to mineralization was temperature sensitive (k_2 values of 0.99 × 10^{-3} h^{-1} and 1.66 × 10^{-3} h^{-1} at 4 and 20 °C respectively). The mineralization of glucose and amino acids was best described using Michaelis-Menten kinetics; there was a

low affinity for both C substrates by the microbial community (K_m = 4.07 ± 0.41 mM, V_{max} 0.027 ± 0.005 mmol kg^{-1} h^{-1}). In conclusion, our results suggest that in these C limiting environments the flux of labile, low MW DOC through the soil solution is extremely rapid and relatively insensitive to temperature. In contrast, the turnover of C incorporated into higher MW microbial C pools appears to show greater temperature sensitivity.

Keywords: Biodegradation; Carbon cycling; Climate Change; Dissolved organic matter; Dissolved organic nitrogen; Global warming; Mineralization; Respiration

Chapter 5

The acclimation of soil microorganisms to temperature change in UK grassland soils

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All the experimental work and the majority of the written work in this paper was undertaken by Elizabeth Boddy. Dr Paul Hill assisted in establishing the field plots and I am grateful for his proof-reading. I thank Professor Erland Bååth for enabling me to visit his laboratory in Lund University and taking the time to teach me how to undertake phospholipid fatty acid analysis. I thank my supervisors for their continued support and guidance.

5.0. Abstract

Despite the large quantity of research examining the impact of temperature on soil organic matter decomposition there is still little consensus on whether an increase in soil CO_2 efflux will be observed with elevated temperature (Kirschbaum, 2004; Knorr et al., 2005). The aim of this study was to understand the factors regulating C turnover in three contrasting grassland soils under a range of temperatures (4 to 20 °C) and whether this turnover showed an acclimation response. Our results showed that temperature significantly altered the microbial PLFA profile, with greater relative concentration of monounsaturated fatty acids at lower temperatures (e.g., 18:1ω7 and 16:1ω7) and greater relative concentrations of saturated fatty acids at higher temperatures (e.g., 15:0 and 16:0). Significant changes in soil solution chemistry were also observed during the incubation with concentrations of DOC, phenolics and P increasing, NO_3^- decreasing and glucose and amino acid concentrations remaining constant. Over the same period, soil respiration rates remained unchanged, indicating that although microbial membrane composition and soil solution chemistry had changed, total system fluxes of $^{12}CO_2$ from the soil remained unchanged. ^{14}C-glucose mineralization was described by a double first order exponential equation and appeared to be temperature sensitive with more $^{14}CO_2$ evolved at 20 °C than at 10 °C, but with no evidence of acclimation. First phase exponential coefficients (k_1) remained insensitive to temperature change and also did not acclimate. The main effect of temperature was to alter the partitioning of ^{14}C into pool a_1, with more ^{14}C allocated to respiration rather than immobilization at higher temperatures. In conclusion, our results indicate that changes in the composition of microbial membranes occur rapidly due to

temperature changes, but that changes in functionality due to temperature were not apparent.

Keywords: Acclimation; Biodegradation; Carbon cycling; Dissolved organic matter; DOC; DON; Mineralization

5.1. Introduction

The uppermost metre of the world's soils contains approximately 1500 Gt C and represents the largest store of organic C in the terrestrial biosphere, being three times larger than that present in the supporting vegetation (Kirschbaum, 2000; Rasse et al., 2005). If climate change stimulates the mineralization and mobilization of this soil C store there is potential for a large positive feedback. There is an ongoing debate, however, over how large the impact of climate change will be on soil CO_2 efflux (Davidson and Janssens, 2006). Warming might increase both the rate of decomposition and net primary production, which could potentially result in only small changes in atmospheric CO_2 concentration. However, Fang and Moncrieff (2001) state that the efflux of soil C is highly sensitive to changes in surface temperature and relatively small changes may have a major influence on the magnitude of soil efflux. For accurate prediction of climate effects on terrestrial C cycling requires a clear understanding of the effects of temperature on the microbially mediated release of CO_2 from soil organic matter (SOM).

The soil microbial biomass is highly active, especially within the rhizosphere where root exudation (up to 10% of a plant's net fixed C) is dominated by low molecular weight (MW) compounds such as sugars, amino

acids and organic acids (Jones, 1999; Kuzyakov and Jones, 2006). The turnover of low MW compounds by microorganisms contributes substantially to the total CO_2 efflux from soil (van Hees et al., 2005). Identifiable low MW compounds (forming <10% of the dissolved organic matter in soil) are recognized as the primary energy source for soil microorganisms. Little is known, however, about the effects of temperature on the concentrations of low MW compounds in soil (van Hees et al., 2005).

Soil contains many thousands of organic compounds all of which can be expected to exhibit unique kinetic mineralization properties. These differences in relative turnover rate can be ascribed to: (1) differences in their physicochemical properties (e.g. charge, solubility, size), which will subsequently affect their transport rates and sorption in soil (Jones and Edwards, 1998), and (2) differences in the range of microorganisms capable of taking them up and their subsequent pathways of metabolism. Consequently, if soil C is considered as one homogeneous pool then the dynamics of smaller pools can be masked and their temperature dependency may be masked. Temperature may only affect a small fraction of the total soil C pool and therefore there is the need for a compound specific approach (Davidson and Janssens, 2006).

Temperature affects most of the major processes in the C cycle, including the initial fixation of C in photosynthesis and the allocation of C between roots and shoots, root growth and respiratory losses of C by plants, and the decomposition of SOM by soil microorganisms. Respiration can only proceed under the constraints of the available substrates (Fitter et al., 1998). In response to temperature change, an initial increase in soil SOM decomposition has been observed, however, this enhancement in SOM mineralization may be short lived

due to potential changes in either substrate availability or microbial community composition and metabolism (i.e. adaptation and acclimation; Kirschbaum, 2004; Luo et al., 2001). Respiration rates in plants are also known to acclimate to changes in temperature over periods of days to months (Wythers et al., 2005). For the purposes of our study, acclimation is defined as history-dependent rates of key processes, such as respiration or mineralization. Thus the acclimated rate will partly depend on the environmental conditions that preceded measurement rather than simply being a function of the conditions of measurement. Short-term acclimation depends on changes in enzyme activity and gene expression and typically has a time-course of days to a week. Acclimation is a result of co-limitation of biological processes, such as respiration being limited by environmental parameters; and co-limitation enables the most effective use of resources. Soil responses are particularly complex and difficult to predict because of the intrinsic diversity of organisms and heterogeneity in their spatial arrangement. Alterations in soil respiration could be due to acclimation of individual organisms (e.g. by altering enzyme activity and gene expression), or adaptation through changes in microbial community structure.

Using a range of grassland soils and in light of the above discussion, the aims of this study were to determine whether relatively short-term changes in temperature significantly affected (1) the concentration of low MW organic C substrates in soil solution during the incubation, (2) the microbial uptake and partitioning of these substrates, (3) changes in soil microbial community structure.

5.2. Methods

5.2.1. Field sites and sampling regime

Soil was obtained from a series of temperate oceanic agricultural grasslands located at Abergwyngregyn, Gwynedd, North Wales (53°14'N, 4°01'W; Table 5.1). The mean annual soil temperature at 10 cm depth is 11 °C and the annual rainfall is 1250 mm. Soil was sampled from three locations along a 1 km catena sequence and included a Eutric Cambisol developed on glacial till, a Dystric Gleysol developed on glacial lacustrine deposits and a Haplic Podzol developed on Ordovician shales. The Eutric Cambisol field site contained three delineated areas of improved pasture for high-density sheep grazing (> 10 ewes ha^{-1}), three areas that had not received any grazing for 2 y, and three areas that had been covered with a gas and moisture membrane which has prevented plant growth and new C inputs for 2 y. Soil was sampled in April 2005 and 2006 at which time the soil temperature averaged 12.3 ± 0.7 °C across the five sampling sites. Soil was collected from each site from three replicate locations using a stainless steel corer (0-5 cm depth). Discernable roots were removed prior to analysis.

Table 5.1. Characterisation of selected properties of UK grassland soils across a catena sequence (where U.G. denotes un-grazed sward and N.V. non vegetated). Values represent means ± SEM; n = 3. The symbols *, NS (not significant) indicate the significant differences at the $P < 0.05$ level between the grassland field sites.

Field site	Moisture content (g kg^{-1})	pH	Total C (g kg^{-1})	Total N (g kg^{-1})	C-to-N ratio	Soil respiration (mg CO_2 kg^{-1} h^{-1})
Gleysol	35 ± 1	5.7 ± 0.7	43 ± 2	* 4.5 ± 0.3	* 9.6 ± 0.2	3.3 ± 0.9
Podzol	28 ± 1	4.4 ± 0.2	* 104 ± 11	* 9.1 ± 0.8	11.3 ± 0.3	2.0 ± 0.4
Cambisol grazed	32 ± 1	5.4 ± 0.6	31 ± 2	2.6 ± 0.3	* 12.2 ± 1.0	2.9 ± 0.6
Cambisol U.G.	31 ± 1	6.0 ± 0.2	32 ± 4	3.0 ± 0.4	10.9 ± 0.2	3.0 ± 0.6
Cambisol N.V.	* 44 ± 1	4.8 ± 0.6	26 ± 1	2.4 ± 0.2	10.9 ± 0.6	2.6 ± 0.4
	*	*NS*	*	*	*	*NS*

5.2.2. Background soil characteristics

Soil was analysed according to Boddy et al. (2007a) with root free soil analysed for C and N content using a Leco CHN 2000 analyzer (Leco Corp., St Joseph, MI, USA). Soil pH was measured in a 1:1 (v/v) mixture with deionised water. Moisture content was assessed by oven drying at 105 °C for 24 h. Soil respiration was measured in the laboratory using an automated SR1 infra red gas analyser (PP-Systems Ltd., Hitchin, UK).

Soil solution was extracted according to Giesler and Lundström (1993). Briefly, intact soil cores were centrifuged (4000 *g*, 15 min, 20 °C) to obtain soil solution and the collected solutions passed through a Whatman 42 filter paper prior to storage at -20 °C to await chemical analysis (Table 5.2). Soil solution samples were analyzed for dissolved organic C (DOC) and total dissolved N (TDN) using a Shimadzu TOC-V-TN analyzer (Shimadzu Corp., Kyoto, Japan). NO_3^- was determined colorimetrically by the Cu-Zn-hydrazine reduction method

Table 5.2. Characterization of selected properties of the soil solutions across a catena sequence of UK grassland soils. Free amino acids assumes an average C content of 62.4 g mol^{-1} and an average N content of 19.6 g mol^{-1}. Values represent means ± SEM (n = 3). The symbols * and *NS* (not significant) indicate the significant differences at the $P < 0.05$ level between the grassland soil solutions.

Sample site	DOC	DON	Glucose	Total free amino acids		Total phenols	Phosphates	NO_3^-	NH_4^+
	(mg C l^{-1})	(mg N l^{-1})	(mg C l^{-1})	(mg C l^{-1})	(mg N l^{-1})	(mg C l^{-1})	(mg P l^{-1})	(mg N l^{-1})	(mg N l^{-1})
Gleysol	176 ± 37	27.3 ± 3.7	14.4 ± 1.7	3.7 ± 0.1	1.2 ± 0.1	9.1 ± 1.8	1.0 ± 0.3	1.1 ± 0.1	3.0 ± 0.5
Podzol	* 573 ± 73	66.3 ± 12.0	16.9 ± 0.5	4.3 ± 0.3	1.3 ± 0.1	15.6 ± 1.2	0.9 ± 0.4	0.8 ± 0.0	4.3 ± 0.9
Cambisol grazed	130 ± 21	40.4 ± 12.1	17.3 ± 2.7	6.7 ± 4.2	2.1 ± 0.8	20.2 ± 3.6	3.9 ± 1.4	1.0 ± 0.01	3.5 ± 0.4
Cambisol un-grazed	133 ± 33	36.1 ± 16.8	19.2 ± 3.5	6.1 ± 1.2	1.9 ± 0.2	21.8 ± 7.8	4.2 ± 2.6	1.1 ± 0.2	6.6 ± 3.4
Cambisol no vegetation	175 ± 16	81.6 ± 50.3	25.9 ± 7.3	1.8 ± 0.1	0.6 ± 0.1	7.8 ± 2.3	2.7 ± 1.2	1.9 ± 0.3	3.6 ± 0.3
	*	*NS*	*NS*	*NS*	*NS*	*NS*	*NS*	*NS*	*NS*

of Downes (1978) and NH_4^+ by the salicylate-hypochlorite procedure of Mulvaney (1996). Phenolic concentrations were assessed using the Folin-Ciocalteau reagent (Swain and Hillis, 1959). Free amino acids were measured fluorimetrically according to Jones et al. (2003). Glucose was determined spectrophotometrically with a Glucose (GO) Assay® kit (Sigma-Aldirch, MO, USA). Phosphate was determined colorimetrically according to Murphy and Riley (1962).

5.2.3. Changes in microbial PLFAs in response to temperature

Microbial community structure was assessed using phospholipid fatty acid (PLFA) analysis. Briefly, 40 g of soil was incubated field-moist for 21 d at 10, 15 or 20 °C (n = 4). PLFAs were subsequently extracted and analysed using the procedure described by Frostegård et al. (1993). Briefly, 1 g of soil was extracted in a single-phase mixture of chloroform:methanol:citrate buffer (1:2:0.8 v/v/v). The phases were separated using a chloroform and citrate buffer solution. Following extraction, the lipids were fractionated into neutral lipids, glycolipids and polar lipids (the phospholipids) on a silica column using chloroform, acetone and methanol in turn. An internal standard of methyl nonadecanoate fatty acid (19:0) was added. The phospholipids were methylated and separated on a gas chromatograph (GC) equipped with a flame ionisation detector. Peak areas were then quantified and analysed using ANOVAs.

PLFA are designated as per Frostegård et al. (1993) and Herrmann and Shann (1997). The nomenclature follows the pattern A;BωC, with A identifying the total number of carbon atoms, B the number of double bonds, followed by the position of the double bond from the methyl end (ω) of the fatty acid. *Cis* and

trans configurations are denoted by *c* and *t*. Prefixes *a* and *i* indicate anteiso- and iso-branching; *br* indicates an unknown methyl branching position; methyl groups are denoted by Me; and *cy* refers to cyclopropane fatty acids. All solvents used were of analytical grade and glassware was heated to 400°C overnight to remove lipid contaminants.

Data of the peak areas for 25 identified PLFA were transformed to be a percentage of the total peak area for all 25 fatty acids (Table 5.3). Bacterial-to-fungal ratios were calculated using the PLFA 18:2ω6 as a measure of fungal biomass and the sum of fifteen bacterial PLFA to estimate bacterial biomass (*i*14:0, *i*15:0, *a*15:0, 15:0, *i*16:0, 10 Me 16:0, *i*17:0, *a*17:0, *cy*17:0, 17:0, *br*18:0, 10 Me 17:0, 18:1ω7, 10 Me 18:0, and *cy*19:0; Bååth and Anderson, 2003). Fourteen PLFA were identified as varying with temperature change. Saturated PLFAs 14:0, 15:0, 16:0, *i*16:0, *i*17:0, *a*17:0, 17:0, 18:0, *cy*19:0, and 20:0 were expected to increase in concentrations at higher temperature and unsaturated fatty acids 16:1ω5, 16:1ω7*c*, 16:1ω9 and 18:1ω7*c* were expected to increase at low temperature (Pettersson and Bååth, 2003).

5.2.4. Soil solution chemistry responses to incubation

Intact swards ($n = 3$) were collected from the grazed Eutric cambisol field site and placed in climate-controlled chambers (Fi-totron PG660/C/RO/HQI, Sanyo-Gallenkamp Ltd, Loughborough, UK) held at 20°C. At 1 h, 1 d, 3, 7, 14 and 21 d after placement in the cabinets, soil solution was recovered and analysed for amino acids, glucose, NH_4^+, NO_3^-, PO_4^{3-}, TDN, DON, phenolics, DOC and respiration as described above.

5.2.5. Substrate mineralization responses to temperature

Soils were incubated for 0, 7, 14 or 21 d at either 10 or 20 °C. At each time interval, replicate samples of each soil (n = 3) were either:

1) maintained at either 10 or 20 °C (temperature static)

2) switched from 10 to 20 °C for 1 h prior to mineralization (temperature rise)

3) switched from 20 to 10 °C for 1 h prior to mineralization (temperature fall)

Immediately following this, ^{14}C-labelled glucose mineralization was determined according to Boddy et al. (2007a). Briefly, 0.5 ml of ^{14}C-U-glucose (10 nM; 37 kBq ml^{-1}; Amersham Biosciences UK Ltd, Chalfont St. Giles, UK) was added to 5 g of soil contained in a polypropylene container. A vial containing 1 ml of 1 M NaOH to trap $^{14}CO_2$ was then placed next to the soil and the polypropylene container hermetically sealed. After 0.25, 0.5, 1, 2, 4, 8, 12, 20, 28, 36 and 48 h the NaOH traps were removed and the $^{14}CO_2$ trapped in them determined by liquid scintillation counting using a Wallac 1404 scintillation counter (Wallac EG&G, Milton Keynes, UK) and Optiphase 3® alkali compatible scintillation fluid (Wallac EG&G).

Glucose mineralization in soil is biphasic (Chotte et al., 1998; Saggar et al., 1999; van Hees et al., 2005; Boddy et al., 2007a), where substrate mineralization is described by double first order decay with asymptote:

$$S = S_U + [a_1 \times \exp(-k_1 t)] + [a_2 \times \exp(-k_2 t)] \qquad \text{(Eqn. 5.1)}$$

where S is the total ^{14}C-label remaining in the soil, S_U is an asymptote defined from the addition of Na$H^{14}CO_3$ instead of ^{14}C-labelled glucose substrate (Boddy et al., 2007a; i.e. the efficiency of the system at capturing $^{14}CO_2$ evolved within

the soil: 48 ± 2% gleysol, 50 ± 1% podzol, 47 ± 3% cambisol grazed, 54 ± 2% cambisol un-grazed, 39 ± 5% cambisol no vegetation; data not shown), k_1 is the exponential coefficient describing the primary mineralization phase, k_2 is the exponential coefficient describing the second, slower mineralization phase, a_1 and a_2 describe the size of pools with exponential coefficients k_1 and k_2 and t is 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 from the soil. The half-life ($t_{½}$) of the pool a_1 can be calculated as:

$$t_{½} = \ln(2)/ k_1 \qquad \text{(Eqn. 5.2)}$$

(Paul and Clark, 1996). The slower second phase (k_2) of $^{14}CO_2$ production is attributable to the subsequent turnover of ^{14}C immobilized within the soil microbial community. As discussed in Boddy et al. (2007a) we do not know enough about the connectivity between pools a_1 and a_2 so we do not calculate the half time for pool a_2.

5.2.6. Statistical and data analysis

All experiments were carried out in triplicate for mineralization studies and quadruplet for PLFA analyses. All data were analysed using factoral ANOVA (Univariate GLM) to evaluate change across different field sites and following incubation. PLFA data was analysed using indicatory PLFAs to investigate the impact of incubation temperature and the differences between sites, using both factoral ANOVA and principal component (PC) analysis. Mineralized $^{14}CO_2$ data were compared by field site, temperature and incubation interval. Statistical procedures were carried out with the software package SPSS

12.0 for Windows (SPSS Inc., Chicago, IL) with $P < 0.05$ used as the upper limit for statistical confidence. To assess whether acclimation had occurred in the mineralization investigation, comparisons of the 'temperature static' treatment were compared to the two temperature shift treatments ('temperature fall' and 'temperature rise'). Acclimation was said to occur if soils incubated at 10 °C and measured at 10 °C had greater mineralization rates than soils incubated at 20 °C and measured at 10 °C; and soils incubated at 10 °C and measured at 20 °C having smaller mineralization rates than soils incubated at 20 °C and measured at 20 °C.

5.3. Results

5.3.1. Influence of temperature on soil microbial community structure (PLFA)

The three different soils were analysed using Univariate ANOVAs to evaluate the impact of incubation temperature and soil type on microbial variation using 25 PLFAs (Table 5.3, Figs. 5.1 & 5.2). The type of soil had a significant effect on the amount and type of PLFAs isolated ($P < 0.001$). The percentage of bacterial-to-fungal PLFA (a relative measure of the bacterial:fungal biomass ratio) was significantly different between the podzol, gleysol and cambisol, with a greater relative percentage of fungal biomass being found in podzols (average ± SEM; cambisol 0.18 ± 0.010, gleysol 0.16 ± 0.001, podzol 0.22 ± 0.003). Podzols also had a significantly greater percentage of the gram-negative fatty acid *cy*19:0 ($P < 0.001$), and gram-positive fatty acid *i*16:0, but significantly less of the gram-negative 16:1ω5 and gram-positive fatty acid *i*17:0 ($P < 0.001$).

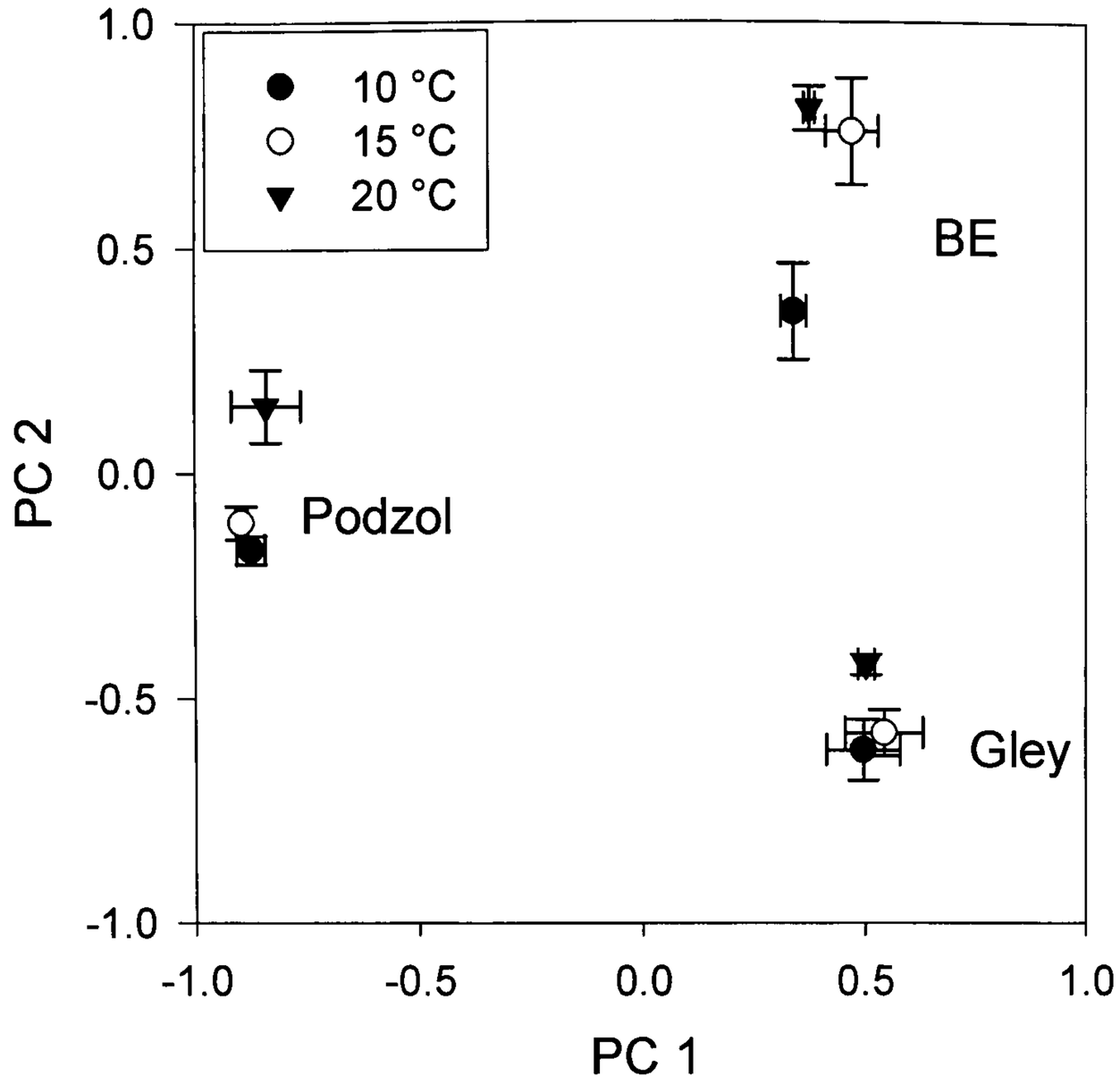


Figure 5.1. Principal component (PC) analysis of PLFA distribution, indicating the mean ± SEM for the different soils types by temperature. Podzol soils can clearly be seen to have different PLFA distribution on the first axis – attributable to the type of soil, and the primary differences between Gleysol and Cambisol (denoted BE) can be seen to be on the second axis attributable to variation in the PLFA distribution by temperature.

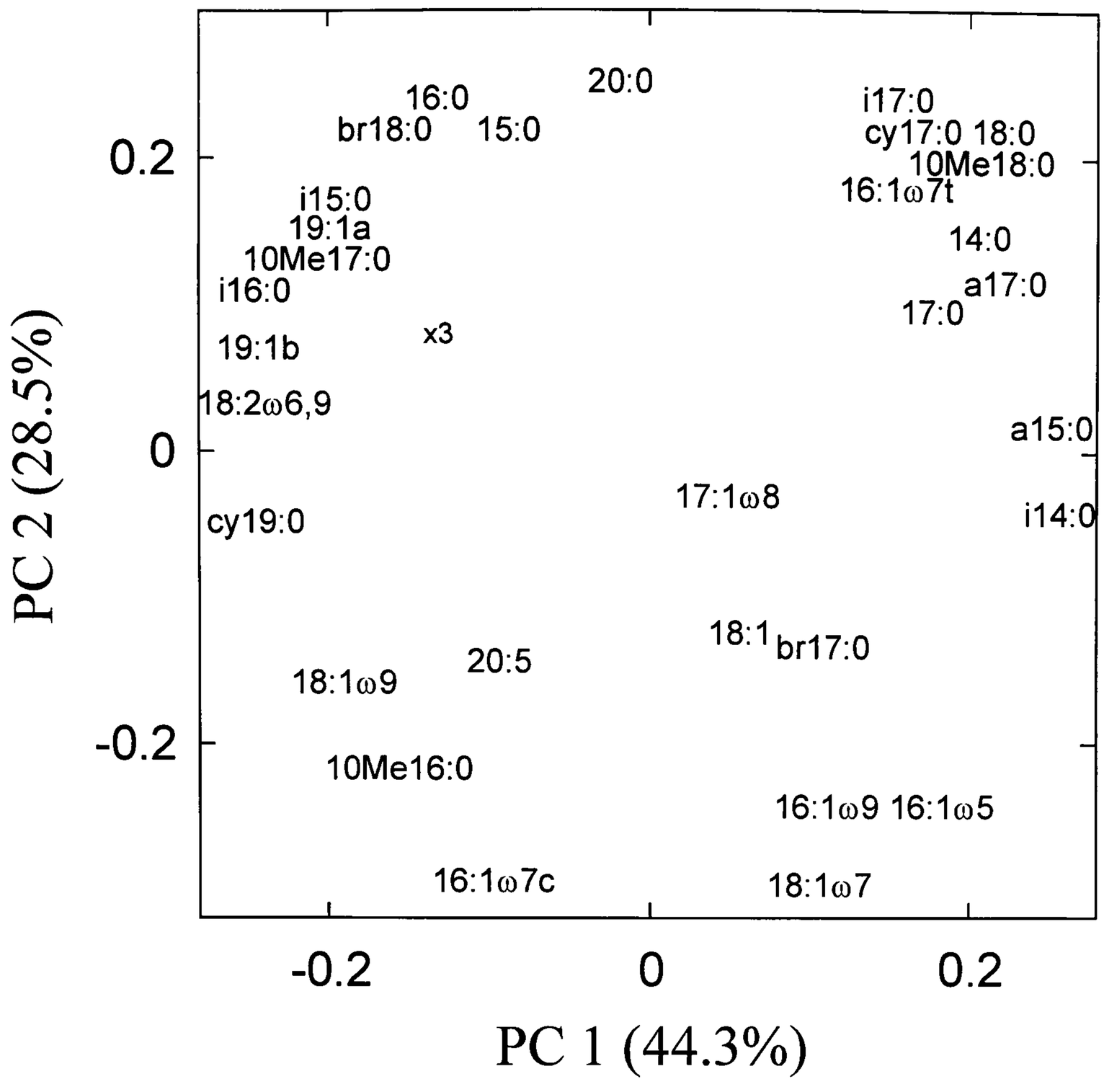


Figure 5.2. The distribution of PLFA following principal component (PC) analysis, with Podzol soil located on the left of the graph (Fig. 5.1.) separated by the elevated fungal population (18:2 and 18:1ω9), whilst the distribution of *cy* 19 and *i*16 and *i*15/*a*15 (*i*15 to the left, *a*15 to the right), with 16:1ω5, *i*14, *a*17, 10Me18 on the right of the axis indicate that pH is determining PC 1 (Bååth and Andersson, 2003). The distribution of monounsaturated fatty acids have the most negative values on PC 2, whilst the saturated fatty acids (14:0, 15:0, 16:0, 18:0, and 20:0) have positive values showing that they increase with temperature.

Table 5.3. The relative percentage (of sum of PLFAs) of selected indicator PLFAs for three soils incubated at 10, 15 or 20 °C for three weeks.

PLFA	Cambisol			Podzol			Gleysol		
	10 °C	15 °C	20 °C	10 °C	15 °C	20 °C	10 °C	15 °C	20 °C
***i*14:0**	1.13 ± 0.02	1.08 ± 0.02	1.06 ± 0.04	0.33 ± 0.22	0.29 ± 0.01	0.31 ± 0.02	0.96 ± 0.01	0.93 ± 0.04	0.87 ± 0.03
14:0	1.12 ± 0.02	1.08 ± 0.02	1.09 ± 0.02	0.94 ± 0.04	0.84 ± 0.04	0.87 ± 0.05	1.31 ± 0.01	1.23 ± 0.05	1.27 ± 0.05
***i*15:0**	7.13 ± 0.05	7.44 ± 0.09	7.56 ± 0.07	8.65 ± 0.24	8.68 ± 0.19	8.79 ± 0.18	8.16 ± 0.27	7.71 ± 0.47	8.31 ± 0.17
***a*15:0**	7.66 ± 0.08	7.69 ± 0.04	7.86 ± 0.13	3.16 ± 0.06	3.09 ± 0.06	3.03 ± 0.03	6.62 ± 0.21	7.12 ± 0.36	7.61 ± 0.09
15:0	0.64 ± 0.01	0.71 ± 0.05	0.80 ± 0.05	0.85 ± 0.03	0.85 ± 0.02	0.90 ± 0.03	0.88 ± 0.06	0.82 ± 0.10	0.86 ± 0.06
***i*16:0**	2.92 ± 0.05	2.94 ± 0.04	2.99 ± 0.02	4.75 ± 0.10	4.88 ± 0.14	4.88 ± 0.10	3.70 ± 0.10	3.62 ± 0.19	3.78 ± 0.02
16:1ω9	1.89 ± 0.04	1.88 ± 0.04	1.84 ± 0.08	1.35 ± 0.13	1.46 ± 0.01	1.16 ± 0.23	1.36 ± 0.06	1.26 ± 0.05	1.16 ± 0.02
16:1ω7*c*	5.66 ± 0.04	5.59 ± 0.05	5.32 ± 0.05	5.74 ± 0.07	5.53 ± 0.08	5.24 ± 0.09	4.54 ± 0.21	3.81 ± 0.07	3.63 ± 0.09
16:1ω5	4.54 ± 0.04	4.49 ± 0.03	4.41 ± 0.04	2.80 ± 0.04	2.76 ± 0.04	2.62 ± 0.04	3.16 ± 0.17	2.92 ± 0.04	2.81 ± 0.12
16:0	12.7 ± 0.11	12.7 ± 0.09	13.2 ± 0.06	14.0 ± 0.11	14.4 ± 0.23	14.2 ± 0.24	13.4 ± 0.27	13.5 ± 0.80	14.6 ± 0.12
10 Me 16:0	6.74 ± 0.09	7.06 ± 0.12	7.04 ± 0.09	7.25 ± 0.11	7.41 ± 0.02	7.35 ± 0.08	6.15 ± 0.09	5.59 ± 0.33	5.88 ± 0.12
***i*17:0**	1.94 ± 0.01	2.05 ± 0.05	2.16 ± 0.02	1.74 ± 0.03	1.84 ± 0.02	2.09 ± 0.13	2.30 ± 0.03	2.61 ± 0.11	2.55 ± 0.04
***a*17:0**	1.92 ± 0.01	2.03 ± 0.12	1.98 ± 0.02	1.30 ± 0.01	1.42 ± 0.13	1.72 ± 0.15	2.07 ± 0.05	2.49 ± 0.22	2.18 ± 0.03
17:1ω8	0.51 ± 0.01	0.56 ± 0.09	0.50 ± 0.03	0.51 ± 0.01	0.51 ± 0.04	0.46 ± 0.08	0.50 ± 0.03	0.72 ± 0.17	0.45 ± 0.01
***cy*17:0**	2.39 ± 0.02	2.48 ± 0.13	2.43 ± 0.01	2.01 ± 0.02	2.11 ± 0.03	2.17 ± 0.09	2.62 ± 0.20	3.07 ± 0.20	3.07 ± 0.03
17:0	0.51 ± 0.01	0.59 ± 0.05	0.55 ± 0.01	0.42 ± 0.01	0.44 ± 0.01	0.50 ± 0.05	0.54 ± 0.02	0.74 ± 0.08	0.57 ± 0.01
***br*18:0**	0.97 ± 0.02	1.01 ± 0.04	0.97 ± 0.02	1.58 ± 0.06	1.60 ± 0.04	1.63 ± 0.12	1.44 ± 0.05	1.75 ± 0.15	1.47 ± 0.04
10 Me 17:0	0.64 ± 0.02	0.63 ± 0.02	0.63 ± 0.02	1.30 ± 0.03	1.35 ± 0.03	1.35 ± 0.07	1.02 ± 0.02	1.03 ± 0.28	1.03 ± 0.01
18:2ω6	1.63 ± 0.05	1.71 ± 0.03	1.62 ± 0.04	3.87 ± 0.14	3.53 ± 0.11	3.56 ± 0.11	2.15 ± 0.19	1.85 ± 0.03	2.25 ± 0.07
18:1ω9	8.21 ± 0.08	7.87 ± 0.09	7.69 ± 0.06	8.73 ± 0.08	8.57 ± 0.14	8.40 ± 0.21	7.89 ± 0.36	7.22 ± 0.23	7.35 ± 0.14
18:1ω7	12.1 ± 0.10	11.4 ± 0.13	11.1 ± 0.12	9.80 ± 0.19	9.28 ± 0.13	9.01 ± 0.20	9.42 ± 0.31	8.45 ± 0.30	8.49 ± 0.15
18:0	2.33 ± 0.04	2.33 ± 0.03	2.47 ± 0.05	2.05 ± 0.03	2.02 ± 0.03	2.20 ± 0.12	2.57 ± 0.06	3.00 ± 0.13	3.01 ± 0.05
10 Me 18:0	3.05 ± 0.05	2.95 ± 0.08	2.99 ± 0.06	1.78 ± 0.05	1.75 ± 0.02	1.74 ± 0.05	4.63 ± 0.10	4.69 ± 0.21	4.82 ± 0.08
***cy*19:0**	7.19 ± 0.14	6.99 ± 0.08	7.18 ± 0.17	9.80 ± 0.21	9.94 ± 0.27	9.80 ± 0.25	6.30 ± 0.48	6.33 ± 0.38	7.18 ± 0.19
20:0	0.33 ± 0.02	0.32 ± 0.01	0.33 ± 0.02	0.37 ± 0.01	0.37 ± 0.01	0.41 ± 0.06	0.41 ± 0.02	0.60 ± 0.16	0.45 ± 0.03

Across the three soil types the main effect of increased temperature on phospholipids was a shift from monounsaturated fatty acids towards saturated fatty acids ($P < 0.05$), although for most PLFAs the effect was minor compared to the effect of the soil types (Table 5.3, Figs. 5.1 & 5.2). In general, there was a greater relative percentages of unsaturated fatty acids such as 16:1ω7*c*, 16:1ω5, 18:1ω7 at 10 °C when compared to 15 and 20 °C ($P < 0.05$; average % ± SEM; 16:1ω7*c*, 5.3 ± 0.4 at 10 °C: 4.7 ± 0.5 at 20 °C; 16:1ω5, 3.5 ± 0.5 at 10 °C: 3.3 ± 0.5 at 20 °C; and 18:1ω7, 10.4 ± 0.73 at 10 °C: 9.6 ± 0.7 at 20 °C). Greater relative percentages of saturated fats were observed at 20 °C than at 10 °C, for example 16:0, *i*17:0, 18:0 and *cy*19:0 ($P < 0.05$; average % ± SEM; 16:0, 13.4 ± 0.4 at 10 °C: 14.0 ± 0.4 at 20 °C; *i*17:0, 2.0 ± 0.1 at 10 °C: 2.3 ± 0.2 at 20 °C; 18:0, 2.3 ± 0.1 at 10 °C: 2.6 ± 0.2 at 20 °C and *cy*19:0, 7.8 ± 1.0 at 10 °C: 8.1 ± 0.8 at 20 °C). There was no difference in fungal-to-bacterial PLFA ratio following incubation at the three temperatures ($P = 0.378$).

5.3.2. Soil solution dynamics in grazed soils over three weeks of incubation

Soil solution extracted at the start of the incubation period showed no statistical differences between soil types neither in the amount of labile DOC recovered (Table 5.2; $P > 0.05$ for glucose and amino acid-C) nor in the amount of DON recovered ($P > 0.05$ for amino acid-N and DON). The only statistical differences were in the total DOC recovered, where the Podzolic soil had more DOC than the other soils (average ± SEM; 573 ± 73 mg l^{-1} compared to 154 ± 14 mg l^{-1}). On average, the total free amino acids represented only 5% of the DON in the 5 soils and most of the DON remained unidentified. The DOC-to-DON ratio of the soil solutions (6 ± 1) was significantly smaller than the C to N ratio

of the total soil (11 ± 0.5; Tables 5.1 and 5.2; $P < 0.05$). Across the five soil types, free glucose represented 10% of the total DOC in the soil solution. Free amino acids also represented a small proportion of the total DOC (3 ± 1%). Consequently, over 85% of the total DOC in the five soil solutions remained unidentified

Over the three-week incubation, soil solution extracted from grazed swards had linearly increasing amounts of DOC (from 129 ± 2 at 1 h to 390 ± 3 mg C l^{-1} at 21 d; $P < 0.001$; $r^2 = 0.76$; Fig. 5.3). Phenolic substances and phosphates also increased in concentration over the incubation period ($P < 0.001$ and 0.027 respectively; with phenolic substance concentrations increasing from 11.4 ± 1.1 at 1 h to 29.0 ± 1.0 mg phenol l^{-1} at 21 d, following a double exponential rise to a maximum fit $r^2 = 0.93$; and phosphate concentrations increasing from 1.6 ± 0.1 at 1 h to 3.5 ± 0.4 mg P l^{-1} at 21 d, following a linear fit $r^2 = 0.83$). In contrast, nitrate concentrations decreased (falling from 0.9 ± 0.1 at 1 h to 0.2 ± 0.004 mg N l^{-1} at 21 d; $P < 0.001$; following a double exponential decline $r^2 = 0.99$). The other measured soil solution parameters – glucose, amino acid, DON, and ammonium showed no significant difference over the incubation ($P > 0.05$, data not shown). Respiration rates remained unchanged throughout the three week period (averaging 8.82 ± 1.30 μg CO_2 g^{-1} h^{-1}; $P = 0.71$, Fig. 5.4).

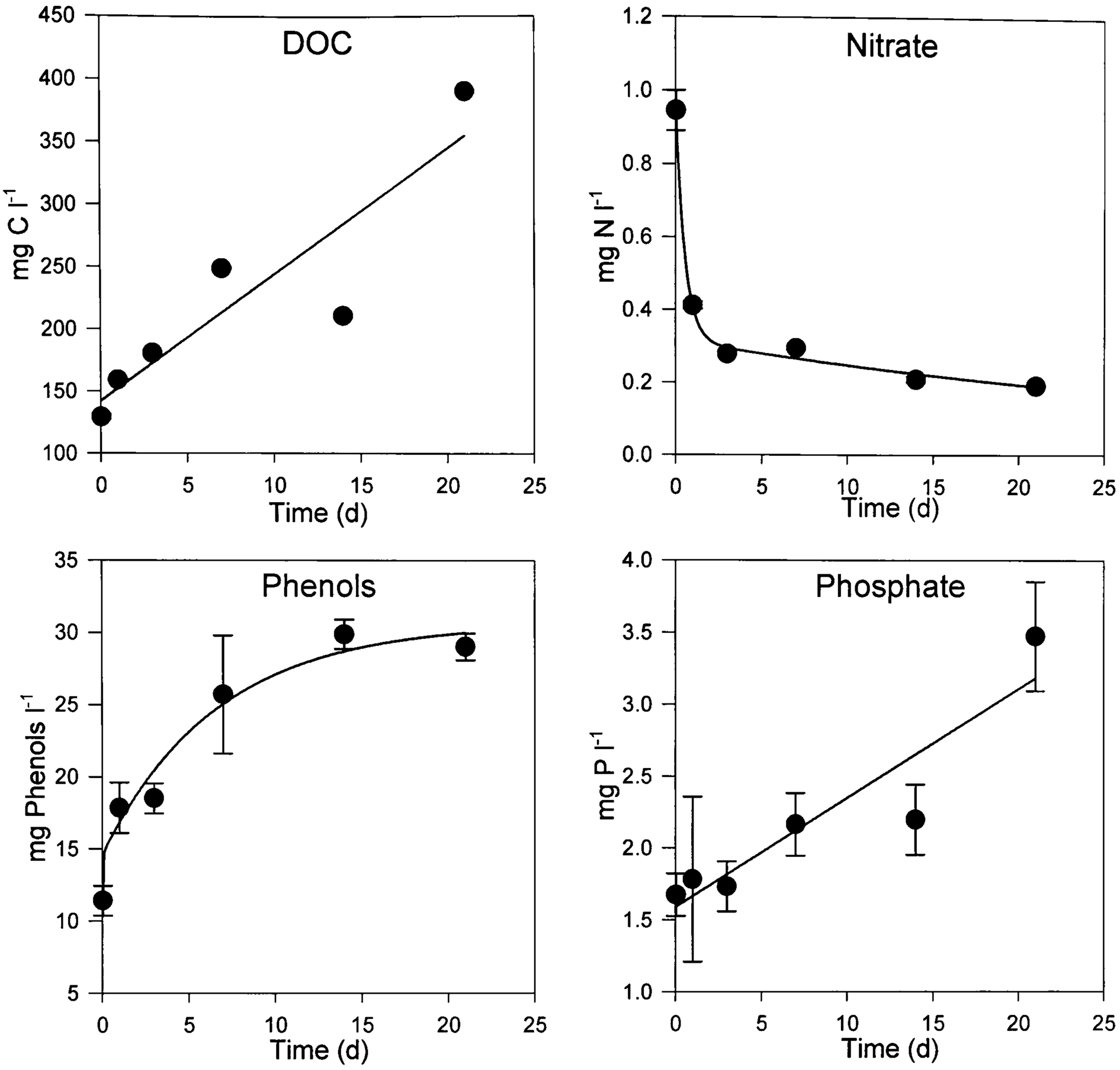


Figure 5.3. Soil solution concentrations of DOC, nitrate, phenolic substances and phosphate after incubation of a grazed cambisol soil incubated for three weeks at 20 °C. Bars are SEM, $n = 3$.

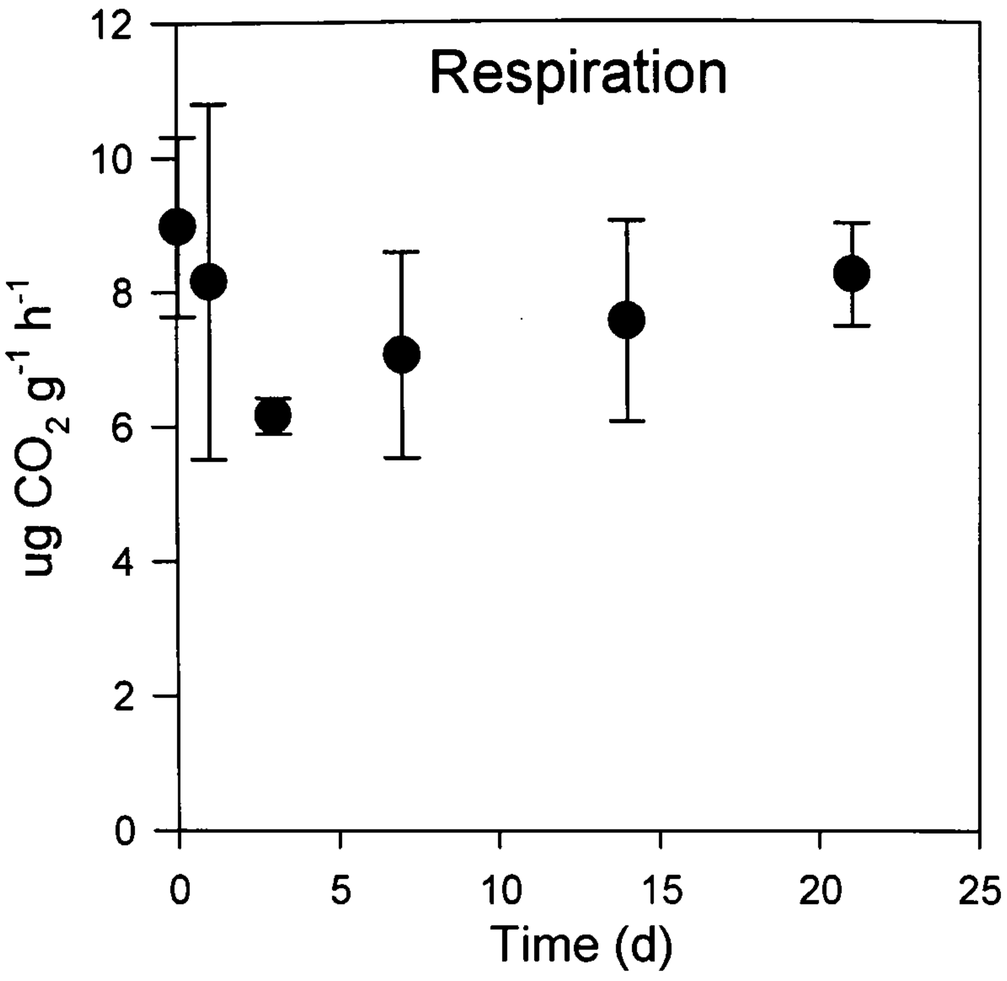


Figure 5.4. Soil respiration measurements from grazed cambisol soil incubated for three weeks at 20 °C, showing no statistical variation over the incubation period. Bars are SEM, $n = 3$.

5.3.3. Acclimation across a catena over three weeks of incubation

A biphasic loss of $^{14}CO_2$ followed the addition of radiolabelled ^{14}C-glucose to the soil surface (Fig. 5.5). A double exponential decay with asymptote model gave a good fit to the experimental data ($r^2 > 0.99$ in all cases, Eqn. 5.1). Overall, there was a close similarity between the dynamics of $^{14}CO_2$ evolution across the five soil types throughout the 48 h incubation period ($P = 0.584$ for mineralized $^{14}CO_2$). ANOVA analysis of the total amount of ^{14}C efflux from the soils at the different incubation temperatures resulted in soils incubated at 10 °C and soils incubated at 20 °C and moved to 10 °C being statistically similar (17.5 ± 0.3 and 16.8 ± 0.3% respectively), but significantly different ($P < 0.001$) from mineralization levels of soils incubated at 20 °C and soils incubated at 10 °C moved to 20 °C, which were statistically similar to each other (20.8 ± 0.3 and 21.4 ± 0.3% respectively). Thus the soils showed no evidence of acclimation as $^{14}CO_2$ release depended on current, not previous, temperature; the only statistical

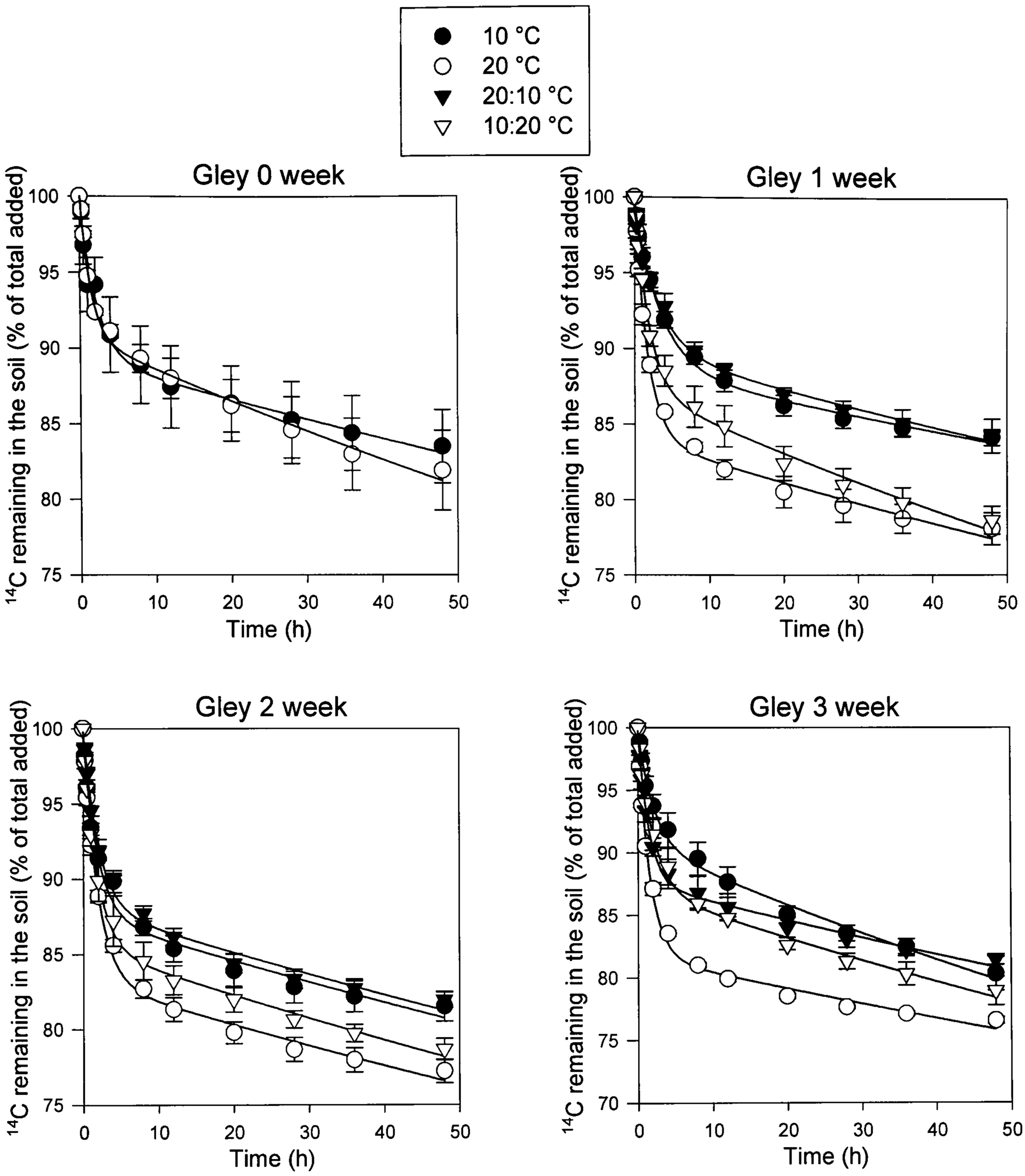
difference came from comparing 10 to 20 °C during measurement of $^{14}CO_2$ evolution. The duration of the temperature incubation period had little effect on the total amount of $^{14}CO_2$ mineralization with only the initial measurement period (t = 0 d) being statistically different ($P < 0.001$) from soils incubated for 1-3 weeks, which were statistically similar to each other (0 week = 16.8 ± 0.5, 1 week = 19.0 ± 0.5, 2 weeks = 19.8 ± 0.4, 3 weeks = 19.7 ± 0.4%). Calculations also indicated that the amount of $^{14}CO_2$ recovered did not conform to a theoretical Q_{10} value of 2. The maximum temperature induced increase in mineralization was seen in the gleysol; however, this only yielded a Q_{10} value of 1.3 from 10 to 20 °C.

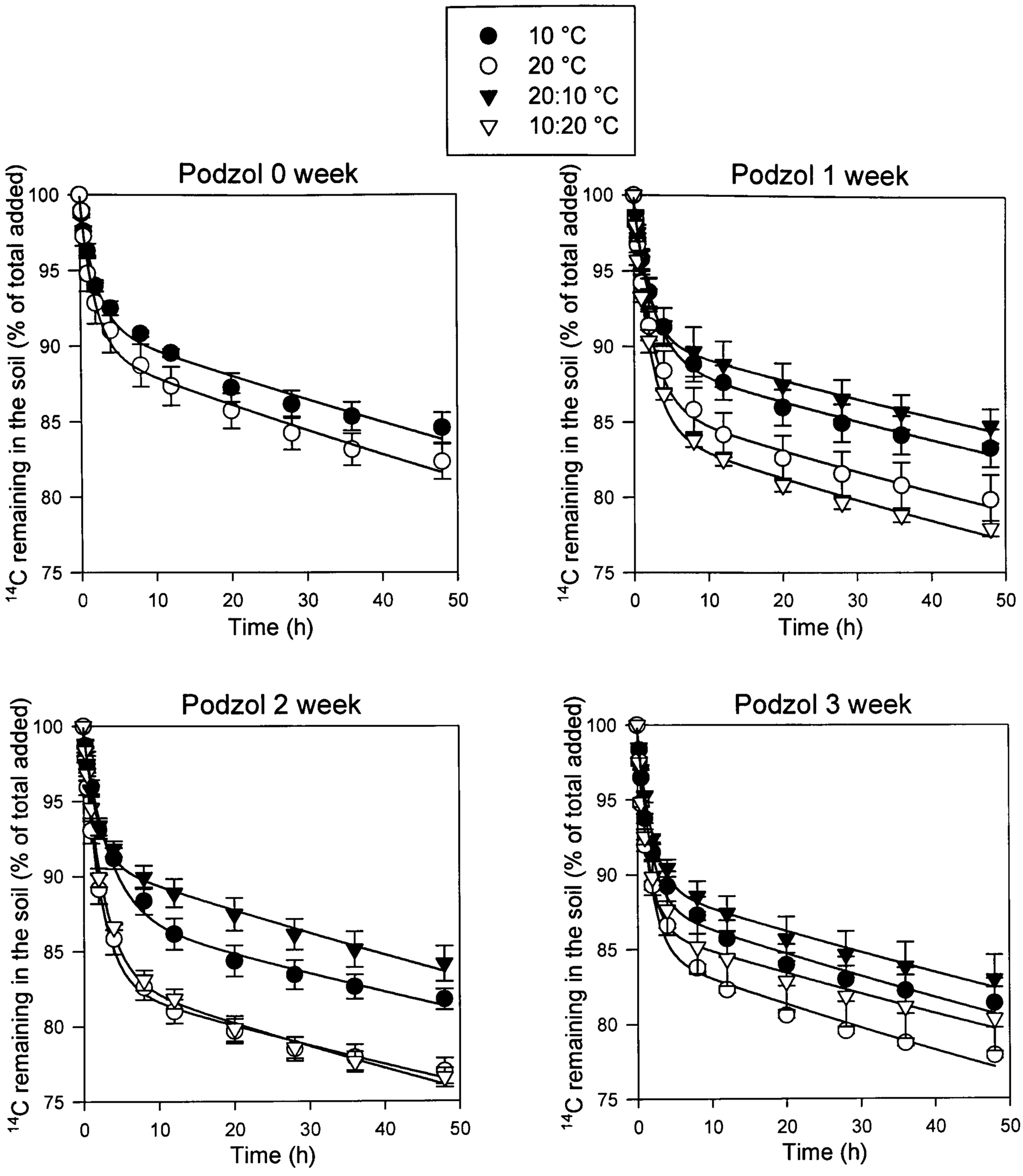
The double exponential model enables us to examine the different kinetic phases of mineralization of the labelled substrate in soil (Table 5.4). Calculations of the half-lives ($t_{½}$; Eqn. 5.2) of the first rapid phase (k_1) of mineralization shows that turnover of glucose in soil was extremely rapid with an average half-life of 1.34 ± 0.04 h. Incubating the soils at different temperatures (10, 20, 20:10, 10:20 °C) had no statistical effect on turnover times and therefore there was no evidence of an acclimation effect on the soils (P = 0.535; 10 °C = 1.5 ± 0.1 h, 20 °C = 1.2 ± 0.1 h, 20:10 °C = 1.4 ± 0.1 h, 10:20 °C = 1.3 ± 0.1 h; Table 5.5). The duration of the temperature incubation period (0 to 21 d) also had no significant effect on the first phase kinetic rates. However, the half-life did vary depending upon the soil type ($P < 0.001$) with gleysol and podzolic soils having statistically faster half-lives for the first rapid phase mineralization of glucose in comparison to the cambisol (gleysol = 1.5 ± 0.1 h, podzol = 1.5 ± 0.1 h, cambisol = 1.4 ± 0.1 h, un-grazed = 1.2 ± 0.1 h, non vegetated = 1.1 ± 0.1 h). The cambisol soils all had statistically comparable half-lives ($P > 0.05$). In comparison to the other soil

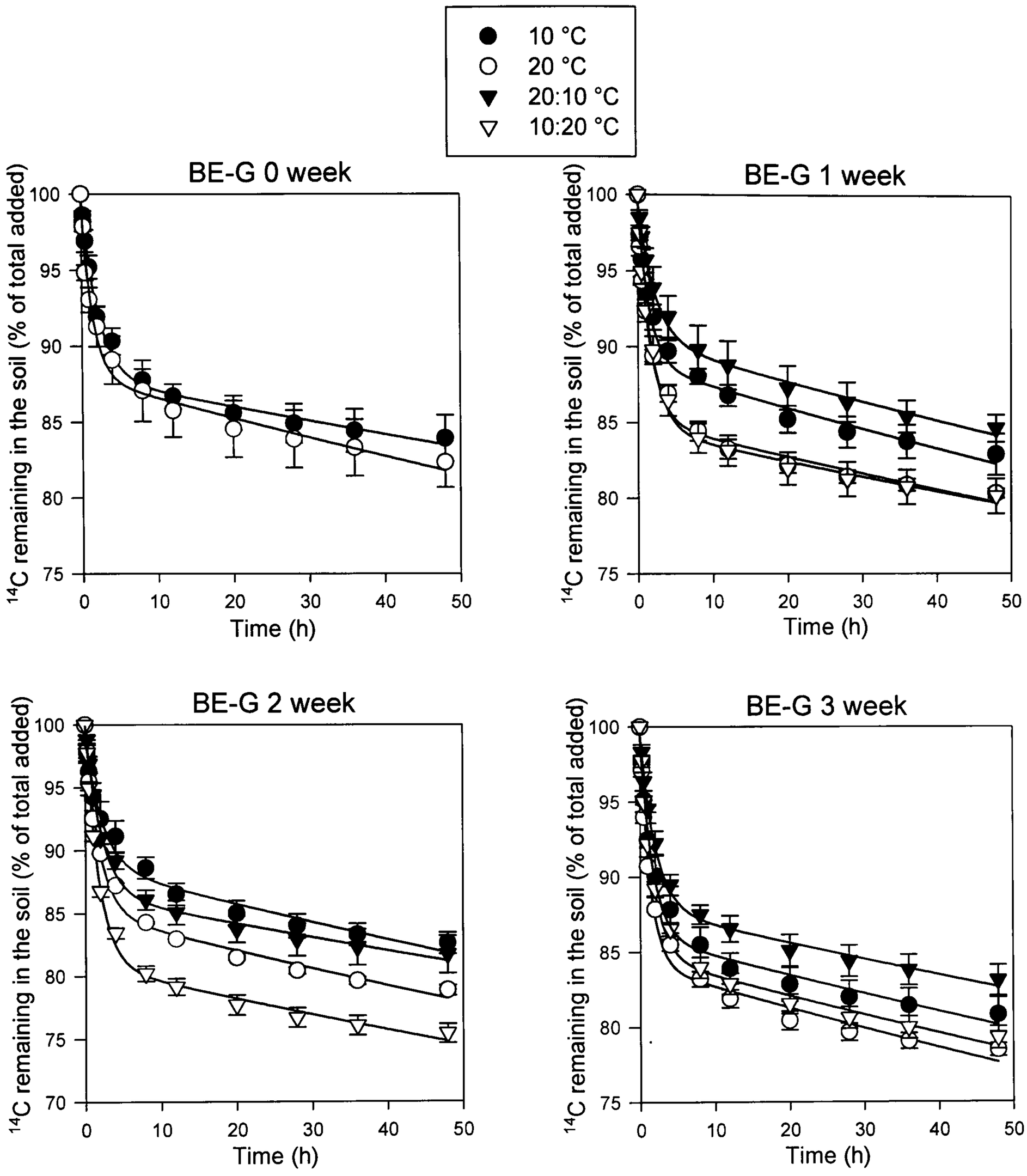
types, the grazed cambisol was statistically similar to the gleysol and podzol soils, whilst the cambisol un-grazed and non-vegetated soils had statistically shorter half-lives than the gleysol and podzol soils.

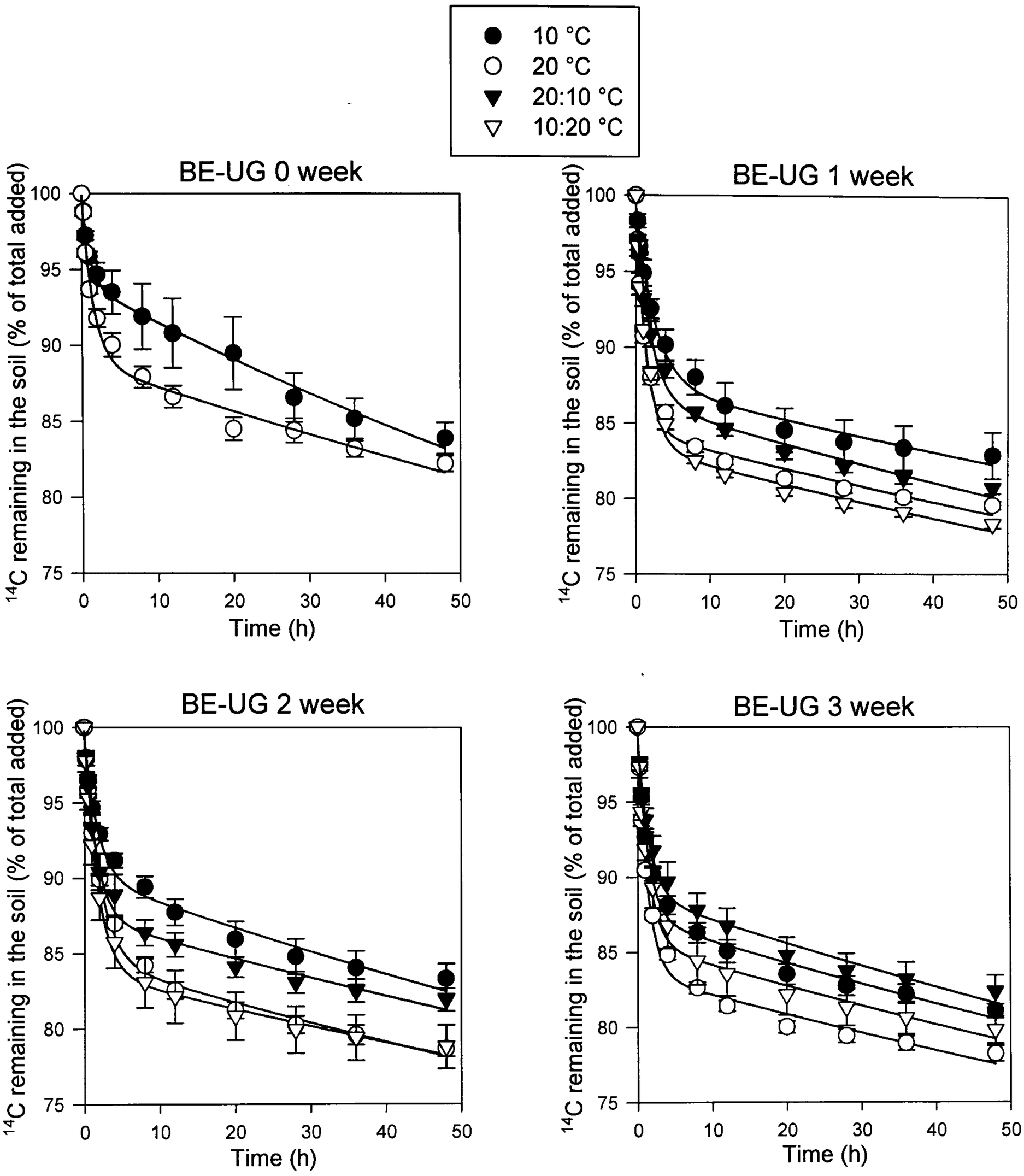
Table 5.5. Example of acclimation and no acclimation responses occurring in the soils (adapted from Table 5.3). The acclimation example is taken from the cambisol non-vegetated soils incubated for two weeks, and the no acclimation example is from the gleysol incubated for one week. Acclimation was said to occur if previous temperatures and not just the current temperature influenced the rate of mineralization. Thus if the rate of mineralization measured at 10 and grown at 10 °C was smaller than the rate of mineralization measured at 10 and grown at 20 °C then acclimation had occurred. Similarly, acclimation was said to occurr if the rate of mineralization measured at 20 and grown at 20 °C was greater than that of the soil measured at 20 and grown at 10 °C.

	Acclimation (from k_2)		No acclimation (from k_1)	
Treatment	Incubated at 10°C	Incubated at 20°C	Incubated at 10°C	Incubated at 20°C
Measured at 10°C	$2.0\times10^{-3} \pm 0.3\times10^{-3}$	$3.0\times10^{-3} \pm 0.3\times10^{-3}$	0.30 ± 0.04	0.30 ± 0.07
Measured at 20°C	$2.7\times10^{-3} \pm 0.3\times10^{-3}$	$4.9\times10^{-3} \pm 0.1\times10^{-3}$	0.58 ± 0.11	0.57 ± 0.11


10 °C
20 °C
20:10 °C
10:20 °C
Gley 0 week
Gley 1 week
Gley 2 week
Gley 3 week
^{14}C remaining in the soil (% of total added)
Time (h)


10 °C
20 °C
20:10 °C
10:20 °C
Podzol 0 week
Podzol 1 week
Podzol 2 week
Podzol 3 week
^{14}C remaining in the soil (% of total added)
Time (h)
100
95
90
85
80
75
0
10
20
30
40
50


10 °C
20 °C
20:10 °C
10:20 °C
BE-G 0 week
BE-G 1 week
BE-G 2 week
BE-G 3 week
^{14}C remaining in the soil (% of total added)
Time (h)


10 °C
20 °C
20:10 °C
10:20 °C
BE-UG 0 week
BE-UG 1 week
BE-UG 2 week
BE-UG 3 week
^{14}C remaining in the soil (% of total added)
Time (h)
100
95
90
85
80
75
0
10
20
30
40
50

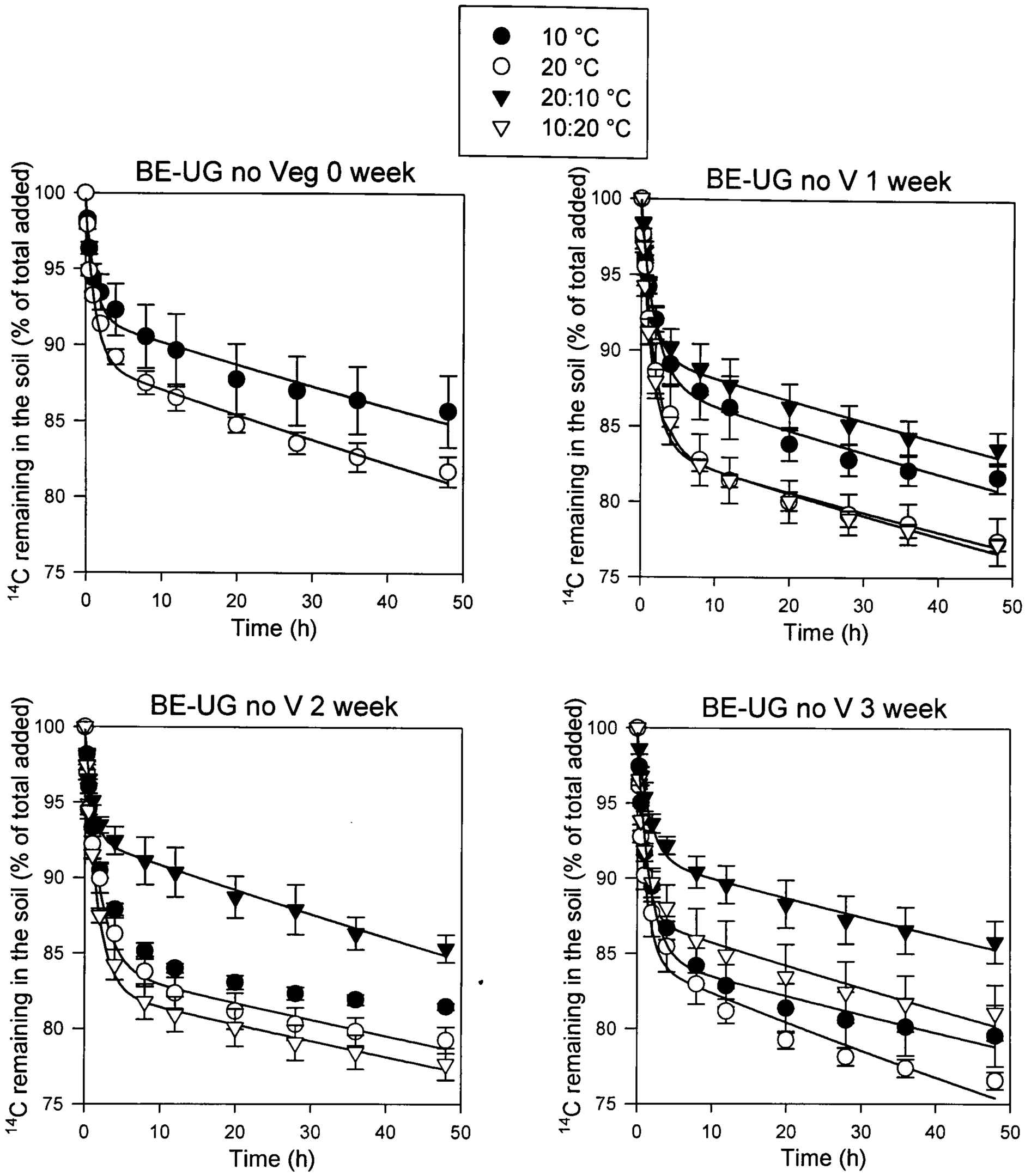


Figure 5.5. Mineralization kinetics following additions of ^{14}C-U-glucose to five agricultural soils (gleysol, podzol, and cambisol – grazed [BE-G], un-grazed [BE-UG] and un-vegetated [BE-UG no V]) incubated at 10 and 20 °C for 1 h, 1, 2, and 3 weeks, before a subset was moved from 10 to 20 °C or 20 to 10 °C to test for acclimation. Mineralized $^{14}CO_2$ was trapped in 1 M NaOH and the percentage of added ^{14}C remaining in the soil plotted over time. Experimental data points are means ± SEM (n = 3) while lines represent fits to a double exponential decay model (see section 5.2 for further details).

Table 5.4. Parameters of the double exponential equation for ^{14}C-glucose mineralization by five agricultural soils, with estimates of the half-life (defined as $0.693/k_1$) of ^{14}C-U-glucose in the soil solution and rate constants for mineralization after incorporation into the microbial biomass (k_2). Pool size estimates for ^{14}C partitioned into pools a_1 and a_2 associated with exponential coefficients k_1 and k_2 respectively are given. Respiration is the total recovered $^{14}CO_2$ at the end of the 48 h incubation period expressed as a % of the total added ^{14}C. For Cambisol soils G represents grazed sward, UG un-grazed sward, and UG no V un-grazed with no vegetation. Values represent means ± SEM (n = 3).

Soil	Temp	Soil solution (a_1)	Soil solution (k_1)	Soil solution half-life (h)	Microbial pool (a_2)	Microbial pool (k_2)	Respiration (%)
Gleysol 0 week	10	10.39 ± 2.22	0.41 ± 0.11	1.95 ± 0.53	38.79 ± 2.27	0.0036 ± 0.0008	16.52 ± 2.42
	20	9.47 ± 0.41	0.72 ± 0.07	0.98 ± 0.10	41.20 ± 0.45	0.0056 ± 0.0017	18.10 ± 2.64
Gleysol 1 week	10	10.81 ± 0.84	0.30 ± 0.04	2.47 ± 0.41	40.81 ± 0.90	0.0026 ± 0.0004	15.85 ± 0.53
	20:10	9.44 ± 2.70	0.30 ± 0.07	2.69 ± 0.84	41.72 ± 0.31	0.0030 ± 0.0007	15.75 ± 1.12
	20	15.74 ± 0.24	0.57 ± 0.11	1.29 ± 0.22	36.08 ± 0.61	0.0041 ± 0.0005	21.92 ± 1.07
	10:20	12.89 ± 2.23	0.58 ± 0.11	1.30 ± 0.30	39.39 ± 2.02	0.0055 ± 0.0012	21.36 ± 0.93
Gleysol 2 week	10	12.31 ± 0.54	0.58 ± 0.16	1.52 ± 0.58	39.45 ± 0.69	0.0038 ± 0.0007	18.49 ± 1.00
	20:10	11.78 ± 0.90	0.52 ± 0.10	1.44 ± 0.32	40.22 ± 0.84	0.0038 ± 0.0003	18.06 ± 0.52
	20	16.46 ± 0.95	0.54 ± 0.10	1.36 ± 0.24	35.40 ± 0.91	0.0043 ± 0.0002	22.79 ± 0.78
	10:20	14.24 ± 1.75	0.54 ± 0.08	1.36 ± 0.23	37.61 ± 1.49	0.0044 ± 0.0011	21.34 ± 0.72
Gleysol 3 week	10	8.87 ± 2.37	0.64 ± 0.17	1.28 ± 0.37	43.10 ± 2.36	0.0062 ± 0.0017	19.73 ± 1.35
	20:10	12.14 ± 1.19	0.69 ± 0.01	1.01 ± 0.02	39.79 ± 1.39	0.0039 ± 0.0007	18.59 ± 0.41
	20	17.66 ± 0.36	0.62 ± 0.04	1.13 ± 0.08	33.90 ± 0.21	0.0040 ± 0.0001	23.48 ± 0.27
	10:20	13.04 ± 0.50	0.47 ± 0.13	1.83 ± 0.64	38.68 ± 0.10	0.0048 ± 0.0008	21.11 ± 1.14
Podzol 0 week	10	8.39 ± 0.31	0.47 ± 0.08	1.55 ± 0.22	44.24 ± 0.11	0.0039 ± 0.0006	15.40 ± 1.01
	20	10.38 ± 1.29	0.50 ± 0.12	1.61 ± 0.47	39.30 ± 1.20	0.0046 ± 0.0007	17.64 ± 1.16
Podzol 1 week	10	10.63 ± 1.20	0.39 ± 0.11	2.10 ± 0.58	39.07 ± 0.97	0.0035 ± 0.0004	16.69 ± 1.26
	20:10	9.36 ± 1.60	0.51 ± 0.06	1.39 ± 0.16	40.50 ± 1.75	0.0032 ± 0.0004	15.22 ± 1.14
	20	13.69 ± 1.83	0.43 ± 0.04	1.65 ± 0.14	36.19 ± 1.40	0.0042 ± 0.0003	20.14 ± 1.67

	10:20	15.08 ± 0.38	0.45 ± 0.04	1.56 ± 0.13	34.57 ± 0.26	0.0047 ± 0.0003	22.07 ± 0.52
Podzol 2 week	10	11.93 ± 1.47	0.30 ± 0.02	2.34 ± 0.13	37.72 ± 1.34	0.0037 ± 0.0003	18.21 ± 0.70
	20:10	8.94 ± 0.99	0.58 ± 0.05	1.21 ± 0.10	41.16 ± 0.85	0.0041 ± 0.0003	15.83 ± 1.18
	20	14.44 ± 0.75	0.55 ± 0.02	1.27 ± 0.05	38.23 ± 0.32	0.0041 ± 0.0004	22.99 ± 0.87
	10:20	16.66 ± 1.27	0.44 ± 0.11	1.76 ± 0.36	33.56 ± 1.28	0.0050 ± 0.0007	23.42 ± 0.64
Podzol 3 week	10	11.90 ± 1.22	0.59 ± 0.10	1.24 ± 0.19	38.04 ± 1.22	0.0044 ± 0.0004	18.61 ± 1.64
	20:10	10.65 ± 1.01	0.55 ± 0.10	1.34 ± 0.22	39.33 ± 1.09	0.0039 ± 0.0005	17.03 ± 1.65
	20	14.64 ± 0.17	0.60 ± 0.03	1.16 ± 0.05	34.95 ± 0.08	0.0051 ± 0.0002	22.10 ± 0.22
	10:20	13.16 ± 1.82	0.73 ± 0.12	1.01 ± 0.16	36.56 ± 1.87	0.0042 ± 0.0005	19.68 ± 2.15
Cambisol-G 0 week	10	11.85 ± 0.55	0.46 ± 0.06	1.56 ± 0.19	46.23 ± 0.45	0.0021 ± 0.0006	16.04 ± 1.52
	20	11.53 ± 1.86	0.73 ± 0.14	1.01 ± 0.17	38.64 ± 1.99	0.0035 ± 0.0001	17.63 ± 1.66
Cambisol-G 1 week	10	10.75 ± 0.42	0.70 ± 0.19	1.22 ± 0.41	41.74 ± 0.50	0.0034 ± 0.0007	17.07 ± 1.37
	20:10	9.26 ± 1.79	0.54 ± 0.18	1.60 ± 0.50	43.50 ± 1.85	0.0031 ± 0.0005	15.34 ± 0.90
	20	13.87 ± 1.11	0.60 ± 0.03	1.15 ± 0.05	38.24 ± 0.82	0.0032 ± 0.0003	19.66 ± 1.30
	10:20	14.80 ± 0.94	0.54 ± 0.04	1.30 ± 0.12	37.55 ± 0.89	0.0029 ± 0.0004	19.85 ± 1.16
Cambisol-G 2 week	10	10.80 ± 0.48	0.49 ± 0.22	2.01 ± 0.65	41.44 ± 0.28	0.0035 ± 0.0005	17.38 ± 0.85
	20:10	13.41 ± 1.10	0.48 ± 0.05	1.46 ± 0.14	39.65 ± 0.85	0.0031 ± 0.0005	18.29 ± 1.48
	20	16.82 ± 0.62	0.45 ± 0.06	1.60 ± 0.19	32.99 ± 0.56	0.0044 ± 0.0004	21.13 ± 0.41
	10:20	18.95 ± 0.51	0.55 ± 0.02	1.26 ± 0.04	34.03 ± 0.59	0.0041 ± 0.0003	24.57 ± 0.76
Cambisol-G 3 week	10	13.58 ± 1.41	0.59 ± 0.12	1.32 ± 0.33	38.93 ± 0.97	0.0032 ± 0.0003	19.18 ± 1.15
	20:10	11.68 ± 0.56	0.49 ± 0.02	1.43 ± 0.07	40.99 ± 0.69	0.0028 ± 0.0003	16.90 ± 1.02
	20	15.46 ± 0.63	0.75 ± 0.07	0.94 ± 0.08	37.23 ± 0.65	0.0039 ± 0.0001	21.53 ± 0.43
	10:20	14.81 ± 0.49	0.60 ± 0.04	1.17 ± 0.07	37.87 ± 0.65	0.0037 ± 0.0002	20.72 ± 0.76
Cambisol-UG 0 week	10	6.64 ± 2.73	1.75 ± 0.70	0.82 ± 0.54	36.54 ± 2.80	0.0058 ± 0.0008	16.07 ± 1.04
	20	10.95 ± 0.95	0.67 ± 0.06	1.05 ± 0.09	37.02 ± 0.94	0.0052 ± 0.0008	17.74 ± 0.52
Cambisol-UG 1 week	10	11.75 ± 1.07	0.38 ± 0.01	1.80 ± 0.04	34.05 ± 1.29	0.0035 ± 0.0004	17.10 ± 1.54
	20:10	12.77 ± 0.41	0.52 ± 0.07	1.42 ± 0.25	32.88 ± 0.32	0.0044 ± 0.0003	19.28 ± 0.36
	20	15.21 ± 0.74	0.77 ± 0.06	0.90 ± 0.06	30.92 ± 0.30	0.0041 ± 0.0003	20.42 ± 0.26

	10:20	15.77 ± 0.45	0.63 ± 0.03	1.10 ± 0.05	29.90 ± 0.28	0.0043 ± 0.0003	21.66 ± 0.28
Cambisol-UG 2 week	10	9.49 ± 0.71	0.62 ± 0.17	1.30 ± 0.37	36.45 ± 1.00	0.0048 ± 0.0006	16.69 ± 1.00
	20:10	12.61 ± 0.77	0.62 ± 0.14	1.26 ± 0.34	33.63 ± 0.61	0.0040 ± 0.0003	18.08 ± 0.75
	20	14.95 ± 0.83	0.50 ± 0.07	1.43 ± 0.18	31.10 ± 0.65	0.0049 ± 0.0001	21.36 ± 0.50
	10:20	16.14 ± 1.57	0.56 ± 0.10	1.32 ± 0.26	30.04 ± 1.77	0.0040 ± 0.0001	21.21 ± 1.46
Cambisol-UG 3 week	10	12.37 ± 0.82	0.76 ± 0.08	0.93 ± 0.09	33.78 ± 0.83	0.0046 ± 0.0005	18.94 ± 0.48
	20:10	10.69 ± 1.28	0.63 ± 0.12	1.17 ± 0.19	35.12 ± 1.27	0.0046 ± 0.0004	17.65 ± 1.11
	20	16.17 ± 0.32	0.75 ± 0.03	0.93 ± 0.04	30.00 ± 0.31	0.0046 ± 0.0002	21.77 ± 0.53
	10:20	13.88 ± 1.82	0.73 ± 0.08	0.97 ± 0.09	32.11 ± 1.80	0.0046 ± 0.0005	20.19 ± 0.91
Cambisol-UG no V 0 week	10	8.16 ± 2.06	1.00 ± 0.30	0.82 ± 0.21	43.39 ± 2.21	0.0035 ± 0.0004	14.28 ± 2.37
	20	11.02 ± 1.18	0.93 ± 0.31	0.91 ± 0.25	59.16 ± 1.30	0.0029 ± 0.0009	18.26 ± 1.00
Cambisol-UG no V 1 week	10	11.54 ± 2.13	0.59 ± 0.16	1.35 ± 0.31	48.68 ± 2.28	0.0032 ± 0.0006	18.35 ± 1.01
	20:10	10.42 ± 1.87	0.79 ± 0.20	0.99 ± 0.21	50.48 ± 1.90	0.0028 ± 0.0003	16.43 ± 1.08
	20	16.50 ± 1.41	0.55 ± 0.14	1.46 ± 0.42	44.18 ± 1.16	0.0031 ± 0.0004	22.58 ± 1.58
	10:20	16.02 ± 0.70	0.71 ± 0.19	1.13 ± 0.31	44.40 ± 0.83	0.0035 ± 0.0002	22.78 ± 0.39
Cambisol-UG no V 2 week	10	14.29 ± 0.82	0.51 ± 0.05	1.40 ± 0.15	46.29 ± 0.70	0.0020 ± 0.0003	18.51 ± 0.22
	20:10	7.03 ± 2.27	1.50 ± 0.67	0.77 ± 0.38	53.16 ± 1.83	0.0030 ± 0.0003	14.65 ± 0.92
	20	15.05 ± 1.43	0.55 ± 0.14	1.40 ± 0.29	44.96 ± 1.28	0.0049 ± 0.0001	20.74 ± 0.88
	10:20	17.15 ± 1.26	0.62 ± 0.05	1.13 ± 0.09	43.48 ± 1.33	0.0027 ± 0.0003	22.33 ± 1.06
Cambisol-UG no V 3 week	10	14.63 ± 1.08	0.59 ± 0.03	1.17 ± 0.05	45.70 ± 1.02	0.0030 ± 0.0007	20.47 ± 2.05
	20:10	9.06 ± 1.45	0.84 ± 0.33	1.36 ± 0.72	51.75 ± 1.54	0.0023 ± 0.0001	14.22 ± 1.41
	20	14.93 ± 1.59	0.75 ± 0.15	1.03 ± 0.26	44.94 ± 1.09	0.0043 ± 0.0007	23.45 ± 0.58
	10:20	12.41 ± 1.92	1.23 ± 0.57	0.79 ± 0.25	41.27 ± 4.44	0.0031 ± 0.0002	18.93 ± 1.84

Estimates of the second phase exponential coefficients (k_2) from the experimental data were much smaller than the first phase coefficients, with k_2 having an average value of $3.9 \times 10^{-3} \pm 0.1 \times 10^{-3}$ h^{-1} compared to a k_1 value of 0.62 ± 0.02 h^{-1} (Table 5.4). In contrast to k_1, incubation temperature did have a significant effect on the second phase of mineralization ($P = 0.001$), with 10 and 20 °C soils having significantly different exponential coefficients ($3.7 \times 10^{-3} \pm 0.2 \times 10^{-3}$ h^{-1} and $4.2 \times 10^{-3} \pm 0.1 \times 10^{-3}$ h^{-1} respectively). Overall, there was no significant acclimation effect, with 10 and 20:10 °C ($3.5 \times 10^{-3} \pm 0.1 \times 10^{-3}$ h^{-1}) being statistically similar, whilst the same lack of acclimation was also seen for 20 and 10:20 °C ($4.1 \times 10^{-3} \pm 0.2 \times 10^{-3}$ h^{-1}). The duration of soil incubation at the different temperatures also had no statistically significant effect on the second phase of mineralization ($P = 0.087$). ANOVA of the five soil types divided the soil into two statistical groups with cambisol grazed and non-vegetated soils ($3.4 \times 10^{-3} \pm 0.1 \times 10^{-3}$ h^{-1} and $3.2 \times 10^{-3} \pm 0.1 \times 10^{-3}$ h^{-1} respectively) having slower release of the ^{14}C in the second phase compared to podzol, gleysol and un-grazed cambisol soils ($4.2 \times 10^{-3} \pm 0.1 \times 10^{-3}$ h^{-1}, $4.3 \times 10^{-3} \pm 0.3 \times 10^{-3}$ h^{-1}, and $4.5 \times 10^{-3} \pm 0.1 \times 10^{-3}$ h^{-1} respectively; $P < 0.001$).

The size of the pools associated with exponential coefficients k_1 and k_2, namely a_1 and a_2 (Table 5.4), were significantly affected by the incubation temperature for both pools. For pool a_1, the results show a clear and statistically significant divide between the % of total ^{14}C partitioned to a_1 at 10 °C (11.1 ± 0.4%) and 20:10 °C (10.6 ± 0.4%), when compared to 20 °C (14.3 ± 0.5%) and 10:20 °C (15.0 ± 0.4%; $P < 0.001$). This provides no evidence for an acclimation response as there was no effect from previous, only of current, incubation temperatures. Total $^{14}CO_2$ recovered from the soils correlated well to pool a_1,

indicating that rising soil temperature increases the relative amount of ^{14}C partitioned into pool a_1 ($r^2 = 0.73 \pm 0.10$; Fig. 5.6). The amount of ^{14}C partitioned to pool a_2 had statistically significant differences between 10 and 20 °C, but there was no significant difference and therefore no acclimation between 10 °C (40.7 ± 0.6) and 20:10 °C (41.6 ± 0.9). There was a significant difference in the calculated size of a_2 between 20 °C (38.3 ± 0.9) and 10:20 °C (36.7 ± 0.7), however, this did not show acclimation as the 10:20 °C value was significantly smaller than 10 and 20:10 °C; for the previous incubation temperature to be having an affect we would expect for 10 and 10:20 °C to be similar. The duration of the soil incubation at the different temperatures had only a significant effect between the 1 h and subsequent incubation periods (with a_1 having a significantly smaller proportion of the ^{14}C pool and the a_2 pool being significantly larger; $P < 0.001$). There was no statistical difference by soil type for pool a_1 ($P = 0.065$), but there were a statistical differences for pool a_2, with the non-vegetated cambisol having the greatest percentage of the ^{14}C pool (47.3 ± 0.8%) and un-grazed cambisol the least (33.1 ± 0.5%), and the grazed cambisol, gleysol and podzol all being statistically similar (39.2 ± 0.6%, 39.0 ± 0.5%, 38.1 ± 0.5%, respectively; $P < 0.001$).

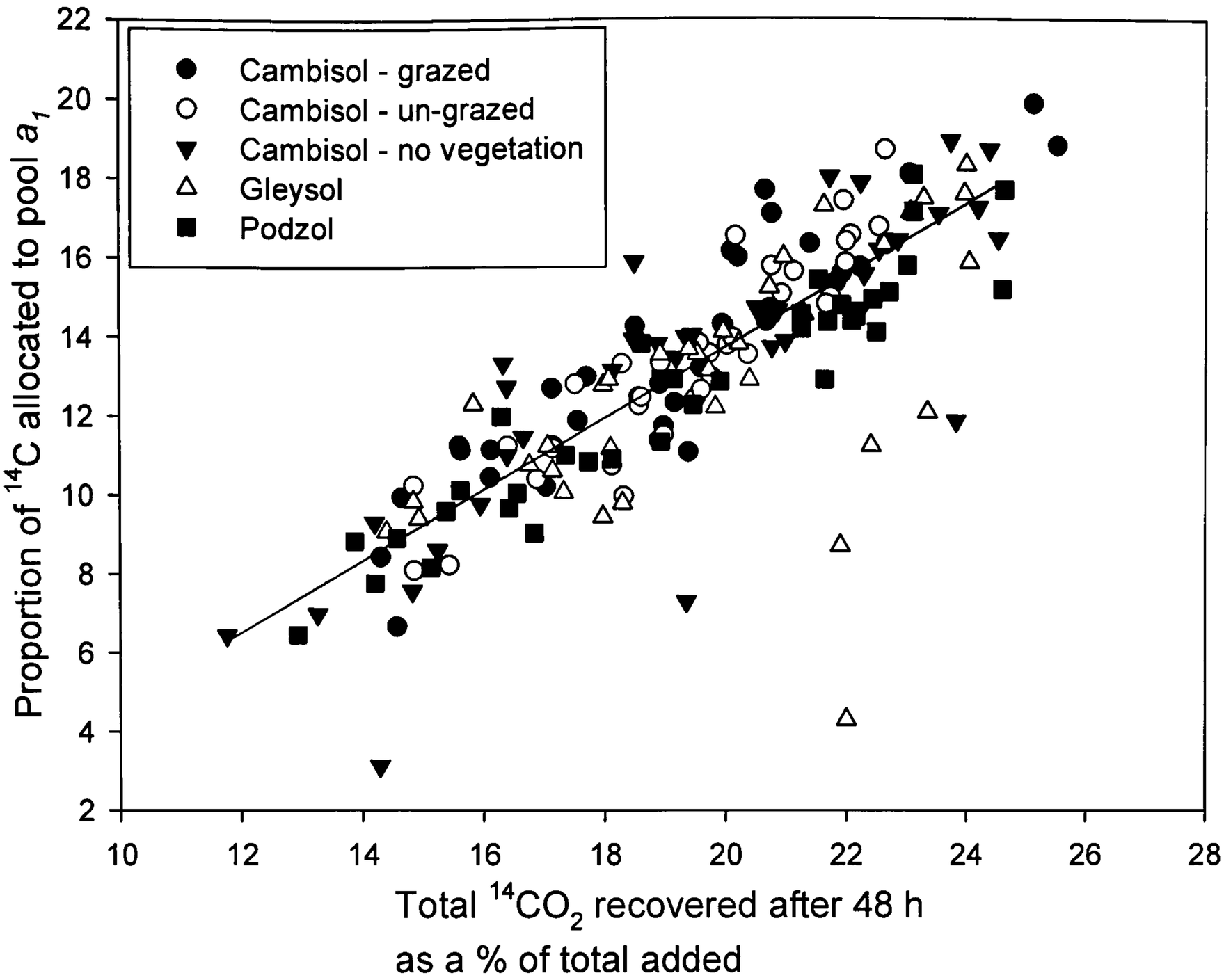


Figure 5.6. Correlation between the proportion of ^{14}C partitioned into pool a_1 and the total $^{14}CO_2$ recovered from the soil surface after 48 h for five agricultural soils incubated at 10 and 20 °C for 0-3 weeks before additions of ^{14}C-U-glucose.

5.4. Discussion

5.4.1. Changes in the microbial community following incubation

As we hypothesized, the type of soil appeared to be the dominant factor regulating the diversity of the soil microbial community, however, incubation temperature also caused a small but significant shift in the PLFA pattern. Consistently, there were more monounsaturated fatty acids apparent at lower temperature, and at higher temperatures saturated fatty acids were favoured. A similar result has also been demonstrated previously in forest soil after increasing the incubation temperature (Petersen and Klug, 1994; Pettersson and Bååth, 2003). This finding could imply either changes in the species composition or, more likely, changes in the structure of the membrane phospholipids of pre-

existing taxa (Perry and Staley, 1997; Pettersson and Bååth, 2003), although the changes are consistent with the phenotypic changes expected (Russell and Fukunaga, 1990). Phenotypic acclimation in the cell membrane occurs also much faster than changes in species composition. This is supported by previous findings in our soils showing that microbial turnover is a relatively slow process (ca. 1-3 months; Boddy et al., 2007a). Pettersson and Bååth (2003) compared the effect of different temperatures on the PLFA pattern and the growth rate response of the bacterial community to different temperatures (temperature adaptation) in a similar study as the present but with a larger temperature interval (5 to 30 ^{o}C). They concluded that the rapid changes in PLFA pattern compared with changes in activity in response to changing temperature were consistent with the hypothesis that the temperature dependent changes in the PLFA pattern were due to phenotypic acclimation and not to species replacement. Therefore it is likely that due to the relatively short period of incubation (21 d) and low temperature range (10 ^{o}C) employed here the PLFA changes in the present study were not due to changes in the species composition of the microbial community, but changes within the cell membrane lipid layer. However, whatever the cause, the soil microorganisms had clearly responded to temperature change.

The different soil types also caused differences in the PLFA profile of the microbial communities. Podzolic soils had a lower pH than gleysols and cambisols and this was associated with a decreased ratio of *i*15:0 to *a*15:0 (Bååth and Anderson, 2003), but the lower concentration of the PLFA 16:1ω5 and higher of cy19:0 in the podzols are also consistent with a lower pH in this soil type. Podzolic soils also had higher levels of fungal PLFAs which is consistent with the theory that fungi are more competitive at low pH, possibly due to their

greater resistence to Al^{3+} which becomes highly soluble at low pH. The incubation periods lasted three weeks, thus some microbial community change could have been expected, however, estimated biomass (from PLFA distribution) only really changed with soil type where fungal biomass was greatest under podzolic soils and least under the improved cambisol pasture. In the latter case this could be due to a suppression of arbuscular mycorrhizal fungi in the cambisol due to the high rates of fertilizer application.

5.4.2. Changes in soil solution chemistry following incubation

The incubation time affected the chemical composition of the soil solution in different ways. Concentrations of DOC, phenols and phosphates all increased over the weeks, while nitrate levels decreased rapidly and glucose and amino acid concentrations remained unchanged. Plants are involved in regulating concentrations of DOC and DON in soil (Khalid et al., 2007). This could be due to direct inputs into the soil from root exudates. Dominant root exudates such as amino acids and glucose showed no difference over the incubation period, so the increase in DOC concentration could be an indirect effect of the plant roots changing the solubility of the soil's native organic matter (Khalid et al., 2007). This is supported in this study and in that of Khalid et al. (2007) by the increase in phenolic concentrations in the soil, which is unlikely to originate from the root exudates (Farrar et al., 2003). DON and ammonium concentrations also did not change over the incubation, but nitrate concentrations fell sharply, probably due to uptake by the growing plants. The soil inorganic N pool has also been shown to decrease on cultivation faster than other organic N pools (Jones and Shannon, 1998).

There was no apparent statistical change in soil respiration rates over the incubation period. Substrate limitation might be a large factor in laboratory-based incubations limiting the response of belowground respiration to temperature change (Hartley et al., 2007). For example, previous studies have shown that soil carbohydrate concentrations fall during three weeks of incubation (Hartley et al., 2006) and that microbial growth and so the activity state of the microbial biomass is dependent on the availability of substrate and nitrogen to the microorganisms (Blagodarsky et al., 1998). Thus although there is evidence to indicate that incubation period induces changes in soil solution, which could change the overall functionality of the soil system, the total CO_2 flux from the soil was unchanged over the three week incubation. However, it must be noted that we did not measure substrate availability at the different temperatures in the different soils over the 21 d incubation period and this needs further investigation before conclusions can be made. In addition, the soils used to measure substrate availability had plants present, whereas the other acclimation experiments were performed in the absence of plants.

5.4.3. Changes in mineralization following incubation

There were differences in the soil microbial community and in the soil solution chemistry following incubation at warmer temperatures. Consequently, we expected mineralization rates to change at the different incubation temperatures. Temperature had a significant effect on the total recovered $^{14}CO_2$ across the five soils with a greater recovery at 20 °C than at 10 °C, but there were no significant differences in the percentage recovery between the different soils and management treatments. The increase in recovery of ^{14}C at 20 °C had a Q_{10} <

2, unlike other studies (Nicolardot et al., 1994; Bekku et al., 2003). The greatest Q_{10} found in this study was 1.3 in the gleysol soil changing from 10 to 20 °C. However, other Q_{10} estimated from ^{14}C mineralization studies show a large variation from 1.6 to 10.8 (Nicolardot et al., 1994). There is doubt about the applicability of Arrhenius plots to describe the temperature dependence of microbial processes (Ågren and Bosatta, 1987; Nicolardot et al., 1994; Rustad et al., 2000; Knorr et al., 2005). The Arrhenius equation was originally used for chemical reactions, whilst the kinetics of organic matter decomposition relies on the combination of many enzymatic reactions occurring in parallel and in series. Many microbial species and enzymatic reactions take place during substrate mineralization and temperature coefficients for microbial growth rate during decomposition are substrate-dependent limiting the applicability of the Arrhenius equation (Davidson and Janssens, 2006). Wythers et al. (2005) also observed that the Q_{10} relationship does not adequately describe the short or long-term response of plant respiration suggesting that new mathematical relationships between temperature and reaction rate need to be established for modelling purposes.

There was no acclimation effect on $^{14}CO_2$ recovered from soils moved from 10 °C to 20 °C or vice versa nor did the incubation period affect $^{14}CO_2$ recovery. Similarly, Jones and Shannon (1998) also observed no significant effect of incubation time on amino acid mineralization rate across four different management regimes (up to 40 d). In addition, the incubation period had no effect after 120 d in soil samples studied by Fang and Moncrieff (2001), suggesting that the lack of response could be due to root removal depleting the available substrate for microbial decomposition. Thus the available evidence suggests that the microorganisms are only reacting to the existing temperatures.

Similar responses have been observed in the reactions of *Escherichia coli* and *Klebsiella oxytoca* to temperature change where no acclimation response is seen (Mellefont and Ross, 2003).

The flux of low MW compounds through the fast pool was extremely rapid (1.34 ± 0.04 h) and was in a similar magnitude to other studies (0.5 to 12 h, van Hees et al., 2005; Boddy et al., 2007a). The half-time for the first phase of mineralization (k_1) demonstrated no significant differences with incubation temperature and therefore no acclimation effect, which is in contrast to Jones (1999) where amino acid half-lives at higher temperatures reduced from 2.9 h at 5 °C to 0.8 h at 34 °C. There was no statistical difference in the three soils under different management at the cambisol site, but cambisols were faster in their mineralization than soils from the gleysol and podzol field sites. This was unexpected as Kuzyakov and Jones (2006) had shown that glucose mineralization was significantly greater in rhizosphere soil when compared to unplanted soil (3 h compared to 5 h for unplanted). Second phase exponential coefficients (k_2) describing mineralization of ^{14}C (assumed to be within the microbial biomass) only showed a significant difference between 10 and 20 °C, with no acclimation, nor was there any effect of the duration of the incubation. Cambisols were all statistically similar across the three management treatments, but had slower turnover times than the gleysol and podzol field sites. Mean residence times (MRT) can be estimated by continuing Eqn. 5.2 for k_2 ($t½ = \ln(2)/k_2$, although this method has limitations as discussed in Boddy et al. (2007). A MRT of 6-9 d for ^{14}C in the soil can be calculated, which is similar to a range of values from other soils varying from 0.6 d to 2.5 y (Saggar et al., 1999; Kouno et al., 2002; van Hees et al., 2005).

Temperature warming caused the coefficient (k_2) to be slightly larger (MRT 8.8 ± 0.5 d at 10 °C compared to 7.1 ± 0.2 d at 20 °C), indicating a shorter turnover time and that microbial turnover was sensitive to temperature change. As shown in Boddy et al. (2007b) the different responses of the two phases to temperature change demonstrate the need to investigate the temperature sensitivity of each C pool within the soil. The labile low MW sugars and amino acid have lower temperature dependence than the ^{14}C incorporated into the microbial biomass. Thus the temperature dependence of soils may be dependent on the pool studied, with larger SOM fractions having different temperature dependencies than the labile pools (Mikan et al., 2002; Barrett et al., 2006; Kirschbaum, 2006).

The distribution of ^{14}C between pools a_1 and a_2 also showed no sign of acclimation, but there was a clear statistical difference between 10 °C and 20 °C with less ^{14}C was allocated to pool a_1 at 10 °C than at 20 °C. The increase of ^{14}C allocated to a_1 with increasing temperature was also well correlated with that of total $^{14}CO_2$ recovery. This was also found in Boddy et al. (2007b) and Nguyen and Henry (2002) where the pool size of a_1 increased with increasing temperature but the rate constant remained the same, indicating more ^{14}C is being partitioned for respiratory processes rather than storage and growth (Fig. 5.6). This could result in the growth efficiency decreasing at elevated temperatures (Apple et al., 2003). The maintenance costs increase with increasing temperature increasing the demand for ^{14}C to be proportioned to pool a_1. There was no difference by vegetation type in pool a_1, but in pool a_2 statistically more ^{14}C was allocated to non-vegetated cambisol soil and the least was in un-grazed cambisol. The length of the incubation had no effect on the distribution of ^{14}C.

Soil warming experiments have shown that after an initial increase in decomposition rates from the soil, that within one to three years the increased efflux returns to pre-warming rates and apparent C pool turnover rates are insensitive to temperature changes. This could be explained by the rapid depletion of the labile OC pool combined to the negligible response of non-labile OC pools under short-term experimental timescales (Knorr et al., 2005). The small effect of temperature on k_2 could be an artefact of the activation energy related to the molecular structure of the OC pool, with higher activation energies with increasing molecular complexity of the substrate. Davidson and Janssens (2006) speculate that the reaction rates are also modified by substrate concentrations and affinities of the enzymes for the substrates. Short-term patterns are dominated by the fluxes into and out of fast turnover pools, whereas long-term changes in C storage are determined by small changes in the flux of C into and out of the slow OC pools (Kirschbaum, 2004). There is a disagreement over the nature of the temperature response of labile and resistant SOM pools to changes in temperature (Fang et al., 2005). One opinion is that labile C decomposition is sensitive to temperature variation whereas resistant components are insensitive (Giardina and Ryan, 2000). Fang et al. (2005) report that SOM decomposition of both labile and resistant organic matter both respond to temperature change.

5.4.4. Conclusions

This study has shown that a short-term response to temperature change is observable in the PLFA composition of the microbial community. Soil solution also changes during the three-week incubation period. However, the incubation

period had little effect on the mineralization rates and no acclimation effect was observed. Taken together, this indicates that although physiological and chemical changes occur rapidly in the microbial community and soil solution, the functionality of the soil takes much longer to adapt to the changing conditions with overall respiration measurements and ^{14}C mineralization remaining un-changed. This effect was also observed by Pettersson and Bååth (2003) where the rate of community adaptation to temperature (estimated as a change in TdR incorporation) was slower than changes in the observed PLFA pattern. The results presented here must also be put in context of the wide daily temperature shifts that occur in these soils (typically a daily oscillation of 3 to 10 °C).

5.5. Acknowledgements

This work was funded by the UK Natural Environment Research Council. We thank Erland Bååth and COST 631 for funding a research visit to Lund University. We thank Jonathan Roberts for helping with the sample analysis.

5.6. References

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Chapter 6

Incubation temperature and priming have different effects on the turnover of discrete SOM pools in UK grassland soils

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All the experimental and written work associated with this paper was undertaken by Elizabeth Boddy. Dr Paul Hill provided the 18 and 6 month labelled soil samples and I am grateful for his proof-reading. I thank my supervisors for their continued support and guidance.

6.0. Abstract

The temperature dependence of soil organic matter (SOM) decomposition is a matter of current scientific debate, with no consensus in the literature. It is important to determine the magnitude of the impact temperature change will have on decomposition rates to assess the potential for positive feedback effects on atmospheric CO_2 concentrations. The argument is complicated by the existence of discrete pools of SOM that differ chemically and in their turnover times. Three plots of an un-grazed Eutric Cambisol grassland soil were labelled *in situ* with either ^{14}C applied as $^{14}CO_2$ to the ryegrass canopy either 18 or 6 or months prior to experimentation, or via the addition of ^{14}C-glucose to the soil immediately before use. Consequently, SOM pools of contrasting lability were differentially labelled with ^{14}C. Samples of each ^{14}C labelled soil were then incubated for 24 weeks at either 4, 10 or 20 °C and their ^{14}C mineralization rates continuously monitored. At 9 weeks into the incubation period, the soil was treated with unlabelled labile C (glucose or amino acid mixture with deionised water as a control) to investigate the impact of priming and temperature on the decomposition of SOM. A triple first order exponential decay model fitted best to the mineralization data from soils labelled at 0 months, whereas a single exponential model was the best fit to data from soils labelled 18 and 6 months prior to incubation. Although SOM mineralization was temperature-sensitive the Q_{10} was < 2, apart from in soils labelled 18 and 6 months previously where Q_{10} > 2 for soils incubated at 4 compared to 10 °C. The main effect of temperature on soils labelled at 0 months was in the relative partitioning of ^{14}C into respiration and immobilization pools rather than their rate of turnover. With increasing temperature more ^{14}C was partitioned into the most rapidly turned over pools (4

°C = 8 ± 1, 20 °C = 18 ± 3) than into the slower mineralizing pools (4 °C = 84 ± 1, 20 °C = 65 ± 7). Increasing temperature had no effect on the fastest rate of mineralization (4.8 ± 0.7 d^{-1}), but third phase exponential coefficients decreased with increasing temperature. Turnover was much slower in soils labelled at 18 and 6 months. Overall, soils labelled 6 months prior to incubation were insensitive to temperature change, whilst soils labelled 18 months previously showed a decrease in the exponential coefficients with warming. We conclude that temperature does affect the turnover of more complex C and is likely to enhance the decomposition of SOM, whilst the turnover of labile C was relatively unaffected. Priming responses with labile C were unaffected by incubation temperature and only affected the mineralization of SOM labelled at 0 months, indicating that the live microbial biomass C pool turns over faster following priming, but that the turnover of bulk SOM is unaffected. We conclude that priming only affects the turnover of the live microbial biomass, irrespective of temperature, but that the turnover of more complex pools of SOM is sensitive to temperature change.

Keywords: Biodegradation; Carbon cycling; Dissolved organic matter; Mineralization; Priming; Soil organic matter; Temperature

6.1. Introduction

Significantly more C is stored in the world's soils than is present in the atmosphere (Rasse et al., 2005). There is the risk of positive feedback occurring if this belowground C is transferred back to the atmosphere following warming-induced acceleration of soil organic matter (SOM) decomposition (Kirschbaum,

2004). Despite much research on the temperature sensitivity of SOM there is still no consensus on SOM responses to global warming (Kirschbaum, 2006). Understanding the temperature dependence of decomposition is particularly difficult because of the diverse number of soil organic compounds, microorganisms and enzymes involved, each exhibiting a wide range of kinetic parameters (Fig 6.1; Davidson and Janssens, 2006; Vanhala et al., 2007).

Temperature is likely to influence extracellular enzyme production, microbial activity and microbial community structure (Kuzyakov et al., 2007). At higher temperatures microorganisms may have a greater capacity to degrade recalcitrant SOM (Biasi et al., 2005). Short-term (48 h) studies have shown, however, that mineralization of low molecular weight (MW) C is relatively insensitive to temperature change, though the turnover of C partitioned into microbial biomass shows a greater degree of temperature sensitivity (Boddy et al., 2007b). The temperature dependence of SOM decomposition is often defined using the Q_{10} value, but this needs to be used with care in soil with its large number of microorganisms and an array of different compounds, each with their own enzymatic reactions and activation energies (Davidson and Janssens, 2006). At present, however, most global climatic change models (GCMs) still employ Q_{10} values as the temperature controller on SOM reactions. While Q_{10} values may represent a gross oversimplification of temperature control on SOM reactions in soil, current evidence has not provided any other universal predictive temperature functions which are superior. This needs to be rectified.

Microbial activity in soils is often limited by C supply, so the release of low MW C from rhizodeposition (up to 10% of a plant's net fixed C) is likely to drive microbial activity and stimulate the decomposition of more recalcitrant

SOM (Kuzyakov, 2002; Dilkes et al., 2004; Kuzyakov and Jones, 2006). Rhizodeposition is dominated by low MW sugars, organic acids and amino acids, which although only present at low concentrations provide a link in the breakdown of recalcitrant microbial and plant necromass (Fig 6.1; Toal et al., 2000; Farrar et al., 2003). Temperature change may increase SOM decomposition either by the direct effect of warming or by an indirect effect via an increase in rhizodeposition through enhanced root exudation and turnover, which may be accompanied by a priming effect on the decomposition of SOM (Kuzyakov et al., 2007).

The mechanisms of priming are poorly understood (Fontaine et al., 2003). Here we consider priming to be a response to low MW C that stimulates the microbial population. This results in an enhanced rate of decomposition of more recalcitrant SOM either because once the added labile substrate is exhausted the microorganisms change substrate or because of the enhanced activity of the microbial biomass enables additional decomposition of SOM (Kuzyakov and Bol, 2006; Kuzyakov et al., 2007). Estimates of priming are often made using either an isotopic tracer or by the difference in CO_2 efflux from the soil with and without substrate addition (Kuzyakov and Bol, 2006). We propose that using a soil that has been previously labelled with a C isotope (^{13}C or ^{14}C) and then adding un-labelled low MW substrate decreases the errors associated with priming investigations and enables the source of the additional efflux to be assessed (Kuzyakov et al., 2007). The use of isotopic tracer has a further advantage: suitable analysis allows preliminary conclusions to be drawn about whether treatments affect all pools of SOM equally.

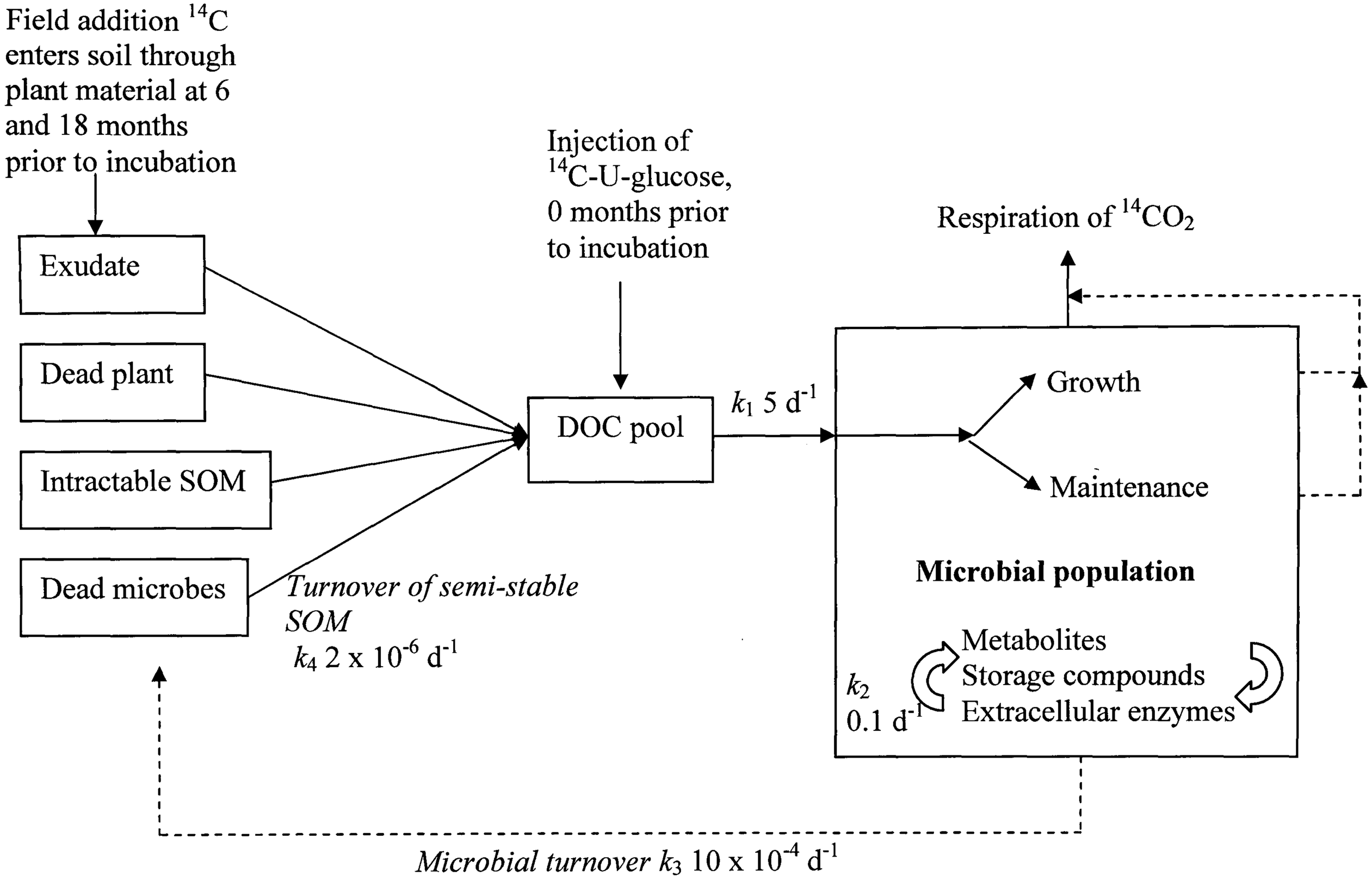


Figure 6.1. Schematic representation of the fate of ^{14}C substrate either added directly to the soluble DOC pool in an un-grazed Cambisol or fed to plant material as $^{14}CO_2$ with subsequent incorporation into the soil for 18 or 6 months prior to soil experimentation. Also shown are the suggested location of the temporal SOM pools described by the exponential coefficients k_1, k_2, k_3, and k_4 and their approximate turnover times. The values attached to k_1 - k_4 will be derived later in this paper.

The aim of this study was to (a) to assess the decomposition of ^{14}C-labelled soils incubated at different temperatures, over a long time period, where the ^{14}C-label is present in different C pools, (b) to assess whether the addition of low MW substrate will cause a priming effect, resulting in additional decomposition of SOM, (c) whether the magnitude of this response will be temperature dependent, and (d) whether the magnitude of this response will vary between specific pools. A model of soil C pools (Fig. 6.1) provides a conceptual framework for our experiments.

6.2. Methods

6.2.1. Field site and sampling regime

Soil was sampled (0-15 cm, $n = 3$) from a temperate oceanic agricultural grassland located in Abergwyngregyn, Gwynedd, North Wales (53°14'N, 4°01'W; Table 6.1 and 6.2). The mean annual soil temperature at 10 cm is 11 °C and the annual rainfall is 1250 mm. The soil was a Eutric Cambisol supporting a sward of *Lolium perenne* L. and *Trifolium repens* L. The field site included three delineated areas that had not been grazed since June 2002. Soil was sampled in November 2005. At this time, the soil temperature at 5 cm depth was 4.6 ± 0.1 °C. Soils were collected from the three subplots and transported to the laboratory in gas permeable polyethylene bags at 4 °C for subsequent analysis within 48 h of sampling.

Table 6.1. Summary of labelling methodologies utilized to evaluate the impact of temperature and priming on C turnover in an un-grazed Eutric Cambisol, including the identification of exponential coefficients from the mineralization of ^{14}C, and their use in the evaluation of priming.

Time before measuring mineralization (months)	0	6	18
Method of introducing ^{14}C to soil	^{14}C glucose applied directly	$^{14}CO_2$ supplied to grass canopy	$^{14}CO_2$ supplied to grass canopy
Number of exponential components identified	3 ($k_{1\text{-}3}$)	1 (k_4)	1 (k_4)
Priming substrates added	Yes	Yes	Yes

Table 6.2. Characterization of selected properties of a Eutric Cambisol un-grazed soil and extracted soil solution. Free amino acids assumes an average C content of 62.4 g mol^{-1} and an average N content of 19.6 g mol^{-1}. Values represent means ± SEM; $n = 3$.

Parameter	
Moisture content (g kg^{-1})	316 ± 5
PH	6.0 ± 0.2
Total C (g kg^{-1})	32.2 ± 3.6
Total N (g kg^{-1})	3.0 ± 0.4
C-to-N ratio	10.9 ± 0.2
Soil respiration (mg CO_2 kg^{-1} h^{-1})	3.0 ± 0.6
DOC (mg C l^{-1})	133.7 ± 33.4
DON (mg N l^{-1})	36.1 ± 16.8
Glucose (mg C l^{-1})	19.2 ± 3.5
Total free amino acids (mg C l^{-1})	6.1 ± 1.2
Total free amino acids (mg N l^{-1})	1.9 ± 0.2
Phenolics (mg l^{-1})	21.8 ± 7.8
Nitrate (mg N l^{-1})	1.1 ± 0.2
Ammonium (mg N l^{-1})	6.6 ± 3.4

6.2.2. Temperature dependent mineralization of ^{14}C

Within each delineated area of un-grazed sward three separate areas were divided in May 2004 (Table 6.1). Pulses of $^{14}CO_2$ were applied to three swards as described in Hill et al. (2007). Acrylic chambers (15 cm × 30 cm × 60 cm; 0.045 m^{-3}) were placed over the soil on a steel frame inserted into the soil, sealed by a strip of closed cell foam. $^{14}CO_2$ was generated by injecting 7.4 MBq $NaH^{14}CO_3$ into a reaction vessel containing dilute HCl. The chambers were sealed and internal stirring fans were turned on to ensure even exposure of the sward. The chambers were left *in situ* for 1 h to permit uptake of the $^{14}CO_2$ into the plants. The next three swards were treated in the same manner in June 2005, whilst the remaining three areas were left un-treated. ^{14}C-labelled material entered the soil as plant exudates and senesced plant material for 18 months or 6 months, so that some of the ^{14}C present at sampling in November 2005 was in medium to high MW pools in the soil (Table 6.1).

On sampling, soils were coarsely sieved to 7 mm and shoots and root material removed. Coarse sieving has previously been shown not to adversely affect microbial activity or substrate availability in this soil (Jones and Willett, 2006). Sub-samples of soil (35 g) were then placed in individual sealed polypropylene containers at 4 °C for 24 h to minimise the effect of disturbance before starting the incubation of 24 weeks at either 4, 10 or 20 °C. 1 ml of 1 M NaOH in an eppendorf tube was placed inside each container of soils labelled 18 and 6 months previously to trap any $^{14}CO_2$ evolved from the soil. This NaOH trap was subsequently replaced 22 times at increasing intervals over the 24 week incubation period. The $^{14}CO_2$ in the 1 M NaOH traps was determined by liquid scintillation counting using a Wallac 1404 scintillation counter (Wallac EG&G,

Milton Keynes, UK) and Optiphase 3® alkali compatible scintillation fluid (Wallac EG&G).

Soil from the unlabelled areas was sieved to 7 mm as before and 35 g samples placed in sealed polypropylene containers at 4 °C for 24 h before being labelled with low MW C as described in Boddy et al. (2007a). Briefly, 0.5 ml of 3.5 kBq ^{14}C-U-glucose was injected onto the soil surface (50 µl, 500 µM glucose; 1 µCi ml^{-1}; Amersham Biosciences UK Ltd, Chalfont St. Giles, UK) and the $^{14}CO_2$ evolution measured as for the soils labelled 18 and 6 months previously (Table 6.1).

6.2.3. Background soil characteristics

Soil was analyzed as per Boddy et al. (2007a; Table 6.2). Briefly, dry root free soil was analyzed for C and N content in a Leco CHN 2000 analyzer (Leco Corp., St Joseph, MI, USA). Soil pH was measured with a BDH Gelplas electrode (VWR International, Lutterworth, UK) in a 1:1 (v/v) mixture with deionized water. Moisture content was assessed by oven drying at 105 °C for 24 h. Soil respiration was measured in the laboratory using an automated SR1 infra red gas analyzer (PP-Systems Ltd., Hitchin, UK). The ^{14}C content of the soil from the three areas was determined following soil oven drying at 105 °C with an OX-400 Biological Sample Oxidizer (RJ Harvey Instrument Corp., Hillsdale, NJ). The ^{14}C content in the 18 month labelled soil was 30.0 ± 14.5 Bq g^{-1} soil whilst in the 6 month labelled soil it was 5.5 ± 2.4 Bq g^{-1}. No ^{14}C (<0.01 Bq g^{-1}) was detected in the un-labelled control soil to which ^{14}C-glucose was subsequently added. The liberated $^{14}CO_2$ from the OX-400 Biological Sample Oxidizer was collected in Oxosol scintillation fluid (National Diagnostics,

Hessle, UK) and then counted using a Wallac 1404 scintillation counter (Wallac EG&G, Milton Keynes, UK).

Soil solution was extracted according to Giesler and Lundström (1993) and analyzed as per Boddy et al. (2007a; Table 6.2). Soil samples were centrifuged (4000*g*, 15 min, 20 °C) to obtain soil solution, the collected solutions passed through Whatman 42 filter paper before freezing at -20 °C to await analysis. Soil solution samples were analyzed for DOC and total dissolved N (TDN) using a Shimadzu TOC-V-TN analyzer (Shimadzu Corp., Kyoto, Japan). NO_3^- was determined colorimetrically by the Cu-Zn-hydrazine reduction method of Downes (1978) and NH_4^+ by the salicylate-hypochlorite procedure of Mulvaney (1996). Total soluble phenolic concentrations were assessed using the Folin-Ciocalteau reagent, calibrated with a phenol standard, according to Swain and Hillis (1959). Free amino acids were measured fluorimetrically according to Jones et al. (2003). Glucose was determined spectrophotometrically with a Glucose (GO) Assay® kit (Sigma-Aldirch, Misouri, USA).

6.2.4. Priming following addition of either glucose or amino acids to ^{14}C labelled soil

After 9 weeks of incubation at 4, 10 or 20 °C, a pulse of un-labelled glucose, amino acid mixture or deionised water as a control was added to the soil surface and the rate of evolution of $^{14}CO_2$ monitored as described before (Table 6.1). The amino acid mixture consisted of 15 equimolar L-isomeric amino acids (alanine, arginine, aspartic acid, glutamic acid, glycine, histidine, isoleucine, leucine, lysine, phenylalanine, proline, serine, threonine, tyrosine and valine). Substrate was added at a ratio of 1 mg C substrate to 1 g C in soil (Lin and

Brookes, 1999; added 0-10 mg C substrate to 1 g C in soil, lower concentrations giving optimal response), resulting in 51 mM of glucose or 68 mM of amino acid were therefore added in 0.5 ml.

6.2.5. Statistical and data analysis

Glucose mineralization in soil is at least biphasic (van Hees et al., 2005; Boddy et al., 2007a) and can be triphasic (Boddy et al., 2007c), and in this study we describe mineralization of ^{14}C by a triple first order decay (Table 6.1):

$$S = [a_1 \times \exp(-k_1 t)] + [a_2 \times \exp(-k_2 t)] + [a_3 \times \exp(-k_3 t)] \qquad \text{(Eqn. 6.1)}$$

where S is the total ^{14}C-label remaining in the soil, k_1 is the exponential coefficient describing the primary mineralization phase, k_2 is the exponential coefficient describing the second, slower mineralization phase and k_3 the third and slowest phase. The parameters a_1, a_2, and a_3 describe the size of pools associated with exponential coefficients k_1, k_2 and k_3, and t is time. The first rapid phase of $^{14}CO_2$ production is attributable to the immediate use of the substrate in catabolic processes and approximates to the depletion rate from the soil. The half-life ($t_{½}$) of the pool a_1 can be calculated as:

$$t_{½} = \ln(2)/ k_1 \qquad \text{(Eqn. 6.2)}$$

(Paul and Clark, 1996). The two slower phases (k_2 and k_3) of $^{14}CO_2$ production are attributable to the subsequent turnover of ^{14}C immobilized within the soil microbial community. As discussed in Boddy et al. (2007a) we do not know enough about the connectivity between pools a_1, a_2 and a_3 to calculate the half time for pools a_2 and a_3.

$^{14}CO_2$ evolution from soil labelled 18 and 6 months previously is described by a single first order decay function (Table 6.1):

$$S = [a_4 \times \exp(-k_4 t)] \quad \text{(Eqn. 6.3)}$$

where S is the total ^{14}C remaining in the soil, k_4 is the exponential coefficient describing the mineralization of ^{14}C from pool a_4. The slow phase exponential coefficient from the mineralization of low MW C (k_3) is likely to be comparable to the exponential coefficient for the mineralization of ^{14}C entering the soil 6 and 18 months previously (k_4).

All experiments were carried out in triplicate. One-way ANOVA was used to evaluate the impact of incubation temperature on the low MW C addition to soil. All other data were analysed using factorial ANOVA (Univariate GLM) to evaluate change following incubation and priming with low MW C. Statistical procedures were carried out with the software package SPSS 12.0 for Windows (SPSS Inc., Chicago, IL) with $P < 0.05$ used as the upper limit for statistical confidence.

6.3. Results

6.3.1. Temperature dependence of turnover of low MW ^{14}C

The evolution of $^{14}CO_2$ from the soil following additions of low MW C in the form of ^{14}C-U-glucose at 0 months best fitted a triphasic model for $^{14}CO_2$ loss over the 24 week incubation period. Previous studies over 48 h have fitted biphasic models (Table 6.1; Fig. 6.2; Boddy et al., 2007a-c), however, the data fitted significantly better to a triphasic model ($r^2 = 0.975 \pm 0.002$ double, $r^2 = 0.997 \pm 0.001$ triple exponential equation; $P < 0.001$). While there was a similar pattern of $^{14}CO_2$ evolution at each of the different incubation temperatures, the mineralization of low MW ^{14}C-U-glucose increased significantly with increasing temperature (24 ± 1, 33 ± 1, and 44 ± 1% ^{14}C mineralized over 24 weeks as a

percentage of the total ^{14}C added at 4, 10 and 20 °C respectively; $P < 0.001$). Although the increases in $^{14}CO_2$ evolution at higher temperatures were significant, the Q_{10} was < 2, with a maximum Q_{10} of 1.4 ± 0.1 from 10 to 20 °C.

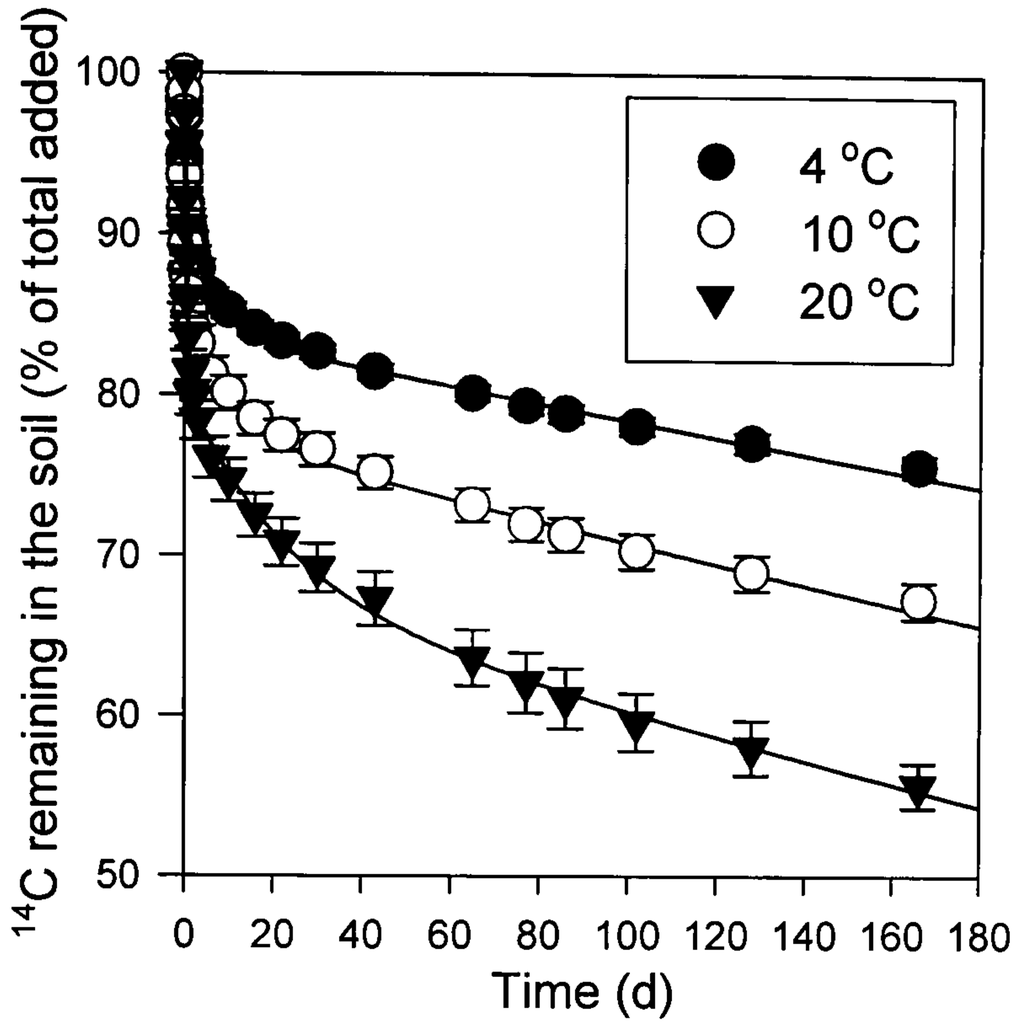


Figure 6.2. Mineralization kinetics following additions of ^{14}C-U-glucose to un-grazed Eutric Cambisol soil, incubated at 4, 10 and 20 °C for 24 weeks. Mineralized $^{14}CO_2$ was trapped in 1 M NaOH and the percentage of added ^{14}C remaining in the soil plotted over time. Bars are SEM, $n = 3$.

6.3.2. Kinetics of mineralization at three temperatures of different ^{14}C-labelled pools

A triple first order exponential equation provided the best fit to the soil labelled with ^{14}C-U-glucose at 0 months, enabling the exponential coefficients to be calculated at different temperatures from the 24-week incubation (Eqn. 6.1; Table 6.3). The impact of incubation temperature was seen on the slower mineralization phases, that of the exponential coefficients for k_2 and k_3 ($P <$ 0.05). There was no effect of temperature on first-phase (k_1) exponential coefficients ($P = 0.520$; averaging 4.8 ± 0.7 d^{-1}, $t_{½}$ 5.4 ± 2.0 h). The slower phase coefficients, thought to equate to the ^{14}C incorporated into the microbial biomass

(describing the metabolism of storage compounds and microbial cell turnover), both showed a significant difference between soils incubated at 4 and 20 °C, however, soils incubated at 10 °C showed no statistical difference from either temperature (average ± SEM; k_2: 4°C = 0.15 ± 0.02 d^{-1}, 10°C = 0.11 ± 0.01 d^{-1}, 20°C = 0.06 ± 0.02 d^{-1}; k_3: 4°C = $6.67 \times 10^{-4} \pm 3.33 \times 10^{-5}$ d^{-1}, 10°C = $9.3 \times 10^{-4} \pm 6.7 \times 10^{-5}$ d^{-1}, 20°C = $13.3 \times 10^{-4} \pm 1.7 \times 10^{-4}$ d^{-1}). The exponential coefficients for k_2 were smaller with increasing temperature, indicating a longer turnover at higher temperatures, whereas the exponential coefficients for k_3 were larger with increasing temperature indicating a shorter turnover time at higher temperatures.

Table 6.3. Parameters of the triple exponential equation for the mineralization of glucose in soil solution after incubation for 24 weeks at 4, 10 and 20 °C. The ^{14}C-U-glucose was added immediately before starting measurements of mineralization. With estimates of the half-life (in days, defined as $0.693/k_1$) of ^{14}C-U-glucose in the soil solution and rate constants for mineralization after incorporation into the microbial biomass (k_2 and k_3). Pool size estimates for ^{14}C partitioned into pools a_1, a_2 and a_3 associated with exponential coefficients k_1, k_2 and k_3 respectively are given. Respiration is the total recovered $^{14}CO_2$ at the end of 24 weeks as a % of the added ^{14}C. Values represent means ± SEM (n = 3), * denotes statistically different results between temperatures at $P < 0.05$.

Parameter	Incubation temperature		
	4 °C	10 °C	20 °C
a_1	8.28 ± 0.93*	13.20 ± 1.28	18.36 ± 2.74*
k_1	5.97 ± 0.49	4.01 ± 0.85	4.44 ± 1.85
$t_{½}$	0.12 ± 0.01	0.19 ± 0.05	0.37 ± 0.26
a_2	7.33 ± 0.68	8.71 ± 0.12	15.65 ± 4.19
k_2	0.15 ± 0.02*	0.11 ± 0.01	0.06 ± 0.02*
a_3	83.9 ± 0.3*	77.7 ± 0.8	65.2 ± 7.0*
k_3	$6.7 \times 10^{-4} \pm 3.3 \times 10^{-5}$*	$9.3 \times 10^{-4} \pm 6.7 \times 10^{-5}$	$13.3 \times 10^{-4} \pm 1.7 \times 10^{-4}$*
Respiration	24.3 ± 0.7*	33.8 ± 1.1*	44.3 ± 1.4*

The ^{14}C distribution between pools a_1, a_2 and a_3 associated with exponential coefficients k_1, k_2 and k_3 was significantly different at 4 and 20 °C,

where a_1 and a_3 were significantly different, but data from soils at 10 °C was statistically similar to 4 and 20 °C (a_1: $P = 0.023$, 4 °C = 8.3 ± 0.9, 10 °C = 13.2 ± 1.3, 20 °C = 18.4 ± 2.7; a_3: $P = 0.044$, 4 °C = 83.9 ± 0.3, 10 °C = 77.7 ± 0.8, 20 °C = 65.2 ± 7.0). Pool a_2 had statistically the same proportion of ^{14}C allocated to it at all temperatures ($P = 0.11$; average ± SEM; 10.7 ± 1.8). Total $^{14}CO_2$ recovered from the soil correlated linearly to pool a_1 and a_3 with increasing soil temperature increasing the relative amount of ^{14}C partitioned into pool a_1 and decreasing the amount allocated to pool a_3 ($r^2 = 0.828$ for a_1 and 0.756 for a_3).

Mineralization from soils labelled with ^{14}C for 6 months and 18 months prior to the incubation showed a best fit to a single exponential decay equation in all cases in comparison to double and triple exponential decay equations (Table 6.4; Fig. 6.3; Eqn. 6.3; $r^2 = 0.927 \pm 0.010$). Although an initial faster phase is shown in Fig. 6.3, this is attributable to disturbing the soil through sampling and highlights the importance of the longer sampling time to truly show mineralization rates. Incubation temperature had no significant effect on the size of the exponential coefficient, k_4, for soils labelled 6 months prior to incubation ($P = 0.235$; average ± SEM; $1.2 \times 10^{-6} \pm 2.0 \times 10^{-7}$ d^{-1}); there was also no significant effect of temperature on the proportion of ^{14}C that remained in pool a_4 at the end of the incubation period ($P = 0.112$; average ± SEM; 99.99 ± 0.001). In contrast, soils labelled for 18 months prior to incubation did have significant differences in the exponential coefficient (k_4) following incubation. Overall, there were smaller exponential coefficients at lower temperatures ($P = 0.029$; 4°C = $5.4 \times 10^{-7} \pm 1.3 \times 10^{-7}$ d^{-1}; 20°C = $1.7 \times 10^{-6} \pm 2.1 \times 10^{-7}$ d^{-1}). Soils incubated at 10 °C were statistically similar to those incubated at 4 and 20 °C ($1.4 \times 10^{-6} \pm 3.0 \times 10^{-7}$ d^{-1}). There was no statistical difference in the distribution

of ^{14}C in pool a_4 for soils labelled 18 months previously and incubated at the different temperatures ($P = 0.087$). Although not statistically different ($P > 0.05$), a greater Q_{10} value was observed in soils labelled 18 and 6 months previously, moving from 4 to 10 °C, than from 10 to 20 °C (4 °C = 2.08 ± 0.36; 10 °C = 1.32 ± 0.14).

Table 6.4. Parameter estimates after incubation for 24 weeks at 4, 10 or 20 °C of the single exponential equation for the mineralization of ^{14}C in soil labelled 18 and 6 months prior to incubation. Soil was initially labelled indirectly with ^{14}C by supplying $^{14}CO_2$ to the plant canopy. Estimated rate constants for mineralization after incorporation into the microbial biomass (k_4) are given. Pool size estimates for ^{14}C partitioned into pools a_4 associated with exponential coefficient k_4 are also given. Respiration is the total recovered $^{14}CO_2$ at the end of 24 weeks as a % of the ^{14}C present in the soil at the start of the incubation. Values represent means ± SEM ($n = 3$).

Parameter	**Incubation temperature**		
	4 °C	**10 °C**	**20 °C**
18 months			
a_4	99.99 ± 0.001	99.99 ± 0.001	99.99 ± 0.001
k_4	$5.4 \times 10^{-7} \pm 1.3 \times 10^{-7}$	$1.4 \times 10^{-6} \pm 3.0 \times 10^{-7}$	$1.6 \times 10^{-6} \pm 2.1 \times 10^{-7}$
Respiration	0.010 ± 0.003	0.023 ± 0.005	0.026 ± 0.003
6 months			
a_4	99.99 ± 0.001	99.99 ± 0.002	99.98 ± 0.005
k_4	$1.1 \times 10^{-6} \pm 1.9 \times 10^{-7}$	$3.3 \times 10^{-6} \pm 1.2 \times 10^{-6}$	$4.6 \times 10^{-6} \pm 1.8 \times 10^{-6}$
Respiration	0.022 ± 0.003	0.056 ± 0.020	0.077 ± 0.029

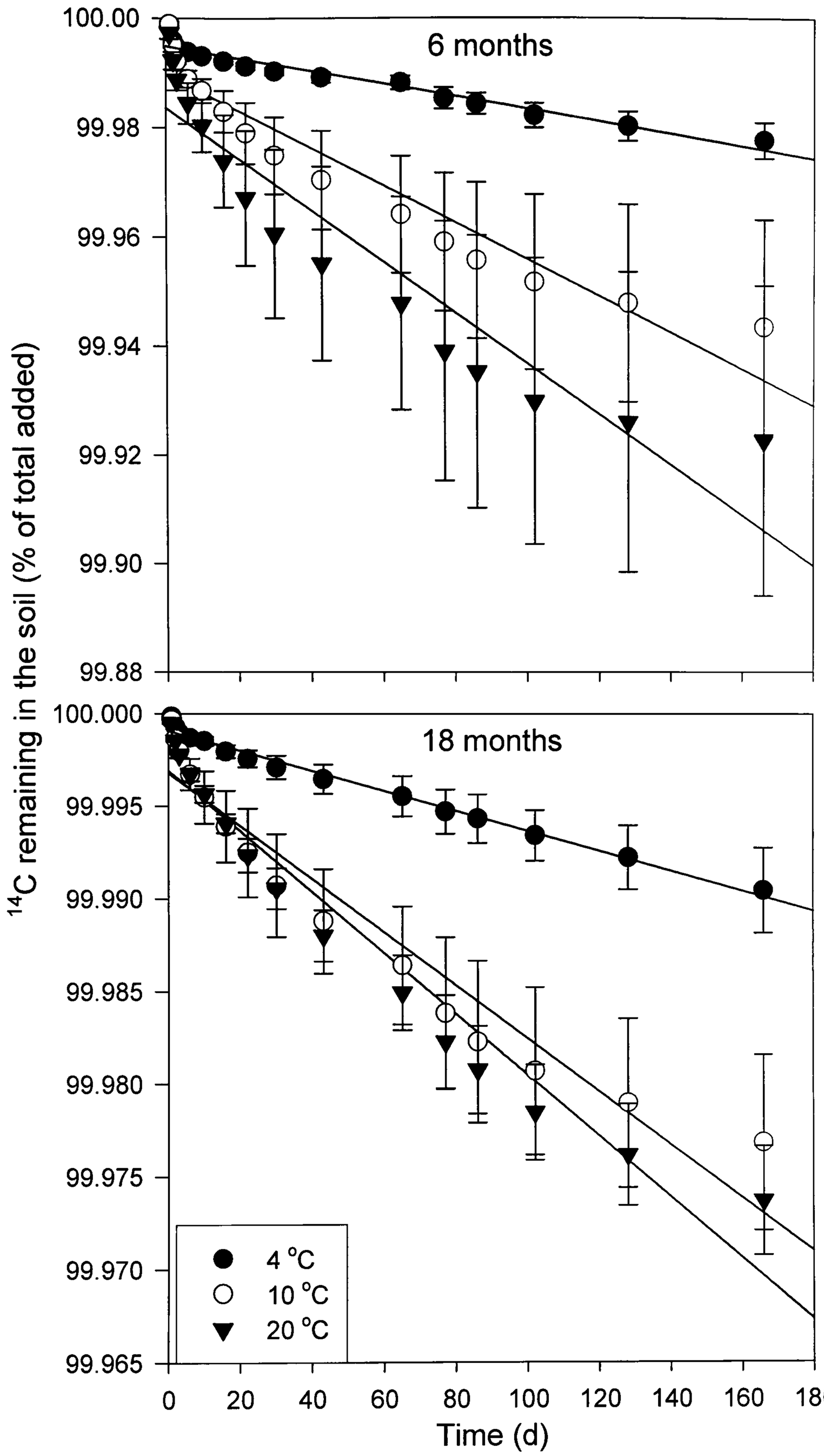


Figure 6.3. Mineralization kinetics following the labelling of soil through the exposure of intact swards to $^{14}CO_2$ either 18 months or 6 months prior to incubation at 4, 10 or 20 °C for 24 weeks. Mineralized $^{14}CO_2$ was trapped in 1 M NaOH and the percentage of added ^{14}C remaining in the soil plotted over time.

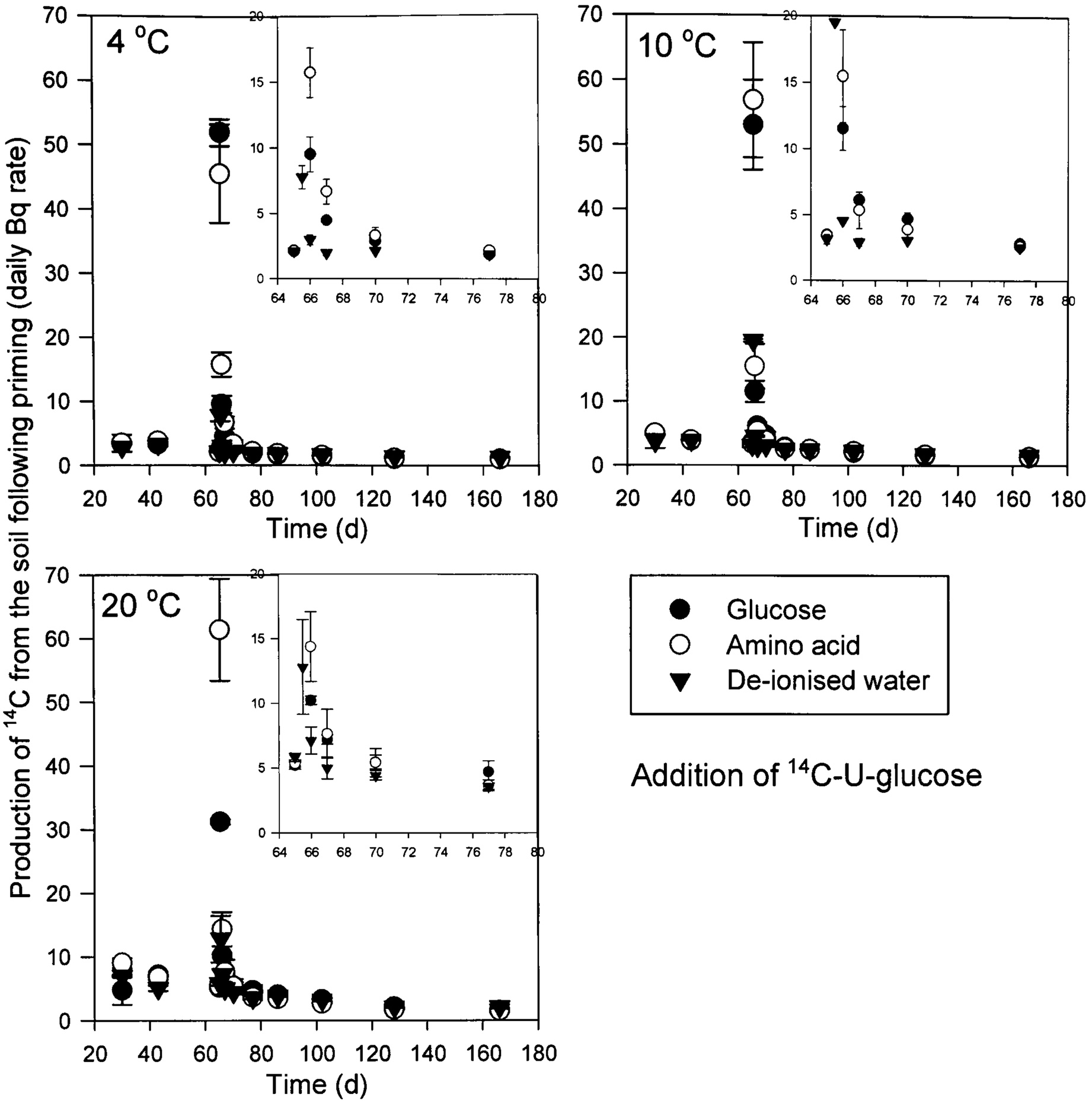
Points represent experimental means ± SEM (n = 3) while lines represent fits to a single exponential decay model.

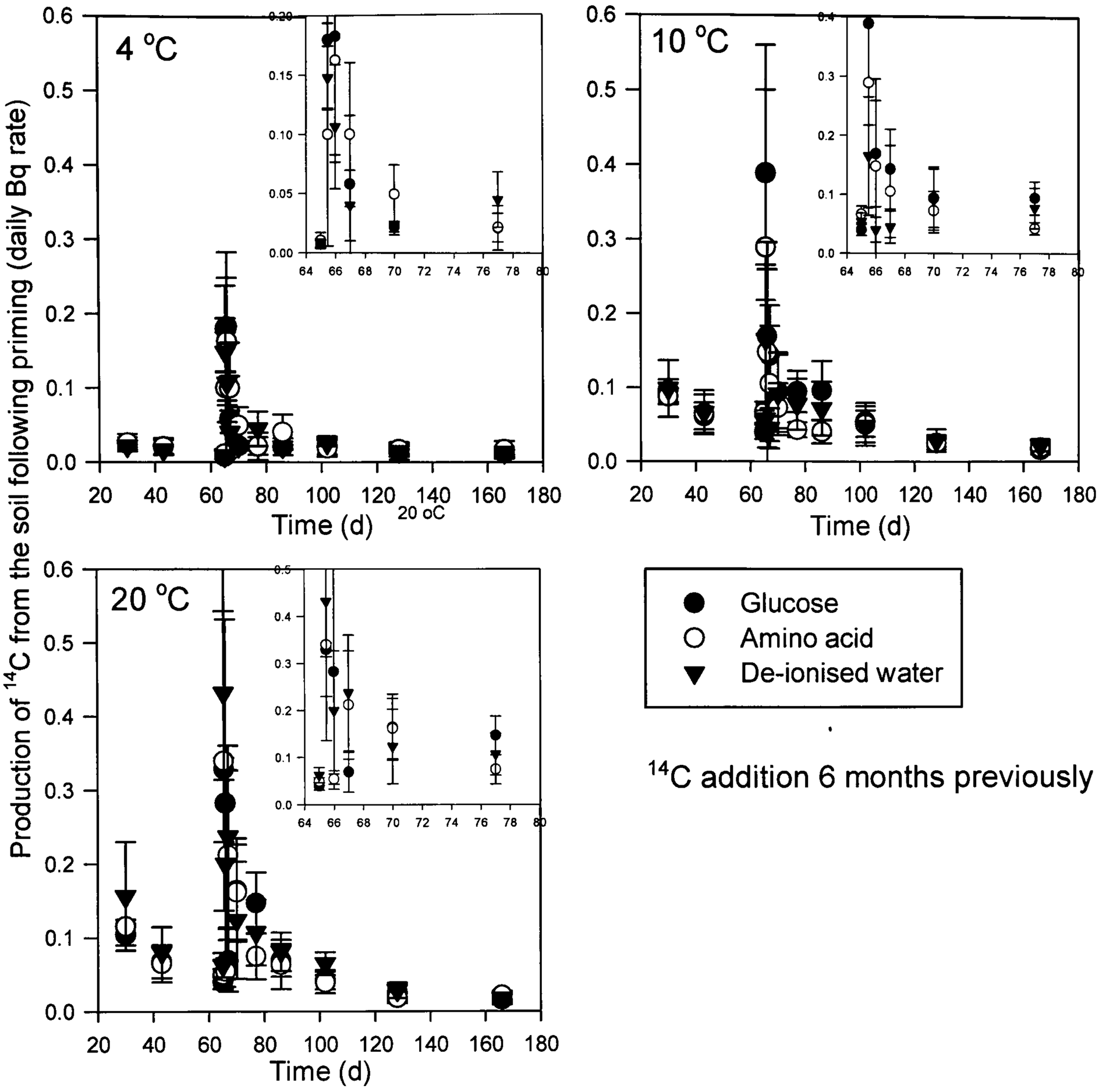
The exponential coefficients k_3 and k_4 for the turnover of ^{14}C in the three plots showed no statistical similarity ($P < 0.001$), with low MW ^{14}C additions at 0 months having statistically shorter exponential coefficients than soils amended with ^{14}C at least 6 months previously (low MW ^{14}C, 0 months: $9.78 \times 10^{-4} \pm 1.10 \times 10^{-4}$ d^{-1}; *in situ* amendments, 6 and 18 months: $2.14 \times 10^{-6} \pm 4.74 \times 10^{-7}$ d^{-1}), indicating a faster turnover of ^{14}C in soils labelled at the start of the incubation with ^{14}C-U-glucose than soils labelled 18 and 6 months previously; soils labelled 6 months previously were statistically similar to those labelled 18 months prior to incubation. The last 64 d of the incubation, when the rate of decline in efflux of $^{14}CO_2$ from the soil surface appears stabilised, demonstrates a clear statistical difference in the incubation temperature between 20 °C and 4 °C for soils amended with low MW ^{14}C at 0 months ($P = 0.028$, 0.04 ± 0.002 % d^{-1} at 4 °C; 0.06 ± 0.009 % d^{-1} at 20 °C). There was no statistical difference by incubation temperature or between soils labelled 18 and 6 months previously. Soils labelled prior to the incubation were statistically slower in the turnover of ^{14}C when compared to soils amended with low MW ^{14}C at the start of the incubation ($P <$ 0.001; average ± SEM; $8.2 \times 10^{-5} \pm 1.2 \times 10^{-5}$ % d^{-1}).

6.3.3. Impact of priming on ^{14}C turnover in different ^{14}C-labelled pools

Change in the daily $^{14}CO_2$ evolution from the three ^{14}C-labelled soils following the addition of low MW C (glucose or amino acid mixture) or deionised water only occurred in the soils amended with ^{14}C-U-glucose at 0

months (Fig. 6.4). The addition of low MW C had no significant effect on the daily $^{14}CO_2$ evolution on field labelled soils at 6 and 18 months; with a short-term (< 1 d) impact of disturbance. However, no significant increase in ^{14}C mineralization rate following the addition of low MW C substrate was seen with mineralization rates similar to that of the added deionised water controls (P = 0.198, averaging 0.46 ± 0.08 Bq d^{-1}). The incubation temperature also had no effect on the magnitude of mineralization following addition of low MW C in field labelled soils, where ^{14}C was present in stable SOM pools. Soil amended with ^{14}C-U-glucose did show statistical differences following the addition of low MW C, though the effect was again short-term, lasting < 1 d. The addition of deionised water had no effect on mineralization rates on soil amended with ^{14}C-U-glucose, but glucose and amino acid mixture both enhanced mineralization rates, 4-5 times that of mineralization rates following addition of deionised water ($P < 0.001$; 41 ± 4 Bq d^{-1} at 0.5 d after priming for glucose, 50 ± 5 Bq d^{-1} amino acid, and 9 ± 2 Bq d^{-1} for deionised water). Incubation temperature had a significant effect on soil amended with ^{14}C-U-glucose following the addition of low MW C, with 4 and 10 °C having a higher mineralization rate than at 20 °C (P < 0.001; 32 ± 7 Bq d^{-1} at 4 °C; 39 ± 7 Bq d^{-1} at 10 °C; 29 ± 7 Bq d^{-1} at 20 °C).


4 °C
10 °C
20 °C
Production of ^{14}C from the soil following priming (daily Bq rate)
Time (d)
Glucose
Amino acid
De-ionised water
Addition of ^{14}C-U-glucose


4 °C
10 °C
20 °C
20 oC
Production of ^{14}C from the soil following priming (daily Bq rate)
Time (d)
Glucose
Amino acid
De-ionised water
^{14}C addition 6 months previously

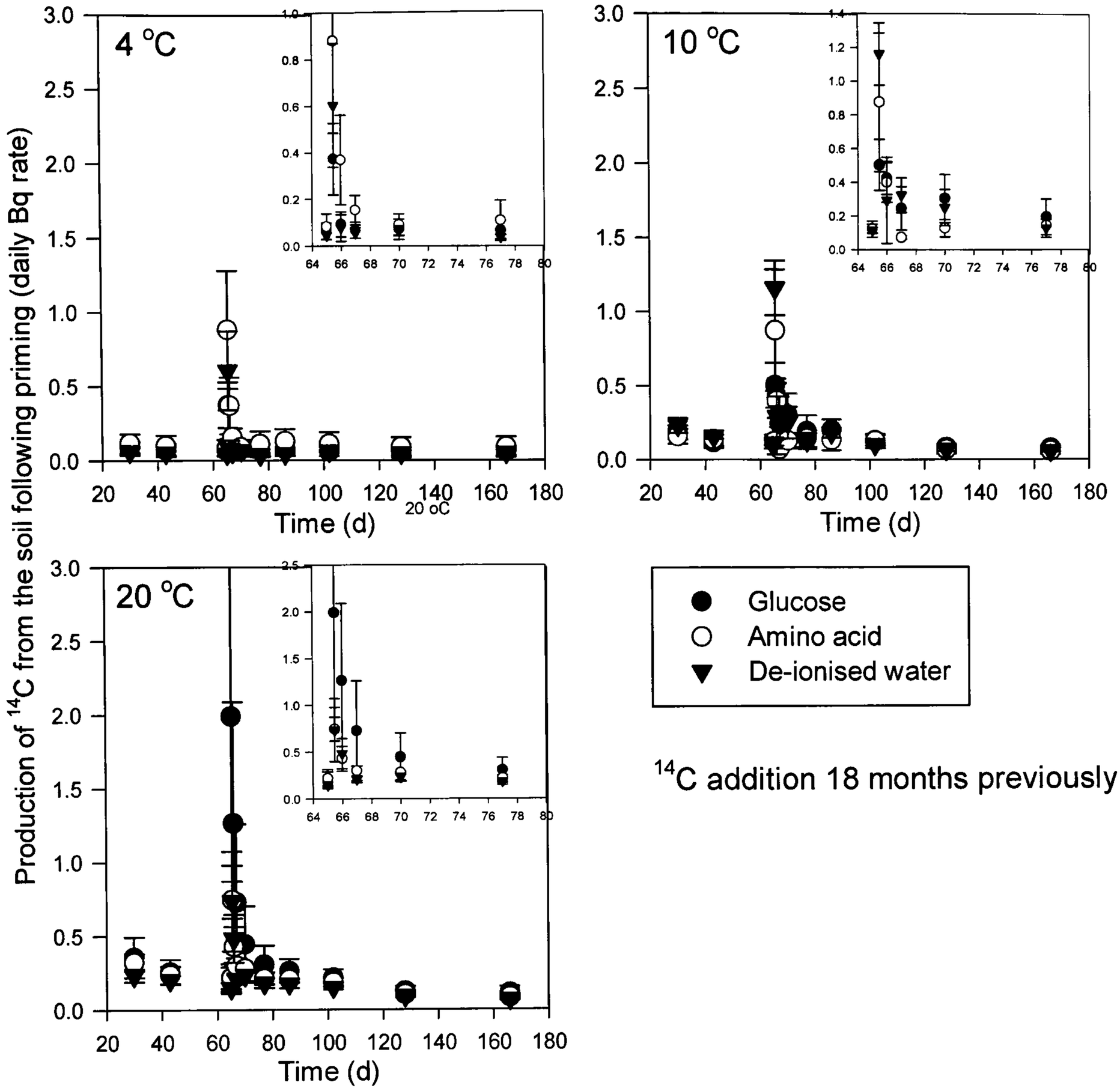


Figure 6.4. Mineralization kinetics following additions of un-labelled labile substrate (glucose, amino acid mixture, deionised water) at 65 d to Eutric Cambisol soils previously incubated at 4, 10 and 20 °C for 9 weeks, and labelled either at the start of incubation with ^{14}C-U-glucose, or 6 months prior to incubation, or 18 months previously. Mineralized $^{14}CO_2$ was trapped in 1 M NaOH and the production of ^{14}C from the soil was plotted over time as the daily Bq rate. Bars are SEM, $n = 3$.

6.4. Discussion

6.4.1. Identification of four temporal SOM pools

The evolution of $^{14}CO_2$ following labelling in the field 18 and 6 months prior to incubation and labelling with ^{14}C-U-glucose at the start of the incubation enabled the identification of four separate temporal SOM pools from the exponential coefficients calculated from the single and triple exponential equations (Fig. 6.1, Table 6.5), with k_1 having the fastest turnover at 5 d^{-1} and k_4 the slowest with a turnover of 2×10^{-6} d^{-1}. Half-lives calculated from k_1 (5.4 ± 2.0 h) gave similar turnover rates for first phase mineralization of low MW substrate in agricultural soils (2.3 ± 0.5 h; Jones et al., 2005), indicating a rapid removal of low MW DOC from the DOC pool. A mean residence time (MRT) can be estimated by continuing Eqn. 6.2 for k_2, k_3 and k_4 ($t½ = \ln(2)/ k_x$). This method oversimplifies the pathways of ^{14}C from soil solution through the microbial biomass, but enables comparisons to other MRT reported. The second phase (k_2) MRT of 6 d is likely to be the turnover of metabolites, storage compounds and extracellular enzymes rather than structural components of the microbial biomass (Saggar et al., 1999; Toal et al., 2000). MRT for the third phase exponential coefficients estimates the turnover of this C pool to be < 2 y, giving a turnover rate which might represent the microbial community, similar to the values reported in forest (0.25 to 2.5 y; van Hees et al. (2005) and ryegrass soils (0.17 to 0.42 y; Saggar et al., 1996; Kouno et al., 2002). The final temporal SOM pool corresponding to exponential coefficient k_4 has a MRT of about 1000 y representing the turnover of semi-stable SOM.

Table 6.5. Summary of four SOM pools differentiated by exponential coefficients from the mineralization of ^{14}C, and their susceptibility to priming and the effect of temperature on the pools. Values represent means ± SEM (n = 3).

SOM pool k (d^{-1})	Mineralization affected by priming?	Mineralization affected by temperature?
4.81 ± 0.67	Yes	No
0.11 ± 0.02	No	Yes (increase turnover)
$9.78 \times 10^{-4} \pm 1.10 \times 10^{-4}$	No	Yes (decrease turnover)
$2.14 \times 10^{-6} \pm 4.74 \times 10^{-7}$	No	Yes (decrease turnover)

6.4.2. Impact of temperature on ^{14}C turnover

Investigations into the temperature dependence of SOM decomposition, and so the respiration efflux from the soil and mineralization of substrates within the soil are often short-term (Boddy et al., 2007a). This study considers the impact of longer-term incubation on the decomposition of SOM by considering the effect of incubating soil labelled with ^{14}C present in different C pools at three temperatures for 24 weeks. We found that more ^{14}C was mineralized at higher temperatures. Total $^{14}CO_2$ evolution from soil amended with ^{14}C-U-glucose at the start of the incubation had a low Q_{10}, similar to the short-term study of Boddy et al. (2007b; 2007c) with a maximum Q_{10} of 1.4. Thus the duration of the incubation did not affect the overall temperature response for the mineralization of low MW ^{14}C to $^{14}CO_2$. Mineralization of ^{14}C in more complex pools had a greater Q_{10}, particularly at low temperatures (Q_{10} of 2.1 for $^{14}CO_2$ recovered at 4 compared to 10 °C, Q_{10} of 1.3 for soils incubated at 10 compared to 20 °C). Bekku et al. (2003) also demonstrated that the greatest respiratory response and so the largest Q_{10} values occurred at low temperatures (< 4 °C) in their Arctic soils. The Q_{10} of mineralization for field labelled soils (18 and 6 months prior to

incubation) was similar to that reported in other respiration studies, with Q_{10} equalling 2 (Nicolardot et al., 1994; Bekku et al., 2003).

First phase mineralization exponential coefficients (k_1) for the turnover of low MW C again demonstrated no significant effect of temperature as was shown in Boddy et al. (2007b; 2007c). The labile fraction was also found to be temperature insensitive in agricultural soils (Vanhala et al., 2007). The exponential coefficients for k_3 followed a similar pattern to Boddy et al. (2007b) where increasing temperature decreased the MRT of the ^{14}C in the microbial biomass, although turnover was much slower here (from 2.8 y to 1.4 y compared to 40 d to 20 d), which may reflect the ^{14}C is partitioned into different microbial pools. MRT for k_3 was similar to microbial turnover MRT of 0.6 d to 2.5 y in forest and agricultural soils (Saggar et al., 1999; Kouno et al., 2002; van Hees et al., 2005). Second phase coefficients (k_2) did not follow this pattern, with the MRT increasing with increasing temperature (4.6 d at 4 ^{o}C to 11.6 d at 20 ^{o}C), which could reflect an increase in the ^{14}C partitioned into storage, metabolites and maintenance at warmer temperatures.

Higher intrinsic temperature sensitivity could be expected for the decomposition of recalcitrant pools, due to activation energies (Fang et al., 2005; Davidson and Janssens, 2006), however, Giardina and Ryan (2000) found no temperature sensitivity of higher MW pools. The MRTs for soil labelled 18 months previously did demonstrate a clear effect of increasing temperature with the MRT calculated from k_4 decreasing from 1700 y at 4 ^{o}C to 400 y at 20 ^{o}C and a similar effect was observed in soils labelled 6 months previously with MRT decreasing from 3500 y at 4 ^{o}C to 1200 y at 20 ^{o}C.

Additions of ^{14}C-U-glucose to soil at the start of the incubation were partitioned into three pools (a_1, a_2 and a_3) associated with exponential coefficients k_1, k_2 and k_3. Pools a_1 and a_3 showed statistical differences in the relative amount according to incubation temperature as was shown by Boddy et al. (2007b), with pool a_1 containing a higher proportion of ^{14}C with increasing temperature and pool a_3 less. There was no change in the partitioning of ^{14}C to pool a_2. As discussed in Boddy et al. (2007b) it is likely that pool a_1 increases with the increasing maintenance costs at higher temperatures, the DOC pool is limiting and therefore does not supply enough C for growth, hence the proportion of ^{14}C allocated to pools a_2 and a_3 does not increase. Kuzyakov et al. (2007) reported that the rates of microbial turnover was greatest at warmer temperatures, with less ^{14}C present in the microbial biomass, hence the decline in ^{14}C partitioned to pool a_3. There was no statistical difference in the partitioning of ^{14}C in field labelled soil 18 and 6 months prior to incubation.

Mineralization in soils labelled at 0 months compared to 6 and 18 months was significantly different, with 0 months best fitting a triphasic model and 6 and 18 month labelled soils best fitting a single phase exponential decay equation. This is primarily an artefact of the sampling time, multiple exponential equations have resulted from $^{14}CO_2$ feeding – resulting in ^{14}C-labelling of the soil through senescence of shoots and roots and exudation from roots (Hill et al., Unpubl.). With soils labelled 6 and 18 months prior to incubation sampling of $^{14}CO_2$ did not commence at the start of labelling as with soil labelled at 0 months, thus the initial rapid mineralization of added substrate is not recorded and therefore a single exponential equation provides the best fit to the data (Hill et al., 2007).

6.4.3. Impact of priming on ^{14}C turnover

The addition of labile C can have a priming effect on the decomposition of litter in soils (Kuzyakov et al., 2007). However, there was no priming effect where the ^{14}C label was in complex semi-stable C pools. A priming effect was only observed where the ^{14}C label was present in the labile C pool. The incubation temperature had no effect on the magnitude of the priming effect. Priming is likely to occur in soils amended with ^{14}C-U-glucose as the amount of C added to the DOC pool could be expected to have an effect on $^{14}CO_2$ efflux from the soil for approximately 3 h (Boddy et al., 2007a), whereas the increase in $^{14}CO_2$ efflux from the soil had a duration of over 1 d. The source of the primed C could be from an increased turnover of the living microbial biomass C rather than the enhanced decomposition of SOM C as the effect was only short-term and was not observed in the soils where the label was present in more complex pools. Thus the increase in efflux of $^{14}CO_2$ from the ^{14}C-U-glucose amended soil can be explained as pool substitution within the microbial biomass rather than a change to decomposition of stable SOM (Dalenburg and Jager, 1981; Brant et al., 2006; Kuzyakov and Bol, 2006).

The response of the microbial biomass to the addition of easily mineralizable substrate is both very fast and persists less than a few hours (Kuzyakov and Bol, 2006; Boddy et al., 2007a). It is possible that as the first measurement of the increase in $^{14}CO_2$ efflux from the soil was taken 12 h after priming the maximal response might have been missed, though the duration of the effect lasting over 1 d is measured. The addition of labile C can have a maximal affect over 2-3 d with the rate returning to the basal rate between a few days to weeks; preferential substrate utilization of labile C is thought to occur

before the mineralization of other available substrate (Kuzyakov and Bol, 2006). This is consistent with the substrate in field ($^{14}CO_2$ labelled at 6 and 18 months) labelled soil being in semi-stable SOM pools.

SOM consists of dead and living material, with the dead SOM consisting of fresh residues, lignin fractions, OM sorbed to colloids and old humus (Dalenberg and Jager, 1989). Small changes in priming could be because soil samples are devoid of fresh residues – other than the turnover of the biomass, that lignin fractions turnover is so small as to be hard to measure, and colloids and old humus are largely unavailable or inert (Dalenberg and Jager, 1989). Thus biomass can be the only source of C available to be primed (Wu et al., 1993). The small effect of priming observed in this Cambisol could also be an artefact of the soil fertility. Large priming effects have been observed in nutrient poor soils and where more complex compounds have been added (Brant et al., 2006; Kuzyakov et al., 2007) and there is a link between the magnitude of priming and the C: N ratio of the soils (Hamer and Marschner, 2005b).

The addition of low MW compounds had little or no effect on SOM mineralization (Fontaine et al., 2003) as shown here, when compared to higher MW compounds, such as ryegrass, cellulose or straw. Priming of SOM is likely to depend on the dynamics of SOM-degrading microorganisms, so the increase in energy from the addition of low MW C would not affect these groups, as the labile C would provide substrate for *r*-strategists rather than *k*-strategists that generally decompose complex organic compounds found in SOM (Fontaine et al., 2003). However, Hamer and Marschner (2005) indicated a positive priming effect from repeated substrate additions, indicating that more than biomass turnover had to be occurring, but they indicated that the repeated additions of

alanine raised pH, which could have been responsible for the increased mineralization of SOM by increasing availability and enhancing microbial activity.

6.4.4. Conclusions

This study has enabled the identification of four temporal SOM pools, which are affected in different ways by temperature and priming (Table 6.5). For climate change impacts more attention must be given to each of the major pools of SOM, with the greatest effects observed in more complex C pools. The longer term study enables a three component model to be fitted to the low MW C data rather than the usual two parameter model, which enabled some differentiation between the mineralization of low MW C for catabolic processes, storage and microbial biomass turnover to be made. The turnover of ^{14}C present in more complex SOM pools was shown to be very slow. The impact of temperature change was observed mainly on the relative partitioning of ^{14}C into biomass versus maintenance and on the turnover of the microbial biomass with the soil showing a clear impact of temperature on the turnover of C present in the biomass. This study has also shown that the duration of the incubation does not change the overall efflux of $^{14}CO_2$ from the soil, when compared to short-term studies; the magnitude of the effect is still the same. Priming was only observed to affect ^{14}C still within the living microbial biomass pool. Thus even if warming stimulates the plants into enhancing rhizodeposition there is unlikely to be a great effect on the bulk SOM either by temperature or priming as the pools were shown to be minimally affected in this study.

6.5. Acknowledgements

This work was funded by the UK Natural Environment Research Council. We thank Alice Gray for providing the 6-month labelled soil. We also thank Jonathan Roberts for helping with the sample preparation.

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Chapter 7

General discussion

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7. General discussion

7.1. Overview of thesis

As detailed in Chapter 1, this thesis has aimed to evaluate the hypotheses that soil respiration is governed by recent inputs from plants, and that rising temperatures will result in an increased respiratory loss of soil organic carbon (OC). Broadly, the aims were:

1) to review the current research on stored soil OC, its cycling within soil, and the loss of respiration-C from soils.
2) to further our knowledge of soil respiration, the number of experimentally measurable pools of C within soil and their turnover rates under different temperature regimes.
3) to evaluate the areas requiring additional research (discussed at the end of this Chapter).

The temperature dependence of soil organic matter (SOM) is currently a matter of considerable scientific debate, with no consensus being reached on the magnitude of the effect on stored OC (Kirschbaum, 2004; Kirschbaum 2006). Neither is there a consensus on the number of pools required to adequately characterise SOM turnover within soils, with models varying from one pool to 200 (Giardina and Ryan, 2000; Blagodatsky and Richter, 1998) and turnover times estimated from 0.5 h to 6000 y (Knorr et al., 2005; Boddy et al., 2007). To this end, this thesis evaluated 8 hypotheses throughout Chapters 2-6, which are discussed in depth in the individual Chapters. All hypotheses were satisfied and in this discussion I consider the implications of my research.

7.2 Implications of research

7.2.1. Methodological concerns

Chapter 2 highlighted an important research issue in that the significance of respiration measurements between the UK and Svalbard field sites depended entirely on the type of measurement made. Measurements by area gave significant differences between the biomes, whereas measurements by weight – considering only soil from the active O horizon, had no significant differences by biome type. Respiration is expected to differ by biome type (Raich and Schlesinger, 1992), however, as shown in this thesis this can be an artefact of sampling methodologies. Chapter 3 also highlighted the importance of *in situ* experiments to accurately portray C dynamics. The turnover of low MW substrate was nearly twice as fast in the field as compared to laboratory mineralization (20-40 min in the field compared to 40-60 min in the laboratory).

7.2.2. Dependence of soil respiration on recent C additions

The overall aim of the thesis was to establish whether soil respiration is dominated by recent C inputs from plants, and to see whether changing temperature will affect the respiratory loss of stored OC from the soil. The decline in soil respiration measurements over time with the absence of new inputs of C from plants and the rapid uptake mechanisms for the mineralization of low molecular weight (MW) C substrate demonstrated that soil respiration is indeed dominated by recent C inputs from plants, a significant component of which is labile compounds, in particular low MW sugars and amino acids (Farrar et al., 2003; van Hees et al., 2005; Fig. 7.1). Svalbard soils responded by a five-fold increase in respiration on the addition of glucose and 3 fold increase on the

addition of glutamate (Chapter 2), further demonstrating the dependence of soil respiration on the additions of labile substrate. Microorganisms can only access soluble C and consequently it is the rate of supply of C substrates to the soil solution that drives respiration (van Hees et al., 2005).

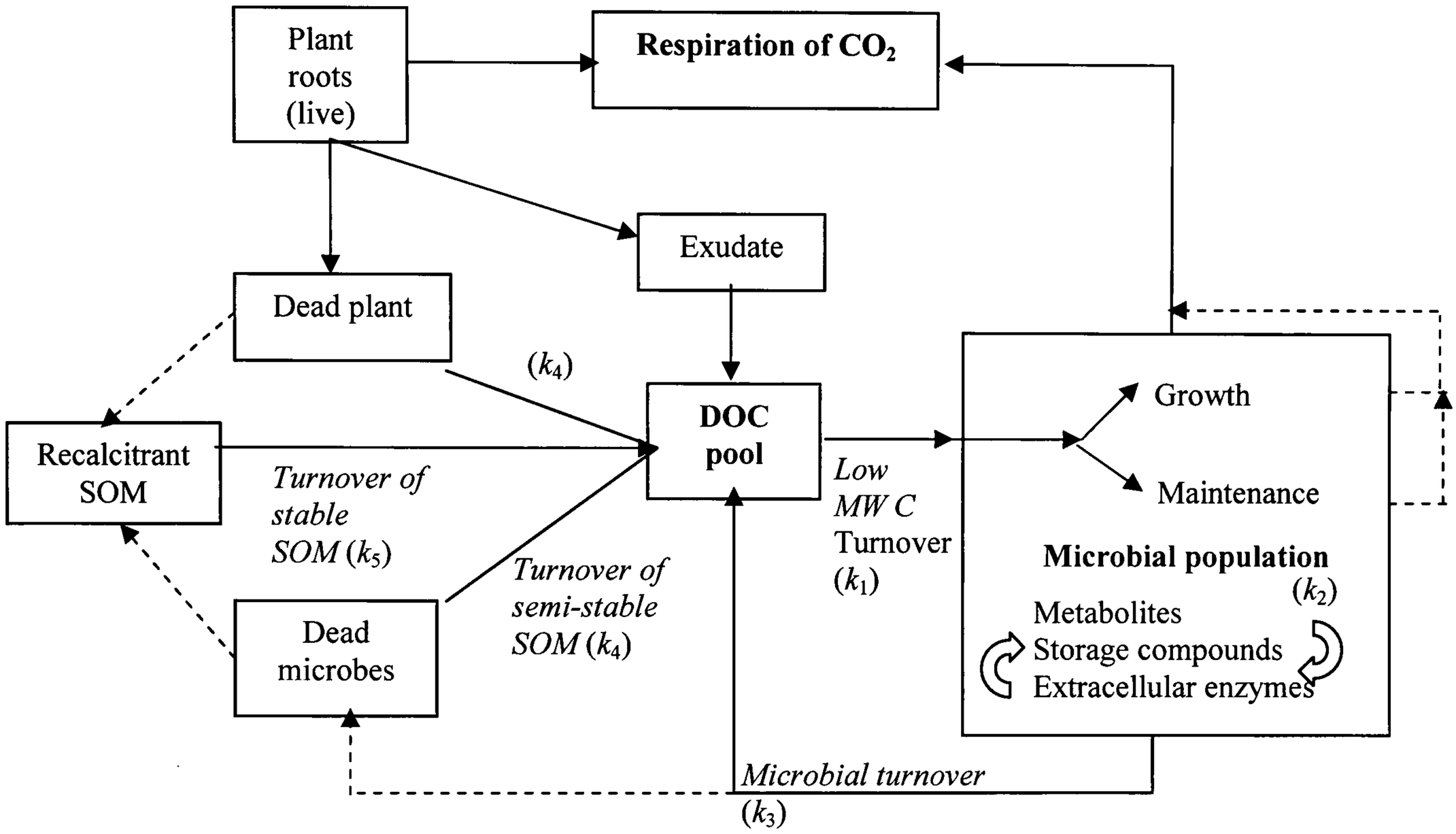

Figure 7.1. Schematic of the fate of C entering the DOC pool, indicating the suggested locations of five SOM pools differentiated by exponential coefficients (k_1, k_2, k_3, k_4, and k_5, values in Table 7.1) from the mineralization of ^{14}C and decline in measured CO_2 over time.

7.2.3. Additional controls on soil respiration

Soil respiration is also controlled by the contribution of roots to the total measured efflux of CO_2 at the soil surface (Ward and Strain, 1999; Fig. 7.1); UK grassland soils were estimated to have a 40% contribution of roots to total efflux (Chapter 2). Svalbard respiration rates reduced to 20% on removal of plant material, indicating a greater dependence on recent, labile, inputs of C from plants. Root respiration is generally estimated to contribute approximately 50%,

though this is highly biome dependent, with roots contributing up to 93% in Arctic tundra (Buchmann, 2000; Raich and Tufekcioglu, 2000; Lee et al., 2003).

However, Chapter 3, 4 and 5 found no impact on soil respiration or low MW mineralization by the type of vegetation studied. There is contrasting evidence on whether surface vegetation influences belowground processes (Raich and Schlesinger, 1992; Raich and Tufekcioglu, 2000). Since roots will vary with the surface vegetation, and soil respiration is dominated by recent inputs from plants, then belowground processes will depend closely on surface vegetation. Soil microorganisms are C and N limited (Chapter 2; Blagodatsky et al., 1998; Galicia and Garcia-Oliva, 2004; Pennanen et al., 2004) and metabolism of added substrate resulted in large increases in respiration rates (Chapter 2) and added labile-^{14}C was mineralized extremely rapidly both in the field ($t½$ 0.5 h, Chapter 3) and laboratory ($t½ < 2$ h, Chapter 3-5). This suggests that differences in root composition would not impact on heterotrophic belowground respiration where the microorganisms are substrate-limited.

A proportion of the total measured CO_2 efflux is also from other soil OM pools (Ward and Strain, 1999; Fig. 7.1), In the absence of fresh inputs respiration declined over time, stabilising after 7 months at < 2 μg CO_2 g^{-1} h^{-1}, indicating that the turnover of a higher MW C pool accounted for approximately 25% of the total respiration of an intact soil core at the start of the experiment (Chapter 2). Kutsch and Kappen (1997) proposed that soil respiration could be modelled by three dominant processes: 1) root and rhizosphere respiration, 2) respiration of low MW SOM, 3) respiration of high MW SOM. Thus, if roots provide an average contribution of 40% and high MW C pools 25%, 35% of respiration

measured at the soil surface must be microbial respiration of recent inputs of C to the soil (Chapter 2).

7.2.4. Turnover of C in soil

Chapters 2-6 evaluated the cycling of OC stored in soil. As discussed previously, there is little consensus on the number of pools sufficient to estimate the turnover of C in soil and the turnover times of C in these pools (Giardina and Ryan, 2000; Knorr et al., 2005; Boddy et al., 2007). Hill et al. (2006; Appendix 1) suggested that it is only when data from a range of experimental and observational systems can be reconciled that we can begin to understand how soil microorganisms respond to changing soil conditions. In this thesis two methodologies were investigated to estimate turnover times of stored soil OC. Initially measurements of $^{12}CO_2$ efflux were taken and secondly the dynamics of ^{14}C were followed via two labelling methodologies (either direct additions of glucose to the soil surface or through labelling of plants and translocation of labelled material to the soil). These three experimental practices enabled us to identify five discrete turnover times for SOM, which we attribute to physical pools of SOM, but we know little about the sizes of these pools (Table 7.1), with the three methodologies giving similar estimates for the turnover of C assumed to be within the microbial biomass and the turnover of the microbial biomass itself. The rapid half-times reported both *in situ* for UK grassland soils (< 0.5 h, Chapter 3) and the turnover of low MW C in Arctic soils (< 1.5 h) are faster than previously recorded (Jones and Kielland, 2002; Henry and Jefferies, 2003). The rapid turnover times for labile C in the soil supports previous studies suggesting that the bottleneck in soil C and N cycling is the breakdown of high MW

molecules rather than the utilization of their low MW breakdown products (Jones et al., 2004; Jones et al., 2005; Boddy et al., 2007).

Table 7.1. Summary of five SOM pools and their mean residence time (MRT) for C in pools calculated from exponential equations, with MRT calculated from k_x using MRT = ln(2)/k_x. Values are averaged from calculations in Chapters 2-6 from UK grassland and Arctic soils.

MRT	Pool	Chapter
0.4 – 5 h	Mineralization of low MW DOC	3, 4, 5, 6
10 – 55 d	Internal cycling of storage compounds, extracellular enzyme breakdown	2, 4, 5, 6
1.2 – 2 y	Microbial biomass	2, 6
1000 y	Semi-stable SOM	6
> 5000 y	Recalcitrant SOM	2

7.2.5. Temperature dependence of SOM turnover

The second focus of this thesis (Chapters 4-6) was to evaluate the impact temperature change could have on the respiratory loss of CO_2 from soils. For the purposes of this thesis we focused on the metabolism of low MW C that had been radiolabelled with ^{14}C enabling its turnover in soil and the microbial community to be estimated (Chapters 4-6; Table 7.2). Generally a Q_{10} value of 2 or greater is assumed to occur on warming (Nicolardot et al., 1994; Bekku et al., 2003). Throughout Chapters 4-6 the greatest Q_{10} values recorded for low MW mineralization was 1.5 for the Svalbard soils. Though mineralization of ^{14}C in more complex pools had a greater Q_{10}, particularly at low temperatures (Q_{10} of 2.1 for $^{14}CO_2$ recovered at 4 °C). However, other Q_{10} values estimated from ^{14}C mineralization have shown large variation from 1.6 to 10.8 (Nicolardot et al., 1994). There is doubt about the applicability of Arrhenius plots to describe the temperature dependence of microbial processes because of the large number of

microorganisms and compounds, each with their own enzymatic reactions and activation energies (Ågren and Bosatta, 1987; Nicolardot et al., 1994; Rustad et al., 2000; Knorr et al., 2005).

Table 7.2. Summary of five SOM pools differentiated by exponential coefficients from the mineralization of ^{14}C and decline in measured $^{14}CO_2$, and their susceptibility to priming and the effect of temperature on the pools. Values are estimated in the laboratory from Chapters 4-6, priming response and temperature dependence of k_5 (estimated in Chapter 2) was not determined, denoted by N.D.

SOM pool *k*	**SOM pool 4 °C (MRT)**	**SOM pool 20 °C (MRT)**	**Mineralization affected by priming?**	**Mineralization affected by temperature?**
k_1	1.4 h	1.4 h	Yes	No
k_2	37 d	19 d	No	Yes
k_3	2.8 y	1.4 y	No	Yes
k_4	1700 y	400 y	No	Yes
k_5	> 5000 y	> 5000 y	N.D.	N.D.

In Chapters 4-6 mineralization of low MW (k_1) showed no sensitivity to temperature change, although in previous studies this had been reported (2.9 to 0.8 h from 5 to 34 °C respectively; Jones, 1999). As before, where the soils were shown to be C and N limited (Chapter 2), the lack of sensitivity to temperature change in the labile pool could be due to the high demand for these substrates (Jonasson et al., 2004; Kirschbaum, 2006). Temperature sensitivity was shown in all higher MW coefficients. Kuzyakov et al. (2007) reported that the rates of microbial turnover were greatest at warmer temperatures, which was observed here in the decrease in k_3. Partitioning of ^{14}C into pools associated with the exponential coefficients (a_1 to a_4) also showed sensitivity to temperature change. In all cases, more ^{14}C was partitioned into a_1 and subsequently less ^{14}C partitioned into later pools (a_3, a_4). This could be due to increased maintenance

costs at higher temperatures resulting in additional ^{14}C being used for respiratory processes rather than storage and growth, which might also then decrease the growth efficiency at elevated temperatures with less C being partitioned to growth (Nguyen and Henry, 2002; Apple et al., 2006).

Chapter 5 investigated whether the soil microbial community would acclimate to temperature change, i.e. whether mineralization is dependent on past environmental conditions or whether it is a response to the current conditions. Acclimation is expected to occur, as it is a result of co-limitation of biological processes. The acclimatory response in soil would see an alteration in gene expression to maintain metabolism at the historic conditions. Phospholipid fatty acid (PLFA) analysis enabled changes in the microbial community to be observed, with more saturated fatty acids being found at higher temperatures. It is probable that this was due to phenotypic acclimation occurring and not due to species replacement (Pettersson and Bååth, 2003). Thus changes in composition of microbial membranes might occur rapidly due to temperature changes. However, there was no evidence of acclimation in the mineralization of ^{14}C nor in the total respiratory $^{12}CO_2$ measured from the soil surface over time, and therefore changes in these functionalities due to temperature were not observed.

As the microbial community is predominantly C limited, and therefore changes in rhizodeposition and litter in an elevated CO_2 atmosphere, and increased temperatures, could result in additional CO_2 efflux from the soil (Allan and Schlesinger, 2004). There is much debate on whether increased additions of low MW substrate from enhanced rhizodeposition will enhance the degradation of higher MW SOM (Zhang et al., 2005). Chapter 6 considered whether priming would affect the turnover of stored C in soils, and found that there was no

priming effect where the ^{14}C label was in complex semi-stable C pools. Priming was only observed where the ^{14}C label was present in the labile C pool and the incubation temperature had no effect on the magnitude of the priming effect. Thus it is unlikely that additional low MW substrate will cause an increase in the decomposition of high MW C in soils.

7.3. Further work

Although extensive research has been undertaken on assessing the contribution of plants to soil microbial respiration and the impact of temperature change on SOM degradation there is still a clear requirement for further research, particularly considering the fate of specific compounds in soil. Although root exudates are known to be dominated by low MW C, particularly sugars and amino acids, in this investigation they were shown to contribute < 5% of dissolved organic carbon (DOC) and contributing < 25% of basal respiration (Chapter 4), with low MW compounds contributing 35% of basal respiration (Chapter 2). There is obviously much work required on analysing the individual constituents of DOC and the turnover of these compounds in soil.

Chapters 4-6 highlighted the importance of higher MW compounds when considering temperature sensitivity and turnover, particularly structural compounds, and other complex polymers (such as celluloses and lignin), which have been little studied (Benner et al., 1984; Tuomela et al., 2001). Table 7.1 estimated five discrete pools, but identified no pools with turnover times between 2 and 1000 y, soil contains many thousands of organic compounds all of which can be expected to exhibit unique kinetic mineralization properties, and so much

more work is needed in this area, particularly considering higher MW compounds.

Soil is a complex and dynamic biological system and the assays available, such as the ones undertaken in this thesis, consider total respiration and mineralization giving overall rates, but do not allow identification of the microbial species involved (Nannipieri et al., 2003). There is a need for additional research considering the role of individual species, labelling live biomass and following the turnover of ^{14}C from specific communities as well as specific compounds.

Short-term processes are dominated by the fluxes into and out of fast turnover pools, whereas long-term changes in C storage are determined by small changes in the flux of C into and out of the slow OC pools (Kirschbaum, 2004). The importance of longer-term experiments was highlighted in Chapter 6, with additional soil C pool identified and the temperature sensitivity of higher MW C established. With the rate of degradation of higher MW C in soils being thought of as the bottleneck in soil microbial productivity rather than the utilization of their low MW breakdown products (Jones et al., 2004; Jones et al., 2005; Boddy et al., 2007), there is the need for longer-term research focusing on well defined and more recalcitrant pools of C and how small changes in their turnover will effect total efflux of CO_2 from the soil.

Finally, Chapter 3 established the importance of *in situ* experiments, with mineralization rates being nearly twice as fast in the field as in the laboratory. For accurate modelling of future soil C turnover to be undertaken, more research into C cycling and temperature dependence needs to be undertaken in the field.

7.4. Summary

UK and Svalbard soils had similar respiration rates per gram of O horizon and rates of metabolism of low MW C (with an exponential decay coefficient k_1 averaging < 1.5 h in the laboratory). However, cycling of C through the microbial biomass was significantly slower in Svalbard soils than in UK grassland soils, though when Svalbard soils were incubated at 20 °C, turnover was similar to UK field rates. Respiration measurements expressed on an area basis were significantly different, with the UK having much greater respiration rates, highlighting the importance of the sampling methodology when considering results. Both soils were substrate limited, indicating that respiration rates were dominated by recent contributions of labile C from plants.

Temperature changes did cause an increased respiratory loss of SOM, but not to the magnitude expected with the Q_{10} value being < 2. Temperature dependence of SOM degradation was shown to be C pool dependent; with mineralization of labile substrate-C insensitive to temperature, but microbial biomass-C turnover and mineralization of higher MW SOM fractions increased with elevated temperatures. Partitioning of ^{14}C changed with elevated temperatures, with more C utilized for respiration rather than growth, indicating a possible decrease in efficiency of growth at higher temperatures.

Further research is needed in the temperature-dependence of SOM degradation, particularly turnover of high MW SOM, in the laboratory and field.

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Appendix 1: Book Chapter

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Chapter 15

Carbon Partitioning and Respiration - Their Control and Role in Plants at High CO_2

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Ecological Studies, Vol. 187
J. Nösberger, S.P. Long, R.J. Norby, M. Stitt, G.R. Hendrey, H. Blunt
(Eds.) Managed Ecosystems and CO_2
Case Studies, Processes, and Perspectives