The Temperature Response of Soil Respiration from Labile and Stable Carbon

A thesis

submitted partial fulfilment

of the requirements for the degree

of

Master of Science (Research) in Earth Sciences

at

The University of Waikato

by

KRISTYN BAILEE NUMA

THE UNIVERSITY OF
WAIKATO
Te Whare Wānanga o Waikato

2020

Abstract

The effect of temperature on the decomposition of labile and stable carbon (C) in soil is a critical factor in understanding soil C dynamics, particularly under a global warming scenario. Despite the temperature response of soil respiration being well-studied, the temperature sensitivity of the labile and stable C pools in soil remains unclear. Here, I conducted a laboratory-based incubation experiment to separate the temperature response of these two C pools. Soil was incubated at 18 discrete temperatures (~8-52°C) for five hours with and without added C substrates. After the incubation period, respiration rates were determined by measuring carbon dioxide (CO_2) production from the soils. Previous studies have attributed differences in the temperature sensitivities between C pools to substrate availability. Therefore, the labile C pool was measured as the CO_2 derived from the added C substrates (450 mM C) to the amended soil (i.e. high C availability), and the stable C pool was represented by the decomposition of soil organic matter (SOM) measured directly from soil without added C (i.e. low C availability).

I applied the macromolecular rate theory (MMRT) to respiration rates (R_S) derived from labile and stable C decomposition, to describe their relationship with temperature. I found in all cases, the temperature response of the decomposition of labile C substrates (labile pool) was well fitted with MMRT (i.e. $\Delta C_P^{\ddagger} \neq 0$), resulting in clear temperature optima (T_{opt}) and inflection (T_{inf}) points. While previous studies have been able to fit SOM-R_S (stable pool) with MMRT, the equation in this study collapsed to the exponential-like Arrhenius equation (i.e. $\Delta C_P^{\ddagger} = 0$), with no calculable T_{opt} or T_{inf} points.

Representing the labile C pool, five simple C compounds, a substrate comprising a wide variety of C compounds (yeast extract), and a complex, long-chained glucan (dextran) were added separately to soil and incubated. The temperature response of the simple C compounds (glucose, glutamine, arginine, maltose, and lysine) and yeast extract were largely the same, with average T_{opt} and T_{inf} points of 37°C and 22°C, respectively. Dextran behaved similarly to the recalcitrant SOM as it could not be fitted with MMRT; thus, no T_{opt} and T_{inf} parameters were derived. This behaviour was attributed to the

high complexity and molecular weight of the compound, reducing its metabolic compatibility with the soil microbes and increasing its physiochemical protection by adhering to soil surfaces. Additionally, I also determined whether the temperature response of these two pools varied with different soil properties by incubating three soils (allophanic, gley, and organic) with and without added glucose. Results suggested that between three very different soils, the temperature response of the decomposition of glucose (labile C) and SOM (stable C) was remarkedly similar, only varying by 3°C (T_{opt}=35°C, T_{inf}=18-21°C).

A final preliminary experiment determining the soil priming effect was undertaken using a newly developed isotopic analysis method, where ^{13}C labelled glucose was added to soil (allophanic). The results showed that within this study, priming enhanced the native SOM decomposition by 30% and had a similar temperature response to the labile C pool. Priming-R_S was well fitted to MMRT suggesting that the C made available by priming was a labile C source for decomposers. T_{opt} and T_{inf} points for priming-R_S (29-31°C and 14-15°C, respectively) were slightly lower than the T_{opt} and T_{inf} observed for labile C compounds. Additionally, in both relative and absolute terms, the temperature sensitivity of C decomposition induced by priming was, generally, lower than for the added C substrates and SOM. These results suggest that the C made available by priming must be more labile and physically accessible to microbes compared to the labile C compounds and SOM.

Overall, this study found that the temperature sensitivity of the stable C pool was higher in relative terms (i.e. Q_{10}) and lower in absolute terms (i.e. first derivative) compared to the labile C pool. The temperature responses of these two pools were *not* the same. MMRT accurately described the temperature-respiration relationship for labile C decomposition, whereas the Arrhenius function better described this relationship for stable C. MMRT describes typical reaction rate responses from systems where biological processes dominate (in this case the biological degradation of soil C by microbes), and is a typical response of the temperature-respiration relationship when substrate supply is high in soil. The Arrhenius function, on the other hand, accurately describes chemical reaction rates, which in this case would be physiochemical processes such as, sorption/desorption and diffusion that transport the

protected C to the microbes for decomposition. An Arrhenius-like behaviour is commonly exhibited when substrate supply is low for the soil microorganisms. Since the temperature response of the labile and stable C pools did not significantly differ between labile substrate types and soil properties, it could be suggested that a two-pool soil C model might be sufficient for the prediction of soil C dynamics. This two-pool model could also be extrapolated to larger ecosystem models, potentially leading to improved accuracy of climate and soil C storage projections. To determine the adequacy and appropriateness of a simple two-pool C model, further measurements of the temperature response of these two pools from a wide distribution of soils from different locations and under various conditions needs to be undertaken.

Acknowledgements

There is a long list of people I would like to thank for helping me throughout this past year, but I will list just a few here. First and foremost, a massive thank you to my supervisor Professor Louis Schipper. Louis has been an encouraging and supportive supervisor, and I feel very honoured to have worked on such an exciting topic that he has developed. I have enjoyed our many discussions on the possibilities of what new data meant, and I am very grateful for the invaluable skills I have developed as your student. Thank you for always being willing to answer my "quick questions" and for getting my drafts back to me very quickly – it really helped keep the stress levels down towards the end!

A big thank you to Noel Bates for being so willing to help with any laboratory needs I had, no matter how big or small I could always count on you. I also appreciated your friendly greetings on the early mornings I was tediously weighing soil.

This thesis would not be possible without help from Jasmine Robinson. I am deeply thankful for all of her support throughout my thesis and for always being willing to help when I needed it – making my life a lot easier! I would particularly like to thank her for the time she gave up to help me during my priming experiments, I could not have done it without you, so thank you. I would also like to extend my gratitude towards Professor Vic Arcus and Professor Susan Schwinning for your invaluable discussions and ideas that really helped shape my thesis. A big thank you to Dr Jordan Goodrich for reviewing chapters for me and for providing assistance on anything Matlab or statistical analysis related.

To everyone in the Waiber research group – thank you for your friendly encouragement and support throughout my thesis. I would particularly like to thank Georgie Glover-Clark for her friendship over the past two years. Georgie, I have been very fortunate to have had you by my side during my masters, your dedication and passion for your research was inspiring and I look forward to seeing what you do next!

Gracias a mi pareja, Jacko for your mathematical explanations, listening to all my presentation practises, and hearing me rant on about how excited I was about new data. Also, thank you for putting up with stressed Kris – that deserves an award itself.

I was fortunate enough to receive funding from the New Zealand Agricultural Greenhouse Gas Research Centre for this project, as well as receiving the University of Waikato Masters Research Scholarship. Also, thank you to DairyNZ for allowing me to collect soil samples from Scott Farm.

To all my friends and family, thank you for your continuous love and support over these years and for pretending to understand/be interested in my thesis. Lastly, I dedicate this thesis to my Mum and Dad. I don't know how I could ever repay you two for these past five (or 23) years. I love you so very much, and I am eternally grateful to have the best parents in the world.

Table of Contents

List of Figures

List of Tables

Chapter 1

Introduction

1.1 Background

Globally, soil carbon (C) is the largest actively cycled terrestrial C pool (Janzen, 2004). The amount of C stored in soil is the result of the balance between C inputs (photosynthesis), where atmospheric C is fixed by photoautotrophs, and C outputs (respiration), where C is returned to the atmosphere as carbon dioxide (CO_2). The exchange of C between soil and the atmosphere is an integral part of the global C cycle, as CO_2 is one of the primary regulators of the Earth's climate (Bleam, 2011). Anthropogenic activities, such as land-use changes, or changes in climate (e.g. moisture and temperature changes), have disturbed this balance by increasing the amount of C outputs through the process of soil respiration. Microbial respiration is one of the main pathways soil C is released as CO_2, where microorganisms in the soil decompose soil C (or soil organic matter (SOM)) retaining a portion for their biomass while releasing the rest as CO_2 to the atmosphere.

Broadly, there is a minimum of two distinct C pools in soil, the labile and stable C pools. The labile C pool comprises simple C compounds that are highly available to microbes for decomposition (Von Lützow & Kögel-Knabner, 2009). The stable C pool contains organic C that is largely physiochemically protected from decomposition or harder to degrade due to their high chemical complexity (Von Lützow & Kögel-Knabner, 2009).

Like most biochemical reactions, respiration is temperature-dependent (Sierra, 2012). Typically, the Arrhenius function is used to explain the temperature-respiration relationship in soil, which produces an exponential increase in respiration rates with increasing temperature. However, biochemical reactions inherently have a temperature optimum (T_{opt}) where reaction rates decline past this point. In the past, this decline in rate has been attributed to enzymatic denaturation. However, temperatures at which enzymatic denaturation usually occurs are higher than what is typically observed in soil (Schipper *et al.*, 2014). The macromolecular rate theory

(MMRT) proposes an explanation for the decline in soil respiration rates above the T_{opt} in the absence of denaturation and attributes it to the thermodynamic properties of enzymes (Hobbs *et al.*, 2013). MMRT directly predicts the T_{opt} point from temperature-respiration curves, and can also derive the temperature at which the maximum change in rate for one unit change in temperature occurs – the inflection temperature (T_{inf}) (Schipper *et al.*, 2014).

The temperature sensitivity of the stable and labile C pools is a highly debated topic within the literature. The temperature sensitivity of soil C decomposition can be measured in relative and absolute terms. The relative and absolute temperature sensitivities inherently produce opposing behaviours (Sierra, 2012), so it is essential for studies to explicitly distinguish what measure they use, which most studies do not. Frequently temperature sensitivity is reported in *relative* terms using the Q_{10} coefficient (Sierra, 2012). The most supported conclusion in the literature is that the stable C pool is more temperature-sensitive, in *relative* terms than the labile C pool (Von Lützow & Kögel-Knabner, 2009). This conclusion is a cause for concern as the stable C pool contributes over 70% of the total soil C pool (Sanchez *et al.*, 1989). If the sizeable stable C pool is moderately or highly temperature-sensitive under a global warming scenario, this could release a significant proportion of CO_2 to the atmosphere causing a potential positive feedback loop where the increase in atmospheric CO_2 concentration might result in further warming of the Earth's surface.

Previously, differences in the temperature sensitivity of different C pools in soil have frequently been attributed to the chemical complexity of the decomposing organic C (Fierer *et al.*, 2005; Li *et al.*, 2017), while other studies have attributed the difference to substrate availability (Thiessen *et al.*, 2013; Sulman *et al.*, 2014; Schipper *et al.*, 2019). Conant *et al.* (2011) also attribute the observed temperature sensitivity of soil to the depolymerisation of C and the rate of enzyme production. Schipper *et al.* (2019) explains that if substrate supply is abundant, the biological process of the decomposition of organic C in soil will likely dominate which inherently has a clearly defined T_{opt} and T_{inf} and will not increase exponentially as described by Arrhenius-like equations. On the other hand, if substrate supply is limited the physiochemical processes (i.e. sorption/desorption, diffusion) will dominate, which exhibits exponential Arrhenius behaviour (Schipper *et al.*, 2019).

Priming is a controversial topic concerning soil respiration, where small additions of labile C are considered to increase respiration of existing soil organic matter (including stable forms). These additions could originate from dead and decomposing litter or root exudates (Zhu & Cheng, 2011). Numerous studies have determined the temperature sensitivity of labile C in soil through the addition of exogenous C but importantly have not accounted for the priming effect (Fierer *et al.*, 2005; Yang *et al.*, 2016; Girkin *et al.*, 2020). Priming can be either negative or positive; however, a recent meta-analysis on the effect of priming on soil respiration found that from 94 incubation studies, 70% showed native SOM decomposition increased 48% due to additions of exogenous C (Sun *et al.*, 2019). There are a few proposed mechanisms for positive priming, but in general added labile C increases microbial activity in soil thereby increasing the availability of more stable C (Thiessen *et al.*, 2013; DeCiucies *et al.*, 2018). Currently, priming is not recognised in Earth system models (ESMs) or many other smaller-scale models, which may result in an overestimation of soil C storage (Guenet *et al.*, 2018).

It is crucial to gain a clearer understanding of the temperature response of the labile and stable C pools in soil to assist in developing accurate and reliable climate models and to understand soil C dynamics on a seasonal and diurnal scale, as soil C is an index of soil fertility.

1.2 Aims and objectives

The overarching aim of this thesis was to separate the temperature response of two C pools in soil. The general methodological approach was to add a range of simple C compounds to soil and incubate across a temperature gradient for five hours, measuring accumulated CO_2. The temperature response of the stable C pool was measured by incubating soil with no added C. The difference in respiration rates from soil with and without added C was described as respiration derived from the added substrate (labile pool) and priming.

Specific objectives were:

- To determine if different soil properties influenced the temperature response of the labile and stable C pools in soil with added glucose used as a model C compound
- To measure the temperature sensitivity (in both relative and absolute terms) of the decomposition of a range of C compounds added to soil to determine if the temperature response of soil respiration varied with C type
- To determine the magnitude and temperature response of soil priming when a labile C source was added to soil

1.3 Thesis layout

Chapter 2 is a review of the current literature covering the controls on microbial respiration with a focus on temperature. An in-depth review of the various terminologies used to describe soil respiration's response to temperature is also given, as well as an introduction into the temperature response of the labile and stable C pools.

Chapter 3 describes the full methodologies used in this research to separate the temperature response of the labile and stable C pool from soil, including soil sampling, laboratory methods, data analysis, and describes preliminary experiments undertaken to ensure these methods were reliable.

Chapter 4 presents the main findings of this thesis written in a paper format so it can later be adapted for publication in a peer-reviewed journal. The discussion section of this chapter describes the temperature response of the labile and stable C pool from within three different soil types, as well as the temperature response of a range of C compounds added to soil. This section also presents preliminary findings on the magnitude and temperature response of soil priming.

Chapter 5 summarises the findings from this thesis and relates it to the current literature. It also provides future research needs and recommendations for similar studies.

Chapter 2

Literature Review

2.1 Global carbon cycling

Carbon (C) cycling processes are of great interest because of their contribution to soil quality and their role in mediating the global soil C store (Lal, 2014). Carbon's chemical properties allow for the formation of a wide variety of compounds, including carbon dioxide (CO_2), one of the primary controllers of the earth's climate (Horwath, 2015). The complex mixture of C compounds is in a constant state of creation, transformation, and decomposition (Bolin, 1970), a phenomenon known as the global C cycle. C cycles through four main pools: atmosphere, biota, soil, and the ocean (Janzen, 2004). Therefore, the global C cycle encompasses both marine and terrestrial environments (Bleam, 2011); however, due to the focus of this thesis, this literature review will concentrate on the terrestrial C cycle, and in particular the soil C component.

2.1.1 Anthropogenic influence on climate

Recognition of anthropogenic influence on the global C cycle started in the early 19th Century with French mathematician, Joseph Fourier. Fourier calculated that the Earth's surface temperature was much higher than what the balance of the incoming and outgoing infrared energy suggested. Fourier was the first to introduce the term 'greenhouse effect' in 1824 using an analogy of a glass bowl, where the glass lets in sunlight, but traps infrared radiation emitted from the ground (Rodhe *et al.*, 1997). This led Fourier to ask the question "What determines the temperature of the surface of the Earth?". In 1838, French physicist, Claude Pouillet, speculated that the answer to this question might be water vapour and CO_2. However, it was Irish physicist, John Tyndall, who scientifically demonstrated that water vapour, methane, and CO_2 absorbed infrared radiation in 1859.

Continuing on the work of Fourier, Pouillet, Tyndall and many other researchers in this field, Svante Arrhenius, a Swedish chemist/physicist, put forth the greenhouse effect theory in his 1896 paper titled 'On the Influence of

Carbonic Acid in the Air upon the Temperature of the Ground'. After spending months working through thousands of calculations, he concluded that doubling the atmospheric CO_2 concentration would increase surface temperatures by 5-6°C – only slightly higher than what models suggest now. Arrhenius published a second paper in 1896, titled 'Nature's heat usage', which was one of the first to link human activities, such as fossil fuel consumption, to an increase of the Earth's surface temperature by "somewhat more than one-thousandth of a degree centigrade per annum" (Arrhenius, 1896).

It was not until the mid-20th century that Arrhenius' predictions gained traction. During this time, scientists began to acknowledge the ever-increasing annual atmospheric CO_2 concentrations, associating increasing concentrations to fossil fuel burning and land-use changes. The CO_2 level in the atmosphere has steadily increased from 280 parts per million (ppm) pre-industrial revolution to over 400 ppm now (412 ppm as at November 2019) (Figure 2.1) (NOAA, 2019).

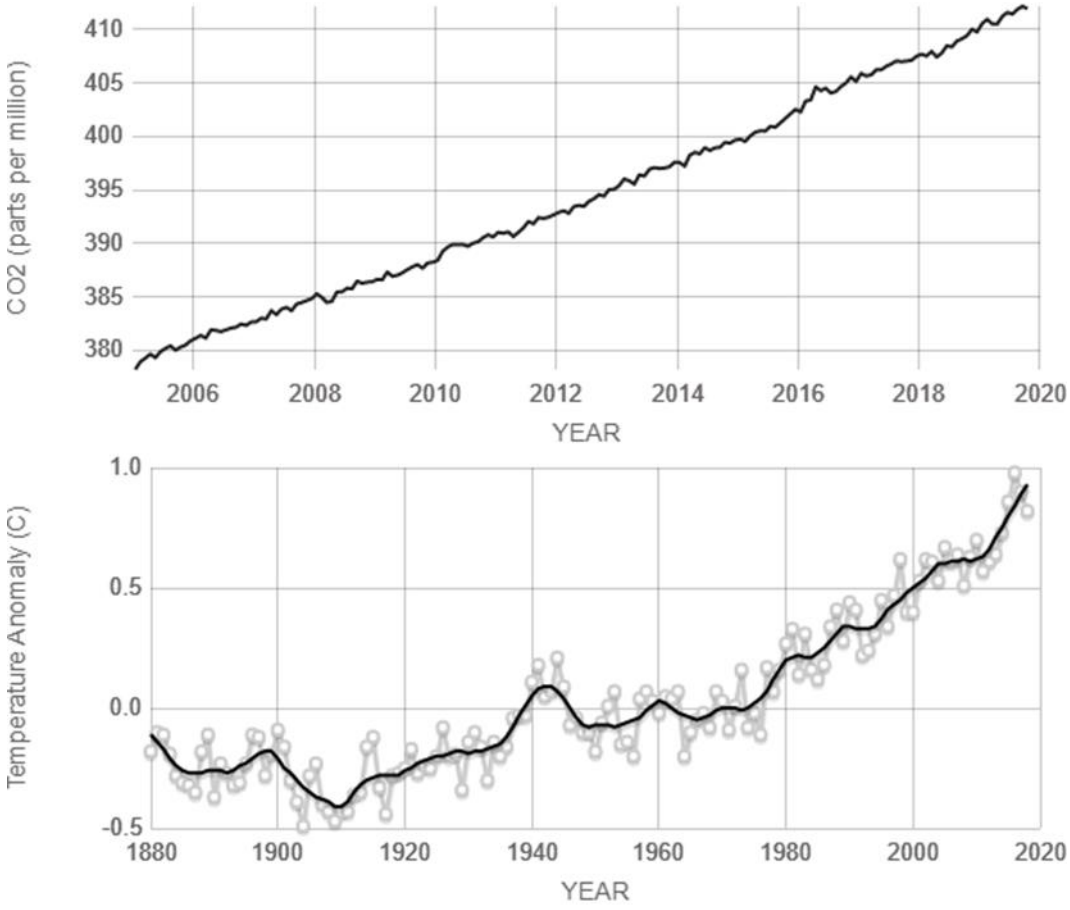

Figure 2.1 (TOP) Monthly atmospheric CO_2 measurements at Mauna Loa Observatory, Hawaii, with the average seasonal cycle removed (NASA, 2019 cited in; NOAA, 2019). (BOTTOM) Temperature anomalies from 1880 to 2020, relative to the 1951-1980 average temperatures (NASA/GISS, 2019)

Given the significant role of CO_2 in regulating Earth's surface temperatures, it is vital to understand the fluxes of CO_2 between the atmosphere and terrestrial ecosystems. Due to the relatively large size of the soil C pool, a small change in its CO_2 fluxes could have a drastic effect on the atmospheric CO_2 pool, and thereby the Earth's surface temperature. The soil-atmosphere C exchange is governed by two major fluxes: photosynthesis and respiration. These fluxes control the balance of C inputs into the soil (photosynthesis) and output to the atmosphere (respiration). This thesis centres on soil respiration and the following section (Section 2.2) will briefly cover soil C cycling and introduce how C enters the soil and, in turn, is released to the atmosphere.

2.2 Soil carbon cycling

Global soils store C mainly as soil organic matter (SOM), which contains at least twice the amount of C stored in the atmosphere, and three times that stored in biota (Bond-Lamberty *et al.*, 2018; Dash *et al.*, 2019). Global soil C levels currently total at around 1500-2000 petagrams (Pg) of C (Dash *et al.*, 2019), and these levels are the result of the balance between C inputs and outputs (Davidson & Janssens, 2006) (Figure 2.2).

2.2.1 Carbon inputs to soil

The soil C cycle begins with the biological fixation of atmospheric CO_2 by primary producers (Bleam, 2011). The prominent primary producers are photoautotrophs which convert CO_2 to organic C compounds through the biochemical process of photosynthesis (Hopkins, 2006). This process is denoted as C uptake (Horwath, 2015):

$$Carbon\ dioxide + Water \xrightarrow{Sunlight+Chlorophyll} Glucose + Oxygen \qquad (2\text{-}1)$$

The organic C can then enter the soil system either directly through root exudates or is transformed into biomass, which enters the soil as litter inputs and is transformed into SOM (Hopkins, 2006; Horwath, 2015) (Figure 2.2). Once incorporated into the soil, SOM is transformed through processes of decomposition, mineralisation, and stabilisation (Luo *et al.*, 2016).

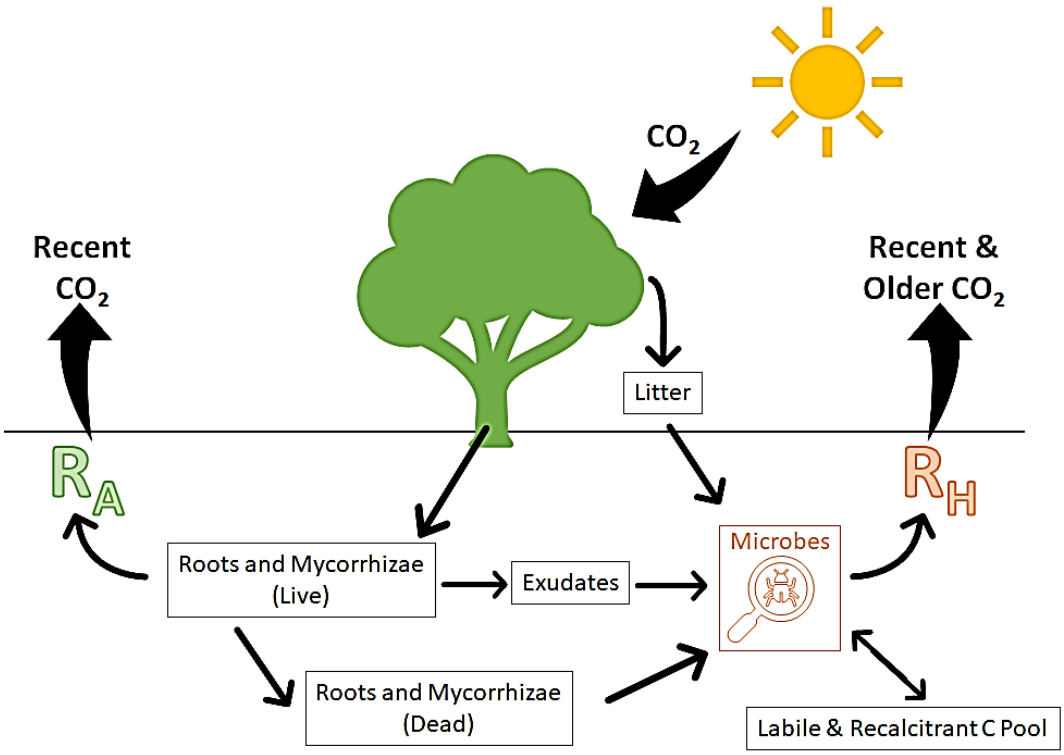

Figure 2.2 Conceptual model of carbon inputs and outputs in soil. R_A represents autotrophic respiration, and R_H is heterotrophic respiration. Adapted from Ryan and Law (2005)

2.2.2 Carbon losses to the atmosphere

C is returned to the atmosphere via two main pathways: (1) Autotrophic respiration (R_A) by plants (including roots) and, (2) Heterotrophic respiration (R_H) by microorganisms within the soil (Brown & Markewitz, 2018) (Figure 2.2). Falkowski *et al.* (2000) also argue for the importance of disturbances (i.e. fires) where large amounts of SOM are oxidised in a short timeframe. Collectively, R_A and R_H make up the components of soil respiration R_S which, as the second-largest C flux, releasing 77 Pg C annually, plays an essential role in the terrestrial C cycle (Li *et al.*, 2018). Researchers suggest that, on average, the magnitudes of R_A and R_H roughly make up 50% each of the total R_S (Ryan & Law, 2005). However, this ratio is not well-defined and is likely variable depending on the ecosystem of interest. Section 2.3 will focus on R_H, also frequently referred to as microbial respiration.

2.3 Microbial respiration

Photoautotrophs utilise the sun's energy by fixing C derived from atmospheric CO_2 through photosynthesis. Heterotrophs, on the other hand, rely entirely on the availability of reduced C compounds as an energy source (Horwath, 2015). Thus, heterotrophs are also known as 'consumers', as they decompose organic material by utilising C from biota, animal, or microorganism origin (Gougoulias *et al.*, 2014). Soil microorganisms are well known for their importance within the global C cycle; however, few researchers have attempted to combine the chemical and microbiological views of C cycling (Kandeler *et al.*, 2005).

When SOM is not physiochemically protected, it is vulnerable to degradation processes such as depolymerisation (Conant *et al.*, 2011). Soil microbes can only access assimilable C (soluble and low molecular weight compounds); therefore, they produce extracellular enzymes to catalyse the breakdown of available SOM (Conant *et al.*, 2011). Heterotrophs utilise this SOM as a substrate for their metabolism. Some of this C is retained for their biomass while the rest is released as metabolites, including CO_2 (Gougoulias *et al.*, 2014).

Microbial respiration is controlled by numerous variables that change with time. Therefore, it is crucial to understand how soil C pools respond to these controls to predict changes in soil C stocks as climate changes and as land is used for different purposes and in different ways.

2.3.1 Controls of microbial respiration

Malik *et al.* (2018) state "soil microorganisms act as gatekeepers for soil-atmosphere C exchange by balancing the accumulation and release of soil organic matter". Therefore, it is important to gain a better understanding of the factors controlling microbial respiration. Section 2.3.1.1 will discuss the influence of C substrate availability on R_H. Sections 2.3.1.2 and 2.3.1.3 will cover the implications of soil moisture content and pH on R_H, respectively.

2.3.1.1 Substrate availability

Microbes cannot always access SOM due to apparent chemical and physical protection from decomposition (Blankinship *et al.*, 2018). SOM comprises a

range of different compounds with varying turnover times ranging from days to millennia (Bol *et al.*, 2009).

In an effort to compartmentalise the characteristic kinetics of the decomposition of different C compounds, various conceptual SOM turnover models have been proposed (Davidson & Janssens, 2006). The most common biogeochemical models of soil C dynamics are CENTURY (Parton *et al.*, 1987) and ROTH-C (Jenkinson *et al.*, 1990). The CENTURY model terms SOM pools of mineral soil from labile (easily accessible/degradable) to recalcitrant (hard to access/degrade) as 'fast', 'slow', and 'passive' pools, while the ROTH-C model terms these pools as 'microbial biomass', 'humified organic matter', and 'inert' (Davidson & Janssens, 2006). There are many other models, but these often have a similar general framework of different C pools.

The most labile pool (fast/microbial biomass) has an average turnover time between 1-10 years and comprises plant residues, root exudates, and microbial/faunal biomass & residues (von Lutzow *et al.*, 2007). Organic-matter binding and physical occlusion within aggregates limit the availability of these otherwise available substrates to microbes (Conant *et al.*, 2011). The turnover time for C substrates from the active pool is orders of magnitude less than those same substrates, which are bound to mineral surfaces (Conant *et al.*, 2011). von Lützow *et al.* (2008) also argue the importance of selective preservation of recalcitrant substrates as a control on SOM turnover; however, this is only relevant in the early stages of decomposition (fast pool) and not for long term stabilisation (slow and passive pools). von Lützow *et al.* (2008) proposed a conceptual model of SOM stabilisation which provides details on the mechanisms governing this complex process (Figure 2.3).

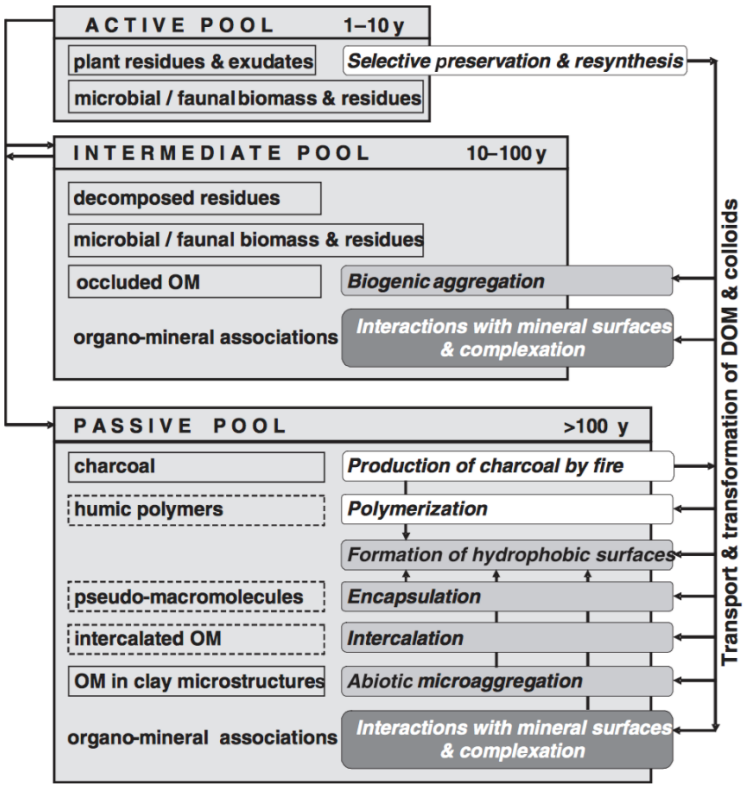

Figure 2.3 Conceptual model of SOM stabilisation proposed by von Lützow *et al.* (2008). The model includes C substrates/material that comprise each soil C pool (dotted boxes indicated known pools that have not yet been directly measured (von Lützow *et al.*, 2008)). The mechanisms behind SOM stabilisation relevant to each pool have been included on the right. These mechanisms are sectioned into three process groups according to Sollins *et al.* (1996): 'white box' = primary and secondary recalcitrance, 'light grey box' = spatial inaccessibility, and 'dark grey box' = organo-mineral interactions (von Lützow *et al.*, 2008). Note: Active = fast pool and Intermediate = slow pool

Essentially, if the majority of extracellular enzymes and decomposers in soil have limited access to SOM due to the mechanisms outlined in Figure 2.3, then CO_2 production will be significantly less (slow and passive pools). However, if a soil has a high proportion of SOM available for decomposition (fast pool), then CO_2 production will be higher.

2.3.1.2 Moisture content

Soil moisture is one of the most important factors controlling microbial respiration, but mechanisms behind this relationship are poorly understood (Moyano *et al.*, 2013). However, it is generally accepted that if soil moisture is too dry or too wet, microbial respiration processes are inhibited for different reasons, thus, slowing respiration rates.

Quantifying the relationship between soil moisture and microbial respiration can be variable as it depends on soil properties (i.e. pore space, bulk density,

and texture) and the expression of the water conditions in soil (Moyano *et al.*, 2012). Soil moisture is described using various approaches; the main two descriptors being soil moisture content (SMC) and soil water potential (SWP) (Cook & Orchard, 2008). SMC expresses the amount of water in the soil, while SWP describes the energy state of the soil water. SMC is expressed relative to the soil mass (gravimetric moisture content, θ_g), or relative to the soil volume (volumetric moisture content, θ_v). The gravimetric moisture content is commonly used in the laboratory, while the volumetric moisture content is more commonly used as a field measure (Moyano *et al.*, 2012). Most soil biologists use the soil's water-holding capacity (WHC) as a method to reflect its SWP (Cook & Orchard, 2008). The methodology for determining the WHC is outlined in section 3.1.1.2, which essentially describes the soil's ability to hold water against drainage. It is a useful measure when considering the SMC of different soil types, as soil properties can affect the soil's WHC. Therefore, it is common in soil biogeochemical experiments that soil moisture content is set to 60% of the maximum WHC instead of 60% SMC. This water content is generally considered to be optimal for many microbial processes. Here, optimal refers to highest rates rather than for any one group of microorganisms (e.g. fully saturated soil would be more beneficial for anaerobes).

Soil moisture has a vital influence on microbial respiration, including controlling the diffusivity of organic C and oxygen (O_2) through the soil to microbes (Figure 2.4). A review of the effects of soil moisture on heterotrophic respiration is given by Moyano *et al.* (2013). Diffusion of C and O_2 are controlled by the relative water saturation (θs), which describes the quantity of water relative to the soil pore-space (Moyano *et al.*, 2013) (Figure 2.4). Soil moisture and water saturation change relative to wet/dry periods.

As soil approaches saturation (i.e. soil pore-space filled with water), the metabolic activity of aerobic organisms decreases as the diffusion of oxygen slows (Moyano *et al.*, 2013). This decrease in O_2 as water fills soil pores limits aerobic respiration and supports anaerobic respiration, which is much slower than aerobic respiration (Davidson & Janssens, 2006). However, the diffusion of C substrates to microbial cells increases in these saturated conditions;

therefore, as shown in Figure 2.4, R_H rates do not reach zero in saturated conditions.

On the other hand, as soil moisture decreases and thus gas-filled porosity increases, the opposite occurs as diffusion of oxygen increases while the diffusion of C substrates slows down (Moyano *et al.*, 2013). As the soil moisture reaches drought levels, the thickness of the soil-water films is reduced, thus inhibiting the diffusion of solutes and extracellular enzymes and lowering substrate availability to the decomposing microbes (Davidson & Janssens, 2006).

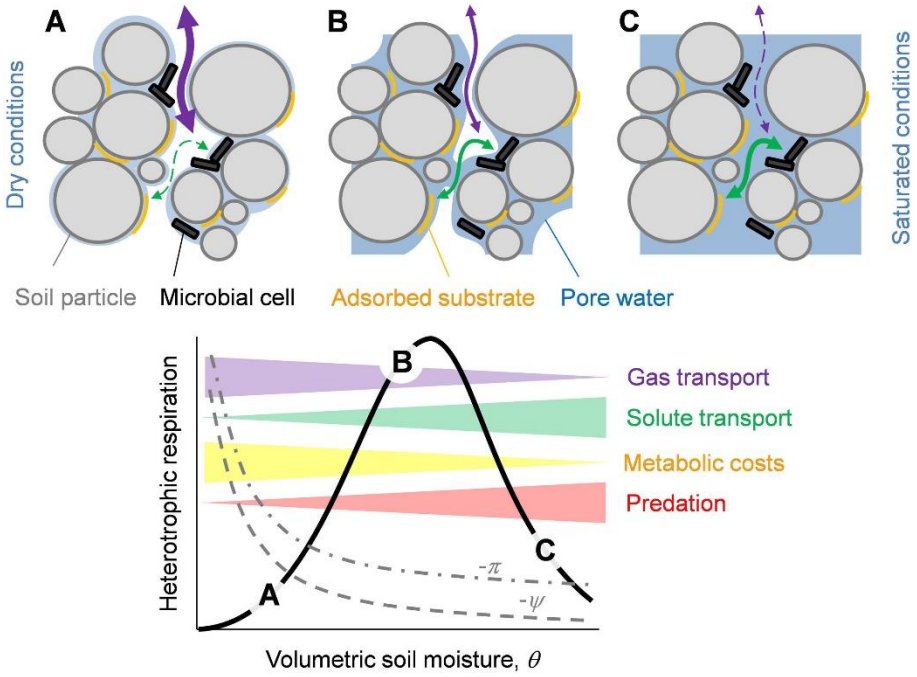

Figure 2.4 (TOP) Schematic illustration of soil moisture effects on microbial activity (Moyano *et al.*, 2013). (BOTTOM) The relationship between R_H and soil moisture governed by gas and solute (including extracellular enzymes) transport, metabolic costs (energy expended), and predation (organism consumes another organism) (Moyano *et al.*, 2013). Ψ describes soil water potential, and π is the cell osmotic potential (Moyano *et al.*, 2013)

The moisture-respiration relationship (Figure 2.4) incorporates other factors not discussed here, such as the metabolic cost of respiration and predation. The net effect of these trade-offs as soil is too wet or too dry is the development of optimal soil moisture for heterotrophic respiration (Figure 2.4, label B), with minimum R_H rates at the lower and higher ends of soil moisture (Figure 2.4, label A and C, respectively). Although there is a general understanding of the processes discussed above, determining the response of the moisture-respiration relationship remains poorly quantified, and simple empirical functions are commonly used to model this relationship (Moyano *et al.*, 2013).

2.3.1.3 pH

Microbial activity, and hence microbial respiration, is also controlled by the surrounding pH of the soil (Malik *et al.*, 2018). pH affects microbial growth and survival by controlling the bioavailability of nutrients and trace elements as pH describes the chemical activity of protons which is a key player for numerous geochemical reactions (Jin & Kirk, 2018). pH is a chemical property known for its essential role in controlling enzyme activity and substrate decomposition by organisms in soil; therefore pH is also linked to soil C storage (Leifeld & von Lützow, 2014).

Microbes can be categorised into three groups based on their optimal growth pH (pH at which maximal growth rate occurs): acidophiles (<5 pH), neutrophiles (5-9 pH), and alkaliphiles (>9 pH) (Jin & Kirk, 2018). One unit deviation from the microbe's respective optimal growth pH can reduce their growth rates by 50% (Jin & Kirk, 2018).

2.4 Temperature response of microbial respiration processes

Constraining the temperature response of microbial respiration in soils is essential to understanding soil C dynamics. Despite this, the temperature-respiration relationship remains a significant source of uncertainty for future climate projections (Liu *et al.*, 2018). There is a multitude of terminologies used when discussing how temperature controls soil respiration, which can often be a source of confusion. To provide some clarity on this complex topic, Box 1 defines the main terms relating to this temperature-respiration relationship that will be used in this thesis. The sections to follow cover a broad overview of the temperature response of microbial respiration processes. Firstly, by discussing the intrinsic and apparent temperature sensitivities of organic-C decomposition (Section 2.4.1). Secondly, the differences between the relative and absolute temperature sensitivities will be defined (Section 2.4.2). A brief synthesis of the application of mathematical models to the temperature response of soil respiration is provided in section 2.4.3, and a simple schematic is also provided to aid in understanding how all of these sections are connected (Figure 2.5).

Box 1: Definitions

The definition of the terms 'temperature dependence' and 'temperature sensitivity' are not always clearly distinguished in the literature, adding to the confusion of this complex research field. Here, these terms are defined as described by Sierra (2012).

- A process (e.g. soil respiration) is deemed 'temperature dependent' if *T* is an explanatory or independent variable in the mathematical representation of that process (Sierra, 2012)
- Sierra (2012) describes 'temperature sensitivity' as the rate of change of a process with respect to temperature while all other variables are held constant. Frequently, Q_{10} is used to describe temperature sensitivity. Q_{10} describes the ratio of rates rather than the rate of change (see section 2.4.2)
- The 'temperature response' of a process simply refers to how a process reacts to changes in temperature. Here, the temperature response of a process is usually associated to the temperature-process relationship

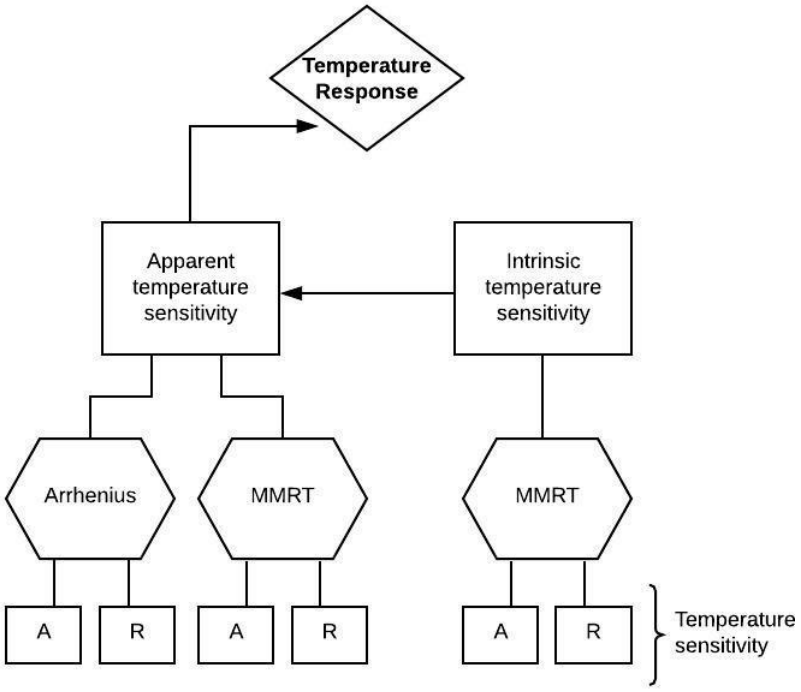

Figure 2.5 Simple schematic illustrating the relationship between the observed temperature response, intrinsic and apparent temperature sensitivities (T_{sens}), mathematical functions applied to the temperature-respiration relationship (MMRT is macromolecular rate theory), and two measures of temperature sensitivity of the decomposition of soil carbon. A is absolute, and R is relative temperature sensitivities.

2.4.1 Intrinsic and apparent temperature sensitivities

Like all chemical and biochemical processes, microbial respiration is dependent on temperature (Davidson & Janssens, 2006). The study of the temperature dependence of organic-C decomposition is crucial to understanding climate change feedbacks under anticipated global warming (Dash *et al.*, 2019). The decomposition of organic-C has been described as having both 'intrinsic' and 'apparent' temperature sensitivities (Davidson & Janssens, 2006; Conant *et al.*, 2011; Dash *et al.*, 2019) (Figure 2.6).

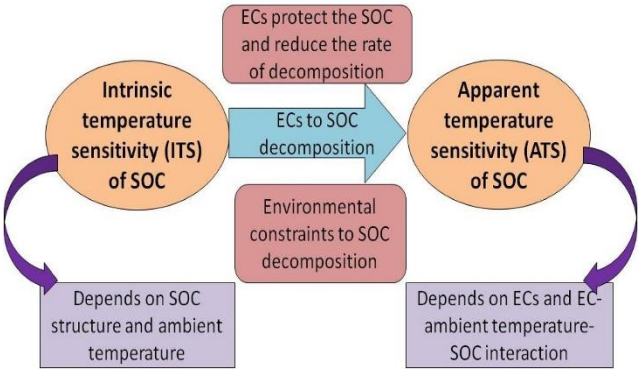

Figure 2.6 Schematic of the intrinsic and apparent temperature sensitivities of the decomposition of soil organic carbon (SOC) (Dash *et al.*, 2019). EC stands for environmental constraints

The intrinsic temperature sensitivity of respiration is a function of the decomposability of a molecule (based on its inherent kinetic properties), its activation energy, and the ambient temperature (Davidson & Janssens, 2006; Schipper *et al.*, 2014; Dash *et al.*, 2019) when no other external factors (e.g. substrate supply) moderate the temperature response. Environmental constraints (ECs) are processes that modify decomposition rates of organic-C by altering substrate supply, thereby obscuring the intrinsic temperature sensitivity (Davidson & Janssens, 2006). Davidson and Janssens (2006) denote the observed temperature response of decomposition under these ECs as the apparent temperature sensitivity. These processes comprise physical, chemical, biological, and biochemical protections of SOM to decomposition (Dash *et al.*, 2019). Essentially, these ECs slow down the rate of decomposition and, therefore, reduce the intrinsic temperature sensitivity exerting relative stability to the SOM (Dash *et al.*, 2019). However, ECs can also be temperature dependent, and the potential adverse effects of this are also briefly covered in sections 2.4.1.1 through to 2.4.1.3.

Q_{10} functions are commonly used to assess the temperature sensitivity of decomposition (in relative terms, see section 2.4.2) (Sierra, 2012). Vant Hoff's Q_{10} coefficient describes the factor by which rate 'x' (decomposition/respiration) increases with a 10°C rise in temperature (Dash *et al.*, 2019). Typically, Q_{10} has a value of 2 in the context of SOC decomposition. However, substrate availability plays a significant role in determining Q_{10}, which is a source of variability. If substrate supply is abundant, and a temperature optimum is not reached, the reaction rate will follow Arrhenius kinetics (Davidson & Janssens, 2006; Dash *et al.*, 2019). The Arrhenius equation is given here as (discussed in more depth in section 2.4.3.1):

$$k = A\ exp\left(\frac{-E}{RT}\right) \tag{2-2}$$

Where k is the reaction constant, A is a frequency or pre-exponential factor, E is the required activation energy in Joules mol^{-1}, R is the gas constant (8.314 J K^{-1} mol^{-1}) and T is the temperature in Kelvin. Inherently, the Arrhenius equation implies that a substrate of high molecular complexity is characterized by high activation energy and a low decomposition rate (Dash *et al.*, 2019). The intrinsic temperature sensitivity increases proportionally with the molecular complexity of a specific soil organic carbon (SOC) pool (Davidson & Janssens, 2006; Sierra, 2012; Dash *et al.*, 2019).

When substrate supply is limited, the reaction rate is described by Michaelis-Menten kinetics (Davidson & Janssens, 2006; Dash *et al.*, 2019):

$$k = \frac{V_{\max}[S]}{(Km + [S])} \tag{2-3}$$

Where, k is the reaction rate, V_{max} is the maximum rate at a given temperature, $[S]$ is the substrate concentration at the active sites of the enzyme, and Km is the Michaelis-Menten constant. Equation (2-3) implies that reaction rates can be further modified by substrate availability $[S]$ and the affinity of the enzymes to substrates (Km) (Dash *et al.*, 2019). Typically, V_{max} and Km increase with temperature, which can lead to their temperature sensitives neutralising each

other and lowering apparent Q_{10} values (Davidson & Janssens, 2006). Thus, the large variability in Q_{10} values can not only be attributed to substrate quality and temperature, but also the temporal and spatial differences in substrate availability (Davidson & Janssens, 2006).

2.4.1.1 Physical environmental constraints

When organic matter is considered physically protected from decomposition processes, this refers to processes creating a physical barrier that stabilises the SOC (Dash *et al.*, 2019). Aggregation of soil particles and organic matter is the most important form of physical ECs. SOM can become physically bound within soil aggregates, limiting microorganism and enzyme access and reducing oxygen concentration (Davidson & Janssens, 2006) and thus microbial activity. However, aggregate formation is affected by both climate and management practices; for example, raindrops and ploughing can destroy aggregates (Davidson & Janssens, 2006).

Another critical physical EC is the physical protection that arises from water saturation, such as occurs in flooded soils. As discussed in section 2.3.1.2, oxygen diffuses much slower through water-filled pores compared to air-filled pores. Therefore, anaerobic decomposition begins to dominate, slowing the degradative enzymatic pathways (Davidson & Janssens, 2006; Dash *et al.*, 2019). In the context of wetlands and peatlands, anaerobic conditions also inhibit the activity of phenoloxidase resulting in an accumulation of phenolic compounds (Davidson & Janssens, 2006). These phenols inhibit the activity of hydrolase enzymes responsible for decomposition, further reducing decomposition rates (Freeman *et al.*, 2001). Flooding can also be correlated to temperature; as temperatures rise the soil-water film thickness, which C substrates and enzymes diffuse through, can be reduced (Davidson & Janssens, 2006). The relationship between moisture and respiration is covered in section 2.3.1.2. Global wetlands and peatlands store around 400-500 Pg C to a depth of 3 m (Dash *et al.*, 2019). This significant store is only stable if the current anaerobic conditions are sustained. If enough water is evaporated due to increases in temperature, then the new aerobic conditions will expose this otherwise protected C store to more rapid decomposition processes. This

phenomenon might display higher temperature sensitivities than what would be expected from kinetics alone.

When soil water is frozen, the diffusion of organic-C substrates and extracellular enzymes is slowed down significantly, minimising the decomposition rate (Dash *et al.*, 2019). This EC is also temperature dependent, which is of significant concern regarding permafrost. Global estimates suggest there is around 400 Pg C stored to a depth of 3 m in permafrost (Davidson & Janssens, 2006). Increased temperatures could thaw permafrost, exposing a large amount of unprotected C to decomposition processes by removing its major constraint (frozen soil water) (Davidson & Janssens, 2006).

2.4.1.2 Chemical environmental constraints

Chemical adsorption of SOC onto mineral surfaces is controlled by the mineral composition and the types of SOC pools (Dash *et al.*, 2019). This process protects SOC from decomposition through electrostatic or covalent bonds binding them to the mineral (including silt and clay) surfaces (Davidson & Janssens, 2006). The processes of sorption and desorption are also temperature dependent, but little is known about their activation energies (Davidson & Janssens, 2006).

2.4.1.3 Biological and biochemical constraints

The composition of plant inputs into the soil, land-use change induced plant biomass inputs variations, and enzyme-SOC interactions represent the central processes for biological ECs (Dash *et al.*, 2019). These are not major ECs independently; however, biological ECs do play a major role in modifying ECs for SOC decomposition (Dash *et al.*, 2019).

Biochemically protected SOC is associated with condensation and complex reactions within the SOC pools and mineral components in soil (Dash *et al.*, 2019). Unlike chemical protection processes, biochemical ECs are not related to soil texture (Dash *et al.*, 2019).

2.4.2 Absolute and relative temperature sensitivity

Reaction rates, such as decomposition and respiration rates, are temperature dependent. When they are exposed to changes in temperature, this can be expressed in two ways: (1) absolute temperature sensitivity and, (2) relative temperature sensitivity (Sierra, 2012). The section presented here is heavily drawn from a critical paper by Sierra (2012) titled 'Temperature sensitivity of organic matter decomposition in the Arrhenius equation: some theoretical considerations'. This paper has a strong focus on deriving the absolute and relative temperature sensitivities of different reaction rates from the Arrhenius function. Put simply, relative temperature sensitivities refer to the ratio of rates and the absolute temperature sensitivities refer to the rate of change of rates. This section focusses on the temperature sensitivity of decomposition rates (k), rather than respiration rates (R_S). However, if microbes are in steady-state, that is the amount of C consumed is equal to the amount respired, then $k = R_S$.

Sierra (2012) states that it is commonly accepted that the rates of decomposition processes follow the Arrhenius equation (Eq. (2-2)). The absolute sensitivity of decomposition is defined by the partial derivative of the decomposition rate relative to temperature (Sierra, 2012):

$$\frac{\partial k}{\partial T} = \frac{EA}{RT^2} \exp\left(\frac{-E}{RT}\right) \tag{2-4}$$

Where k is the decomposition rate, T is absolute temperature, E is the activation energy, A is the pre-exponential factor, and R is the universal gas constant.

Substituting in Equation (2-2) results in equation (2-4) simplifying to:

$$\frac{\partial k}{\partial T} = k\frac{E}{RT^2} \tag{2-5}$$

The values of these derivatives are always positive, implying that as temperature increases, the decomposition rate will always increase for constant values of the activation energy (Sierra, 2012).

The relative temperature sensitivity of decomposition rates (Sierra, 2012) is defined in logarithmic form as:

$$\frac{\partial \ln k}{\partial T} = \frac{E}{RT^2} \tag{2-6}$$

The relative temperature sensitivity relates to the absolute temperature sensitivity by dividing Eq. (2-5) by *k*:

$$\frac{1}{k} \cdot \frac{\partial k}{\partial T} = k \frac{E}{RT^2} \cdot \frac{1}{k} \tag{2-7}$$

k is cancelled out on the right side of Eq. (2-7), so that:

$$\frac{1}{k} \frac{\partial k}{\partial T} = \frac{E}{RT^2} \tag{2-8}$$

Consequently, the absolute (Eq. (2-5)) and relative (Eq. (2-8)) temperature sensitivities of decomposition rates produce apparently contradictory results.

Absolute temperature sensitivities imply that substrates of higher quality (low *E* and simple molecular structure) are more temperature sensitive than lower quality substrates (Sierra, 2012). Low-quality substrates (high *E* and complex molecular structure) have lower decomposition rates and in relative terms are more temperature sensitive than higher quality substrates (Sierra, 2012). This phenomenon can be defined by comparing the behaviour at the limits of the absolute (Eq. (2-9)) and relative (Eq. (2-10)) temperature sensitivities as the quality of substrate decreases (E→∞):

$$\lim_{n \to \infty} \frac{\partial k}{\partial T} = 0 \tag{2-9}$$

$$\lim_{n \to \infty} \frac{1}{k} \frac{\partial k}{\partial T} = \infty \tag{2-10}$$

The above equations show that as the substrate quality decreases, the absolute and relative temperature sensitivities behave in opposing ways. Figure 2.7a and b show that in relative terms, the temperature sensitivity of decomposition increases linearly towards infinity, while in absolute terms, it decreases exponentially towards zero (Sierra, 2012). These contradictory behaviours are the same for any substrate at any given temperature (Sierra, 2012). This fundamental difference means defining the type of temperature sensitivity as either absolute or relative is critical when describing the behaviour of chemical and biochemical reaction rates.

As mentioned in section 2.4.1, Q_{10} is one commonly used measure of relative temperature sensitivity of the reaction rate of either decomposition or respiration:

$$Q_{10} = \frac{k_{(T+10)}}{k_T} \tag{2-11}$$

Figure 2.7a and Figure 2.8 depict the similarities between the equations (2-8) (relative temperature sensitivity of k) and (2-11) (Q_{10} response to temperature and activation energy) as they both produce comparable shapes as a function of temperature and activation energy.

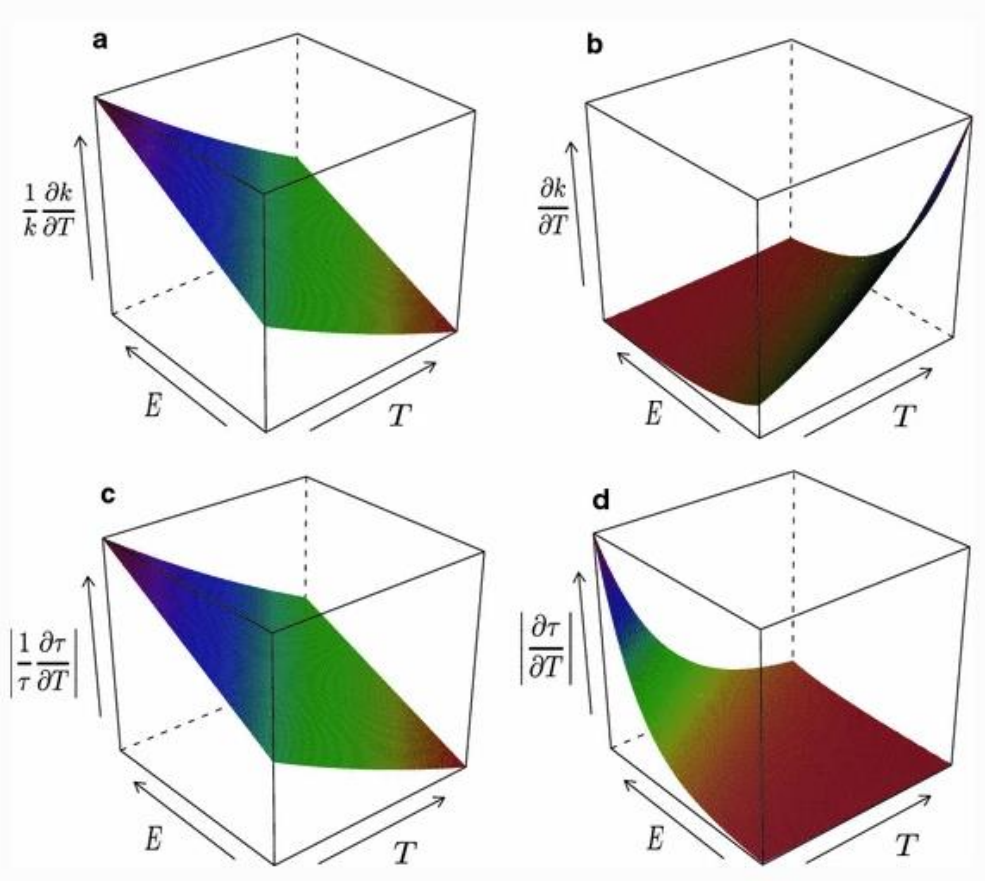

Figure 2.7 Temperature sensitivities of decomposition rates (*k*) and turnover time as a function of temperature (*T*) and activation energy (*E*). (a) Relative temperature sensitivity of *k*. (b) Absolute temperature sensitivity of *k*. (c) Relative temperature sensitivity of τ (turnover rate). (d) Absolute temperature sensitivity of τ (Sierra, 2012). Arrows are moving from low to high values. Vertical bars in (c) and (d) shows that absolute values were used (Sierra, 2012)

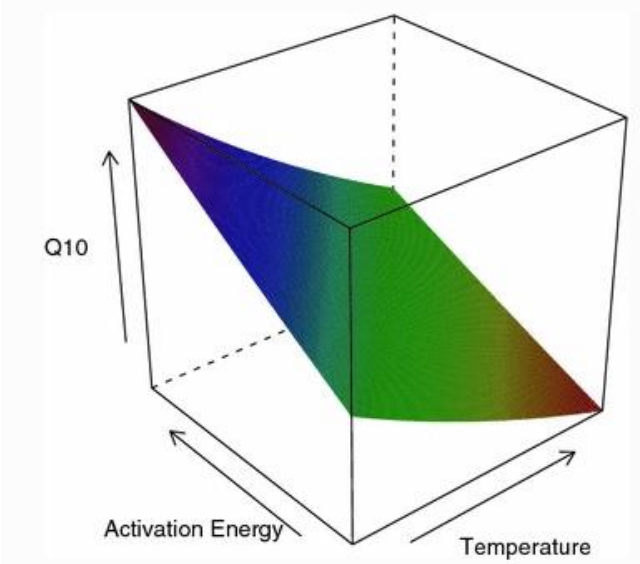

Figure 2.8 Q_{10} calculated from the Arrhenius equation as a function of activation energy and temperature (Sierra, 2012)

The opposing behaviours of the temperature sensitivities in relative (including Q_{10}) and absolute terms can be further demonstrated by their applications to turnover times (mean residence time). Dettmann (2013) states that, in general, a turnover time of a system is defined as "the ratio of the quantity of a material or energy in a system to its outflow rate. It may also be viewed as the inverse of the fraction of material or energy that leaves per unit time".

In this case, the turnover time (τ) is the inverse of the decomposition rate described by Arrhenius:

$$\tau = \frac{1}{k} = \frac{\exp\left(\frac{E}{RT}\right)}{A} \tag{2-12}$$

Therefore, the absolute temperature sensitivity is given by a partial derivative of Eq. (2-12) with respect to temperature:

$$\frac{\partial \tau}{\partial T} = \frac{-E}{ART^2} \exp\left(\frac{E}{RT}\right) \tag{2-13}$$

Equation (2-13) simplifies to:

$$\frac{\partial \tau}{\partial T} = \tau \frac{-E}{RT^2} \tag{2-14}$$

The relative sensitivity can be represented in logarithmic form (Eq. (2-15)) or relative terms (Eq. (2-16)) by dividing the absolute temperature sensitivity by τ:

$$\frac{\partial \ln \tau}{\partial T} = \frac{-E}{RT^2} \tag{2-15}$$

$$\frac{1}{\tau}\frac{\partial \tau}{\partial T} = \frac{-E}{RT^2} \tag{2-16}$$

Sierra (2012) transforms the relative and absolute temperature sensitivities to absolute (positive) values, thus, removing the negative term of the

activation energy (E) in both equations (denoted by vertical bars in Figure 2.7c and d). This results in the relative temperature sensitivity of k and τ both being equal to E/RT^2, and the absolute temperature sensitivity of τ modified to ($\tau(E/RT^2)$). The amendments to these equations of turnover time are highlighted by the similar behaviours of the relative temperature sensitivities of k and τ (Figure 2.7a and c). The opposing behaviours of the absolute temperature sensitivities of k and τ (Figure 2.7b and d) are due to the factors of k and τ themselves. As explained earlier, τ is defined as the inverse of the decomposition rate k. Sierra (2012) states that the opposing behaviour of these absolute temperature sensitivities due to the inverse of k is not necessarily a reflection of biophysical behaviour but rather a mathematical artefact of $1/k$ for low values of the activation energy.

2.4.3 Modelling the temperature-respiration relationship

While numerous studies present a positive relationship between R_S and T, there is no consensus on the exact form of the relationship (Luo *et al.*, 2016). A range of models have been proposed to define this relationship. Here, we discuss the commonly used Arrhenius equation, including the empirical adjustments made by Lloyd and Taylor (1994), and the relatively new model called macromolecular rate theory (MMRT).

2.4.3.1 Arrhenius

In 1889, Arrhenius proposed his equation (Eq. (2-2)) for the temperature dependence of reaction rates based on the early work of van't Hoff (1884). Many mathematical models pertaining to the temperature dependence of soil respiration processes are based on the Arrhenius equation (Lloyd & Taylor, 1994; Fang & Moncrieff, 2001). The Arrhenius equation describes the dependence of a chemical reaction on temperature, which is a function of the energy required by reactants to transform into products (i.e. activation energy) (Schipper *et al.*, 2014). If the activation energy of an organic-C molecule is high (more energy required), then the decomposition/respiration rate will be lower, if the activation energy is low, then the rates will be higher (Sierra, 2012; Schipper *et al.*, 2014).

Despite its high frequency of applications to soil respiration models, Lloyd and Taylor (1994) found that the Arrhenius relationship could not provide an unbiased estimate of respiration rates. Lloyd and Taylor (1994) examined the residuals of multiple datasets and found that the conventional Arrhenius equation underestimated respiration rates at low temperatures and overestimated respiration rates at high temperatures.

An adjustment to the Arrhenius equation was proposed by Lloyd and Taylor (1994), whereby the activation energy for respiration varied inversely with temperature:

$$k = Ae^{\frac{-E_o}{(T-T_o)}} \quad (2\text{-}17)$$

Where E_0 = 308.56 K and is no longer the activation energy of the Arrhenius equation (Fang & Moncrieff, 2001), and T_0 = 227.13 K.

Fang and Moncrieff (2001) tested the Arrhenius derived Lloyd and Taylor equation and found that it behaved very similarly for respiration rates from both farmland and forest soil, but they concluded that the extra parameter of T_0 in Eq. (2-17) did not improve the Arrhenius model significantly.

A vital factor to note about both the Arrhenius and Lloyd and Taylor models, concerning biochemical processes, is that they do not account for the decline in enzymatic activity past the temperature where respiration is maximal (i.e. temperature optimum) (Schipper *et al.*, 2014). This may be the cause of the overestimation of R_S observed by Lloyd and Taylor (1994) from the Arrhenius equation.

2.4.3.2 Macromolecular rate theory

The macromolecular rate theory (MMRT) was developed as a possible explanation for the decline in enzymatic rates above the temperature optimum (T_{opt}) (Hobbs *et al.*, 2013; Schipper *et al.*, 2014; Arcus *et al.*, 2016). This decline in enzymatic rates past the T_{opt} is usually attributed to enzyme denaturation (Hobbs *et al.*, 2013; Schipper *et al.*, 2014). However, enzyme denaturation cannot fully explain the decline in rates above the T_{opt} as this can occur in the

absence of denaturation (Arcus *et al.*, 2016). Typically, most enzymatic denaturation occurs at higher temperatures than are observed in soil (Schipper *et al.*, 2014).

In 1935, the Erying equation was presented as a development on the Arrhenius equation, which are still both used today to give accurate descriptions of the temperature dependence of a wide variety of chemical processes across a broad temperature range (Schipper *et al.*, 2014). The Erying equation substitutes the pre-exponential factor, A for $k_B T/h$ where, k_B is Boltzmann's constant and h is Planck's constant (Schipper *et al.*, 2014). The activation energy (E) is also substituted for the change in Gibbs free energy ($\Delta G^{\ddagger}$), which is calculated from the difference between changes in enthalpy and entropy for the respective reaction ($\Delta G^{\ddagger} = \Delta H^{\ddagger} - T\Delta S^{\ddagger}$). These alterations give:

$$k = \frac{k_B T}{h} e^{\left(\frac{-\Delta G^{\ddagger}}{RT}\right)} \tag{2-18}$$

Which can be transformed by taking the natural log of each side (Schipper *et al.*, 2014) to give:

$$\ln(k) = \ln\left(\frac{k_B T}{h}\right) - \frac{\Delta G^{\ddagger}}{RT} \tag{2-19}$$

The activation energy (E) in the Arrhenius equation essentially describes the energy barrier reactants have to 'jump' over to transform into products (Schipper *et al.*, 2014). This transformation is now denoted as the 'transition state'. An assumption made for the Erying equation is that the components of Gibbs free energy, $\Delta G^{\ddagger}$ (changes in enthalpy, $\Delta H^{\ddagger}$ and entropy, $\Delta S^{\ddagger}$) are independent of temperature (Arcus *et al.*, 2016). This assumption is usually correct for small molecules in solvents; however, not necessarily for macromolecules which almost always mediate biological reactions (Schipper *et al.*, 2014). Arcus *et al.* (2016) argued that because enzymes are flexible macromolecules of high molecular weight and changes in heat capacities ($\Delta C_P^{\ddagger}$), they may be more important during catalysis and need to be accounted for in appropriate equations.

To account for changes in heat capacity during catalysis, Hobbs *et al.* (2013) developed a thermodynamic theory (MMRT) which considers the thermodynamic properties of enzymes. Hobbs *et al.* (2013) showed that MMRT described the temperature dependence of enzyme-catalysed rates with large, negative values for changes in heat capacity ($\Delta C_P^{\ddagger}$). MMRT recognises that the activation energy (E) from the Arrhenius equation (Eq. (2-2)) is itself temperature dependent due to a large negative change in heat capacity associated with enzyme-catalysed reactions (Schipper *et al.*, 2014). This thermodynamic theory can account for the decline in enzymatic rates above the T_{opt}, as it directly predicts the T_{opt} in temperature vs. rate plots, unlike the Arrhenius or Erying equations (Schipper *et al.*, 2014) (Figure 2.9).

Schipper *et al.* (2014) state that during catalysed reactions, there are large and significant changes in heat capacity between different states of enzymes. In particular, the difference in heat capacity between the enzyme bound to the substrate and the enzyme bound to the transition state for the reaction ($\Delta C_P^{\ddagger}$). The following equation shows that large values of heat capacity give rise to an evident temperature dependence of Gibbs free energy:

$$\begin{aligned} \Delta G^{\ddagger} &= \Delta H^{\ddagger} - T\Delta S^{\ddagger} \\ &= \left[\Delta H_{T_0}^{\ddagger} + \Delta C_P^{\ddagger}(T - T_0)\right] - T\left[\Delta S_{T_0}^{\ddagger} + \Delta C_P^{\ddagger}(\ln T - \ln T_0)\right] \end{aligned} \qquad (2\text{-}20)$$

Where $\Delta H^{\ddagger}$ is the activation enthalpy, $\Delta S^{\ddagger}$ is the activation entropy, and $\Delta H_{T_0}^{\ddagger}$ and $\Delta S_{T_0}^{\ddagger}$ are the changes in enthalpy and entropy, respectively, between the enzyme-substrate complex and the enzyme bound to the transition state at a reference temperature (T_0) of (Schipper *et al.*, 2019).

By incorporating the term $\Delta C_P^{\ddagger}$ from equation (2-20) into the Erying equation (2-19) the following equation, coined macromolecular rate theory is produced:

$$\ln(k) = \ln\left(\frac{k_B}{h}\right) - \frac{\Delta H_{T_0}^{\ddagger} + \Delta C_P^{\ddagger}(T - T_0)}{RT} + \frac{\Delta S_{T_0}^{\ddagger} + \Delta C_P^{\ddagger}(\ln T - \ln T_0)}{R} \qquad (2\text{-}21)$$

The heat capacity (C_P) quantifies the temperature dependence of the enthalpy (H) and entropy (S) (Arcus *et al.*, 2016). The MMRT equation assumes that there is a difference in heat capacity between the enzyme-substrate and enzyme-transition state species for enzyme-catalysed reactions, therefore, $\Delta C_P^{\ddagger} \neq 0$ (Arcus *et al.*, 2016). If $\Delta C_P^{\ddagger}$ is nonzero, the reaction rate will deviate from typical Arrhenius behaviour (Figure 2.9) (Schipper *et al.*, 2014). However, if $\Delta C_P^{\ddagger} = 0$, then the non-logarithmic version of MMRT will collapse into the Erying equation (Eq. (2-19)) and produce an Arrhenius-like behaviour (Figure 2.9) (Arcus *et al.*, 2016).

Schipper *et al.* (2014) applied equation (2-21) to the temperature response of a wide variety of soil microbial processes, including respiration, soil enzyme activities, and nitrogen and methane cycling. The study found that MMRT "agreed closely with a wide range of experimental data and predicts temperature optima for these microbial processes". While MMRT provides a useful description of many observations of soil biochemistry with increasing temperature, further work is needed to determine whether the theory has explanatory power or is a useful equation that fits biological processes.

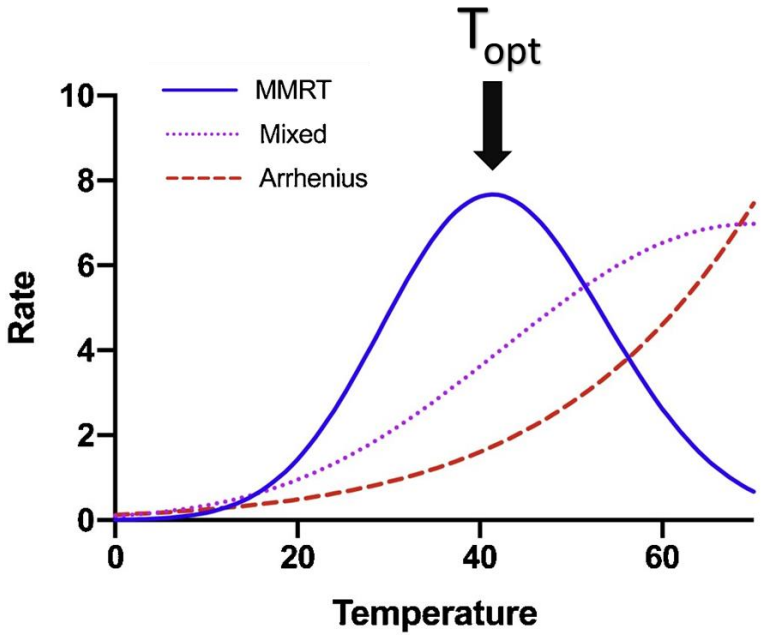

Figure 2.9 Conceptual figure adapted from Schipper *et al.* (2019) showing a typical reaction rate response from biochemical processes where $\Delta C_P^{\ddagger} \neq 0$ (macromolecular rate theory, blue line) and from physical chemistry processes where $\Delta C_P^{\ddagger} = 0$ (Arrhenius function, dashed red line) with increasing temperature. The dotted pink curve represents a 'mixture' of these two responses where the biological system (MMRT) becomes substrate limited the physical chemistry processes response dominates (Arrhenius) (Schipper *et al.*, 2019). The arrow depicts the temperature optimum that is derived from the macromolecular rate theory and notably, is not present in the Arrhenius function

2.5 Temperature sensitivity of different C pools in soil

The concept of fractionating SOM into different C pools was introduced in section 2.3.1.1 when discussing the controls of substrate availability on microbial respiration. Three different fractions of the C pool were described: (1) fast (easily degradable C), (2) intermediate, and (3) slow (hard to degrade C). There are likely many different pools in soil, and so a three pool model is somewhat arbitrary. Here, for illustrative purposes, we simplify these pools into two: (1) labile (readily available and easily degradable (soluble) C) and (2) stable (physiochemically protected and harder to degrade C) (Von Lützow & Kögel-Knabner, 2009; Robinson *et al.*, submitted). There is generally a broad consensus on the existence of these two C pools within soil in the literature; however, there is still confusion surrounding their respective temperature sensitivities.

Over 80% of the organic C that is actively cycled in the global C cycle is stored in soil, with the rest bound in vegetation (Schlesinger, 1995). Up to 75% of this SOM is stored within the stable C pool, and the remaining fraction is stored in the labile C pool (Sanchez *et al.*, 1989). It is crucial to determine how sensitive these pools are to changes in temperature, as this can play a significant role in controlling the magnitude of CO_2 returned to the atmosphere. Contradicting results for the relative temperature sensitivity of C decomposition have been published in the literature, with some studies stating the decomposition of stable C is not temperature sensitive within the temperature range of 5-35°C, others stating the labile and stable C pools responds similarly to temperature, and several concluding that the decay rate of the labile C pool was more temperature sensitive compared to the stable pool (Von Lützow & Kögel-Knabner, 2009). However, the most supported conclusion in the literature reports that the stable C pool is more temperature sensitive than the labile pool (Von Lützow & Kögel-Knabner, 2009; Robinson *et al.*, submitted). If the stable C pool is, in fact, more sensitive to changes in temperature, this could be a cause for concern due to its relatively large size, as the CO_2 fluxes derived from this pool would result in probable sustained C losses (Von Lützow & Kögel-Knabner, 2009).

Robinson *et al.* (submitted) suggested reasons for these different conclusions, firstly the inconsistencies in partitioning methods for separating the respiration of these two pools, and secondly the limited number of incubation temperatures and long incubation lengths. Measuring the temperature sensitivity of soils from long incubation studies (7-700 days) may result in skewed data as soil properties (total C/N and microbial biomass) and population structure may be altered with time (Robinson *et al.*, submitted). Robinson *et al.* (submitted) also suggest that "fitting complex functions to small data sets and deriving sensitivity is susceptible to substantial error". Robinson *et al.* (2017) determined that the confidence in parameter fits begins to decline at 20 incubation temperatures and continues to do so with fewer temperatures. Many studies in the literature only use 3-4 temperatures to calculate temperature sensitivity.

With these causes for substantial error in determining the temperature sensitivity of soil C pools in mind, Robinson *et al.* (submitted) developed an isotopic methodology for accurately separating the temperature response of R_S from two different C pools. This method was used in the research described in this thesis (see section 4.3.2). Robinson *et al.* (submitted) tested this method by adding ^{13}C enriched plant litter to soil and incubating soil samples at 30 different temperatures (2-50°C) over 5 hours. A mixing model was used to separate the temperature response of R_S from the litter (representing the labile C pool) and the bulk SOM (representing the stable C pool). The results aligned with the currently most supported conclusion discussed previously: the decomposition of labile litter was less temperature sensitive (in relative terms, Q_{10}) than the more stable SOM decomposition (Robinson *et al.*, submitted).

2.6 Further research needs

This chapter has highlighted the importance of gaining a better understanding of soil C dynamics and how associated processes respond to changes in temperature. The temperature response of heterotrophic respiration (R_H, microbial respiration) is a popular topic within the literature and is of great interest regarding climate change. It is also essential to determine how R_H responds to temperature to understand soil C dynamics on a seasonal and diurnal scale.

This is a complex area of research, in which we still struggle with contrasting conclusions, inconsistent methods, and confusing definitions. Although valuable recent developments have been achieved, a consensus has yet to be reached on multiple factors. Therefore, this lack of consensus undermines the development of reliable and useful representations for climate models that take into account the complex nature of soil C dynamics.

A primary focus of this thesis is separating the temperature response/sensitivity of respiration from the labile and stable C pool to aid in understanding the influence climate change will have on soil C storage. On a seasonal and diurnal scale, this will aid in understanding soil fertility and C stabilisation processes (for increased storage).

The majority of this research uses the characteristics of physicochemical and biochemical processes to indicate the temperature responses of R_H from the stable and labile C pool, respectively. The absolute and relative temperature sensitivities also need to be calculated to aid in this research. This thesis has investigated the potential soil priming effect, which can be overlooked in temperature-respiration studies and has therefore used isotopic techniques to separate the temperature response from the stable and labile C pools.

Chapter 3

Methodology

3.1 General methods

This chapter describes the general methods for determining the temperature response of microbial respiration from soil with two treatments, (1) amended with carbon compounds (treatment soil), (2) no addition of carbon compounds (control soil). By calculating the difference between these two treatments, we can separate the temperature response of respiration from the labile C pool (i.e. fast, active pool) represented by the added carbon and the stable C pool (i.e. recalcitrant, passive pool) represented by SOM decomposition. An abbreviated version of these methods is also presented in the following chapter, but the intent here is to provide a greater level of detail and a fuller description of some methodological checks. Within section 3.1, section 3.1.1 covers the methods for soil sample preparation, section 3.1.2 describes the addition of C compounds to soil, and section 3.1.3 introduces the temperature gradient block, which is the central laboratory equipment used in this thesis' research. Section 3.1.4 outlines the methods used for collecting CO_2 gas samples and calculating soil respiration rates, and lastly, section 3.1.5 briefly covers curve fitting respiration rate data. The last section (3.2) describes minor preliminary experiments conducted to increase confidence in the reliability of the data produced from the methods outlined in this chapter.

The methods used in this chapter are adapted from methods developed by Robinson (2016) and Robinson *et al.* (2017).

3.1.1 Soil sample preparation

Careful soil preparation was a crucial part of this research to ensure that reliable data was produced. This section covers soil collection methods, measurement of soil moisture content, and preparation of the C compound solutions used for soil amendments.

3.1.1.1 Soil collection

Three soil types (Te Rapa, Te Kowhai, and Horotiu) were collected from a DairyNZ research farm (Scott Farm) in the Waikato region. This site was ideal as the different soil types were in proximity to the laboratory and were subjected to the same climate/weather and land management (Robinson, 2016). Topsoil was collected from each soil type using a bucket sampler (75 mm deep, 20 mm diameter). The soil was transported back to the University of Waikato where it was passed through a 2 mm sieve to achieve homogeneity. The bulk soil was stored at room temperature in a large plastic zip-lock bag plugged with cotton wool to allow for gas exchange (Robinson, 2016). The well-drained allophanic Horotiu soil (Typic Udivitrand; 6.2%C, 0.6%N, soil pH of 6.0, and $\delta^{13}C_{VPDB}$: −26.5 ‰; Robinson *et al.* (2017)) was used for the bulk of this investigation. The poorly-drained gley Te Kowhai soil (Typic Ochraqualf; 5.2%C, 0.51%N, and soil pH of 6.2; Robinson *et al.* (2017)) and the moderately well-drained organic Te Rapa soil (Typic Epiaquoll; 16.5%C, 0.97%N, and soil pH of 5.8) were used to compare the temperature sensitivity of respiration of added glucose from three contrasting soils.

3.1.1.2 Soil moisture content

The rate of microbial respiration is controlled by multiple factors, including the soil moisture content (SMC). Therefore, establishing a consistent SMC during comparative incubation experiments was an essential aspect of the sample preparation. SMC can be analysed through various methods, and this investigation used the gravimetric water content (θg, g g^{-1}) which was determined for the bulk soil.

$$\theta g = \frac{Soil_w - Soil_d}{Soil_d} \quad (3\text{-}1)$$

Where the weight of the fresh soil sample ($soil_w$, g) is weighed before it is oven-dried (105°C) removing water before being reweighed ($soil_d$, g).

The majority of soil biochemistry experiments set the SMC based in part on the soil type's maximum water-holding capacity (MWHC) (specifically 60% of the MWHC). Methods described by Harding and Ross (1964) were used to determine the MWHC for each soil type. Briefly, this involved saturating a

subsample (~4 g) of soil overnight in a clamped funnel with filter paper, so water ponded on the soil surface. The saturated soil was allowed to drain for ~3 hours before being weighed to determine the MWHC ($Soil_w$). A subsample was oven-dried (105°C) overnight, weighed ($Soil_d$, g), and the water content was calculated. The θg was determined for the MWHC using equation (3-1) which was then multiplied by 0.6 to give the θg at 60% MWHC. The subsequent addition of the different carbon compounds (section 3.1.2) (made as solutions) to the soil samples was accounted for when setting the original SMC. That is, the soil moisture content was slightly less than 60% MWHC and was raised to this moisture content following the addition of the C substrate in solution. The ratio of soil to added C solution was 1:0.125, where for each gram of soil, 0.125 g of solution/water was added. Therefore, 0.125 g was subtracted from the θg at 60% MWHC to give the true θg to which the soil was then altered:

$$\theta g_a = MWHC_{60\%} - 0.125g \quad (3\text{-}2)$$

Where θg_a (g g^{-1}) is the gravimetric moisture content before the addition of C solution and $MWHC_{60\%}$ is 60% of the maximum water holding capacity (g g^{-1}).

3.1.2 Addition of carbon compounds

A primary objective of this research was to measure the temperature sensitivity of microbial respiration from soils with different added carbon compounds. The six compounds used in this investigation and their respective properties are described in Table 3.1. Yeast extract (BBL™ Extract of Autolysed Yeast Cells, reference number 211929) was also added to soil and incubated. As yeast extract is a culture media, it does not have a measurable molecular weight and formula. Therefore, it is not included in Table 3.1. It is, however, very soluble in water.

Table 3.1 Carbon compounds added to soil to determine the temperature response of soil and carbon compounds

Name	Molecular weight ($g\ mol^{-1}$)	Solubility in water ($g\ ml^{-1}$)	Type	Molecular formula
D-Glucose	180	0.909	Simple sugar	$C_6H_{12}O_6$
D-Maltose	342	1.080	Disaccharide	$C_{12}H_{22}O_{11}$
Dextran	~480,000	Yes^	Glucan	$H(C_6H_{10}O_5)_xOH$*
L-Arginine	174	0.149	α-amino acid	$C_6H_{14}N_4O_2$
L-Glutamine	146	Yes^	α-amino acid	$C_5H_{10}N_2O_3$
L-Lysine	146	1.500	α-amino acid	$C_6H_{14}N_2O_2$

*^for the amounts added at standard state (25°C and one atmospheric pressure). *see section on dextran below*

3.1.2.1 Amounts of carbon to be added

Each carbon solution was required to have the same number of moles of carbon molecules to allow a fair comparison of the temperature response of their decomposition rates. The carbon molarity was based on the addition of glucose at 75 mM that was adapted from Degens *et al.* (2001). Since there are six C atoms in one mole of glucose (Table 3.1), the equivalent molarity for carbon is given by equation (3-3).

$$[Carbon] = 75\ mM \times 6$$

$$[Carbon] = 450\ mM \tag{3-3}$$

Therefore, each C compound solution had a carbon molarity equivalent of 450 mM C. To determine the C compound concentrations for the different compounds (Table 3.2), the following calculation was used:

$$[Compound] = \frac{450\ mM}{C_n} \tag{3-4}$$

Where C_n is the number of carbon molecules in one mole of the respective compound (e.g. there are 12 C molecules in one mole of maltose; Table 3.1).

Table 3.2 Concentration of carbon compound solutions added to soil to measure the temperature response of R_H. All additions are equivalent to 450 mM C

Carbon compound	Solution concentration (mM) of carbon compound
Glucose	75
Maltose	37.5
Dextran	0.026 *
Arginine	75
Glutamine	90
Lysine	75

** see calculation below*

As discussed above, yeast extract does not have a specific molecular weight or formula, and thus, equation (3-4) cannot be calculated. Therefore, it was assumed to have similar properties to glucose, and as a result, glucose and yeast were treated the same when making up their respective solution (i.e. the weight (g) of glucose added to distilled water was used when making up the yeast extract solution).

Dextran:

Dextran is a complex, branched polysaccharide constructed of many glucose molecules and is characterised as a glucan (Redasani & Bari, 2015). This glucan has chain lengths varying from 3 to 2000 kilodaltons (Redasani & Bari, 2015). Dextran's molecular formula is $H(C_6H_{10}O_5)_nOH$. The dextran used here ($dextran_a$) had a known molecular weight of ~480,000 g mol^{-1} but had an unknown chain length, so equation (3-4) could not be calculated. Therefore, an extra calculation (Eq. (3-5)) was needed to determine $dextran_a$'s chain length so that the number of C atoms was known. The amount of C atoms in $dextran_a$ was calculated by using the molecular weight (504 g mol^{-1}) of a known dextran chain length ($Dextran_b$) which had a molecular formula of $H(C_{18}H_{30}O_{15})OH$.

$$C_a = \left(\frac{MW\ of\ dextran_a}{MW\ of\ dextran_b}\right) C_b \qquad (3\text{-}5)$$

$$= \left(\frac{480{,}000\ g\ mol^{-1}}{504\ g\ mol^{-1}}\right) 18$$

$$= 17{,}142$$

Where C_a is the unknown amount of carbon atoms from $dextran_a$ and C_b is the known amount of carbon atoms from $dextran_b$. MW is the molecular weight of $dextran_a$ (unknown chain length) and $dextran_b$ (known chain length).

To ensure the accuracy of equation (3-5), it was also used to determine the amount of hydrogen and oxygen atoms in dextran$_a$, which was 30,476 and 15,238, respectively. Each proportion of atoms were then multiplied by their respective atomic weights to convert to units of g mol^{-1}, these were added together and equalled a total MW of 479,998 g mol^{-1}.

3.1.3 Temperature gradient block

The central piece of equipment for this research was the temperature gradient block (Figure 3.1). The temperature block was constructed of an aluminium body, which had dimensions of 168 cm × 13 cm × 23 cm. There were 18 evenly spaced holes along the top surface of the block referred to here as 'cells'. The cells have a volume of ~230 cm^3 which allows for up to three sealed Hungate tubes (see below) containing sieved soil samples to be incubated in each cell. To achieve a temperature gradient along the block, one end was cooled using an ice bath circulating antifreeze (Jubalo F10-UC/3), while the opposite end was warmed using a heating element (Shinko ECS series controller & GEWISS GW 44 217 junction box). Two to three hours after the ice bath and heater were switched on, the block stabilised at a temperature range of 8-10°C at cell 1 rising in increments of ~2.5°C to 50-52°C in cell 18. This range is partially dependent on the ambient temperature of the lab.

The temperature block was originally constructed to incubate larger intact soil cores. Therefore, the block had to be slightly altered to allow incubation of Hungate tubes containing soil. A layer of foam mat was fixed to the top of the block, and small cuts were made above each of the cells (Figure 3.2a). The small cuts in the foam allowed the gas-tight tubes (Hungate tubes; Figure 3.2b) to be inserted easily into each cell with minimal gaps to ensure stable temperatures. Since three Hungate tubes (15 ml) can fit in each cell at one time, this allows for up to 54 samples (up to three different treatments or replicates, i.e. 3 × 18) to be incubated during an experiment (Figure 3.2c). A large polystyrene/foam lid was fitted over the top of the inserted tubes to help maintain a stable temperature gradient and allow sampling of headspace gases (Figure 3.1).

Figure 3.1 Temperature gradient block with the polystyrene lid on, the ice bath, and the heating element

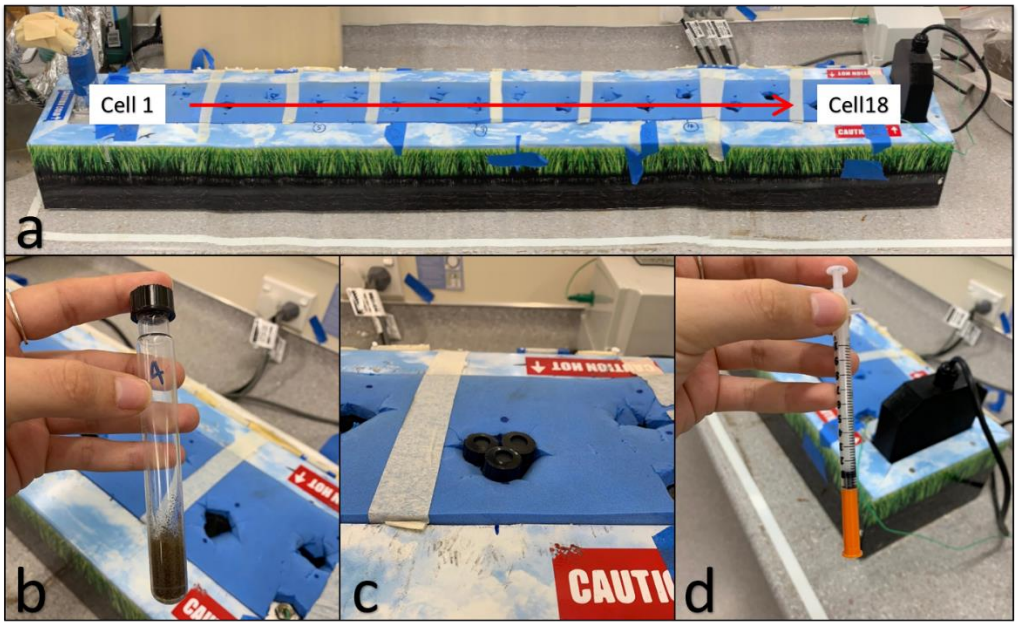

Figure 3.2 (a) Temperature gradient block without polystyrene lid on showing the placement of the cells. (b) 15 ml Hungate tubes with rubber septa (not shown) and screw cap lid. (c) A demonstration of the Hungate tubes during incubation. (d) 1 ml insulin syringe needles used to take gas samples from the Hungate tubes through the septum. After sampling, needles were inserted into a rubber stopper until injection into an IRGA for CO_2 determination

3.1.4 Measuring R_H from soil flooded with C substrates along a temperature gradient

The temperature block was left on overnight to reach stable incubation temperatures. Preliminary work suggested that the block reached stable temperatures after ~2.5 hours.

The next morning a C solution was made up to a concentration equivalent of 450 mM C by adding the appropriate amount of the respective C compound

powder (e.g. glucose) to distilled water. The C solution (0.25 ml) was then pipetted into the bottom of 18 Hungate tubes (15 ml). Soil (2 g ± 0.01) was weighed into these 18 Hungate tubes, which were then sealed with rubber septa and screw caps to ensure there was no gas leakage (Figure 3.2b).

For the control soil, distilled water (0.25 ml) was pipetted in the bottom of 18 15 ml Hungate tubes before the addition of soil (2 g ± 0.01) so that the SMC was the same for each treatment, as this is an important control on R_H (See section 2.3.1.2). As three tubes can fit into each of the cells (Figure 3.2c), two different C solutions could be added to 18 soil samples each, along with 18 control soil samples (e.g. 18 × soil with added glucose, 18 × soil with added maltose, and 18 × control soil). A preliminary experiment showed that the temperature was stable across the length of each cell so that all three soil samples in a cell would be incubating at the same temperature. Following methods from Degens *et al.* (2001), before incubation, each tube was vortex mixed (~3-5 seconds) to ensure the C solution/distilled water was thoroughly mixed through the soil. Since there were 54 tubes to incubate, the start times for each treatment's incubation periods were staggered to minimise potential errors associated with wait times for gas sampling/analysis. During the 5 hour incubation, four blanks were also incubated at room temperature by sealing empty Hungate tubes. The blanks were used to account for the background CO_2 concentration in the laboratory.

After 5 hours from when the first tube was inserted into its cell, insulin syringe needles (Becton-Dickinson and co) (Figure 3.2d) were used to take a 1 ml sample of gas from the headspace in the tube through the rubber cap (septa). If CO_2 production was higher than the highest standard (3% CO_2) then 0.5 ml gas samples were taken instead. Robinson *et al.* (2017) stated that these syringes were ideal due to the needle being welded onto the barrel, which eliminates gas leakage. Because the incubation periods were staggered (18 tubes at a time), it took <10 min to take the gas samples from all 18 tubes. This equated to a maximum bias of <3% of the 5 hour incubation time, so it was decided for this reason that it was not necessary to consider when calculating respiration (Eq. (3-6)). Immediately after each gas sample was taken, the needles were inserted into a rubber bung to reduce gas loss prior to CO_2 analysis using the infrared gas analyser (IRGA). Due to the proximity of the

temperature block to the IRGA, there was minimal travel time from the last syringe being inserted into the bung to the first gas sample being measured (<1 min). A standard curve was produced, typically using 3, 2, 1, 0.8, 0.6, 0.4, and 0.2, and 0.04% CO_2 standards. The highest and lowest standards were determined by the highest/lowest peak of the gas samples. Triplicates of the standard curve were produced, taking the average of the three peaks for the final calculation (3-6). The respiration rate, R_S (µg C g soil^{-1} hour^{-1}) was calculated using equation (3-6) for each gas sample.

$$R_S = \left[\left(\left(\frac{H_s/V_i}{H_{st}/V_i} \right) - \left(\frac{H_b/V_i}{H_{st}/V_i} \right) \right) \times S \times V \times 10^3 \right] \div (ODW \times t) \qquad (3\text{-}6)$$

Where H_s, H_{st}, and H_b are the peak area of the sample (mm^2), standard (1% CO_2, mm^2), and blank (mm^2), respectively. V_i is the injection volume (mL), S is the CO_2 concentration in the standard (1% = 0.01 µg CO_2 mL^{-1} gas) (Robinson, 2016), V is the headspace volume (mL), ODW is the oven-dried weight of the soil (g), and t is the incubation time (hr) (Robinson, 2016).

3.1.5 Data Analysis

The resulting respiration rates for each treatment were fitted using macromolecular rate theory (MMRT). For the majority of the data, the natural log of MMRT (Eq. (2-21)) was used to reduce the high leverage of the high respiration rates at the warmest temperatures (Robinson, 2016; Robinson *et al.*, submitted).

$$\ln(k) = \ln\left(\frac{k_B}{h}\right) - \frac{\Delta H^{\ddagger}_{T_0} + \Delta C^{\ddagger}_P (T - T_0)}{RT} + \frac{\Delta S^{\ddagger}_{T_0} + \Delta C^{\ddagger}_P (\ln T - \ln T_0)}{R} \qquad (3\text{-}7)$$

Where k_B is Boltzmann's constant, T is temperature (K), h is Planck's constant, $\Delta H^{\ddagger}_{T_0}$ (J mol^{-1}) and $\Delta S^{\ddagger}_{T_0}$ (J mol^{-1} K^{-1}) are the changes in enthalpy and entropy, respectively between the enzyme-substrate complex and the enzyme-transition state at a reference temperature (T_0), $\Delta C^{\ddagger}_P$ is the change in heat capacity between the enzyme-substrate complex and the enzyme bound to the

transition state, and R is the universal gas constant (Schipper *et al.*, 2014; Robinson, 2016)

The difference in respiration rates between soil with and without added C was described as the rates derived from the decomposition of the added C compounds. This difference may have also included priming, which is further explored in section 4.3.2. These calculated respiration rates were also fit with MMRT. Consequently, three temperature-respiration curves were produced: (1) total (derived from SOM + 'C compound' [with potential priming], measured from the treatment soil), (2) soil (derived from SOM measured from the control soil), and (3) 'C compound' (respiration calculated from the difference between rates (1) and (2)). Note, 'C compound' is interchangeable with any compounds listed in Table 3.1 (plus yeast extract).

Using MMRT parameters in equation (2-21), the temperature at which the reaction rate is maximal and the change in rate = 0 (referred to as the temperature optimum (T_{opt})) can be calculated from:

$$T_{opt} = \frac{\Delta H^{\ddagger}_{T_0} + \Delta C^{\ddagger}_{P} T_0}{-\Delta C^{\ddagger}_{P} - R} \tag{3-8}$$

The temperature inflection point (T_{inf}) was calculated using equation (3-9) which determines the temperature at which the change in rate is the greatest (Schipper *et al.*, 2014; Schipper *et al.*, 2019).

$$T_{inf} = \frac{\Delta H^{\ddagger}_{T_0} + \Delta C^{\ddagger}_{P.} T_0}{-\Delta C^{\ddagger}_{P} \pm \sqrt{-\Delta C^{\ddagger}_{P.} R}} \tag{3-9}$$

The absolute temperature sensitivity of the derived temperature-respiration curves was determined by calculating their first derivative (change in the rate of the respiration curve, $\frac{\partial R_s}{\partial T}$), while their relative temperature sensitivity was calculated using van't Hoffs Q_{10} coefficient (Schipper *et al.*, 2014; Robinson *et al.*, 2017).

3.2 Preliminary methodology checks

To ensure accurate and reliable data were produced, preliminary experiments were undertaken to provide confidence in the main methodologies outlined in this chapter. These experiments were designed to address three different objectives:

- Section 3.2.1 answers whether the C compound concentration of the added substrate to soil was sufficient to ensure the soil was not carbon-limited
- Section 3.2.2 determines whether CO_2 production from soil (treatment and control) was linear for the incubation length to determine whether microbial growth occurred during the incubation period
- Section 3.2.3 examines the linearity of the temperature gradient of the block

3.2.1 Determining substrate concentration was non-limiting

A preliminary experiment was designed to ensure the concentrations of the C compound solutions added to soil were sufficient so that by the end of the experiment, there was still a high concentration of substrate remaining. In addition to the 450 mM C concentration of glucose, five other concentrations of glucose (120, 210, 300, 600, and 780 mM C) were also added to soil and incubated to see how a wide range of concentrations performed over 5 hours. These soils were incubated at 18 different temperatures (8-52°C) in the temperature block over 5 hours. A relatively high percentage of the added C being respired during the incubation would mean that the respiration from the treatment soil (added C) may have become limited by the concentration of added substrate, particularly at high temperatures.

The minimum and maximum percentages of the added C that was respired from Horotiu soil with varying concentrations of added glucose is given in Table 3.3. The minimum represents the lowest percentage of C respired from the added glucose for each concentration, typically from soil incubated at low temperatures (~8°C). The maximum represents the highest percentage of C respired from the added glucose for each concentration, typically from soil incubated at high temperatures (~52°C).

The results (Table 3.3) provided confidence for using the 450 mM C concentration derived from Degens *et al.* (2001), as the maximum amount the added C (from glucose) respired during the 5 hour incubation was only 15%. This meant that it was unlikely that respiration of glucose was carbon limited for the subsequent experiments even at high temperatures.

Table 3.3 Basic statistics (min and max) of the percentage of C added that was respired from six C concentrations of glucose (120 -780 mM of C) over a 5-hour incubation at 18 different temperatures (8-51°C). The 450 mM C glucose concentration is underlined as this was the standard concentration used for all six carbon compounds. The minimum for each concentration is typically from cell 1, while the maximums are typically from cells 15-18.

	Percentage of added glucose respired:					
	120 mM (%)	210 mM (%)	300 mM (%)	<u>450 mM (%)</u>	600 mM (%)	780 mM (%)
Minimum	8	6	5	3	1	1
Maximum	56	39	25	15	12	9

It was essential to ensure the treatment soil was not C limited for two main reasons (1) to ensure the treatment soil was a fair representation of the labile C pool, where C availability is high, and (2) to determine an accurate temperature response from the individual C compounds. A highly cited paper (395 citations) by Fierer *et al.* (2005) incubated soils amended with carbon solutions at five discrete temperatures (10, 15, 20, 25, and 30°C) for up to 52 days. Fierer *et al.* (2005) set each carbon solution concentration (seven in total of different chemical complexities) to 120 mM C. This solution was added to 5 g dry weight equivalent of soil, which equated to a solution to soil ratio of 1:0.1. Considering the relatively low concentration of the carbon solutions added to soil (120 mM C) and the much longer incubation length (~250 times longer than the incubation length used here) it is likely that carbon was limited in the experiment conducted by Fierer *et al.* (2005) and likely became limiting more quickly at higher temperatures. For comparison, Table 3.3 depicts that a glucose solution with a concentration of 120 mM C had a minimum and maximum percentage of the added C consumed of 8% and 56%, respectively within 5 hours. Since the maximum value is from soil incubated at 46°C and the highest incubation temperature in Fierer *et al.* (2005) was 30°C, the percentage of added C (120 mM C) consumed was given for 30°C = 34%. It is important to note that the equivalent percentages for the added C consumed

for the experiment conducted by Fierer *et al.* (2005) would likely be substantially higher due to the longer incubation length. In this case, it could be suggested that the incubated treatment soils in Fierer *et al.* (2005)'s experiment were carbon limited, thereby producing temperature-respiration relationships that are not an accurate representation of a non-substrate limited reaction rate.

3.2.2 Determining CO_2 production was linear over 5-hours

Preliminary work conducted by Robinson *et al.* (2017) suggested that an incubation length of five hours was adequate to ensure enough CO_2 production at lower temperatures that could be easily detected using an IRGA. A potentially limiting factor of the methods described in this chapter is whether the soils were incubated long enough for microbial growth to occur. Microbial growth would mask the exact temperature response of the labile and stable C pools. Consequently, it was important to determine whether growth occurred during the five hour incubation as growth represents an adaption to temperature, and here the objective was to determine the temperature response of soil as collected.

An experiment was designed to investigate whether the CO_2 production was linear over six hours to demonstrate minimal microbial growth during the five hour incubation period. The experiment was undertaken twice, first with soil not amended with carbon compounds (control soil), and secondly with soil amended with glucose (450 mM C). Each experiment was conducted over two days, as six batches of 18 soil samples were incubated for 1, 2, 3, 4, 5, and 6 hours (i.e. day one – 2×18 soils were incubated for 1, 2, and 3 hours). The LINEST function in Excel was used to derive the statistics of respiration-incubation length relationship at each temperature. The closer the R^2 value was to 1, the more linear the CO_2 production was with respect to incubation length. Two example plots are shown in Figure 3.3, showing the worst (left) and best (right) linear fits of respiration at 18 different incubation temperatures for each hour over 6 hours.

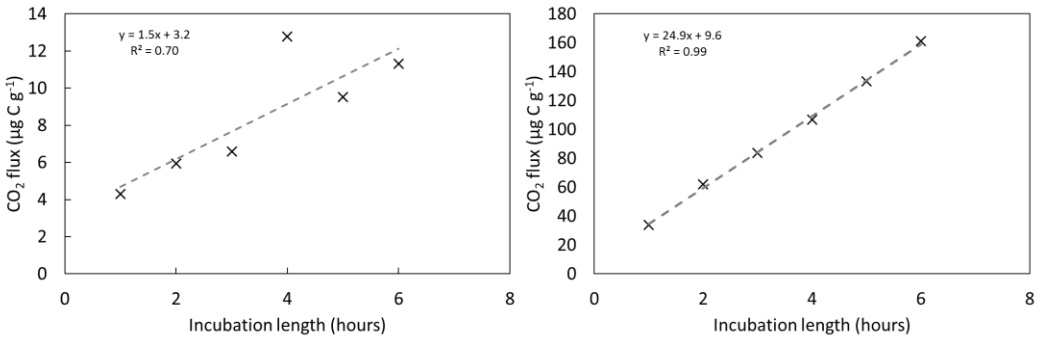

Figure 3.3 Example plots of respiration rates (CO_2 flux, µg C g^{-1}) for six different incubation lengths (1, 2, 3, 4, 5, and 6 hours) at two temperatures: (left) ~8°C for soil with no added C, and (right) ~25°C for soil with added C (glucose)

The vast majority of the R^2 values of CO_2 concentration with time were >0.90 (Figure 3.4). The consistent linearity suggested minimal microbial growth within the six hour incubation. Growth would have resulted in an upward curving line as microbes were able to accelerate decomposition. The lowest R^2 value was ~0.70 produced from the control soil in cell 1 (~8 °C) (Figure 3.3). However, the lowest R^2 value seems to be caused by an abnormally high R_S from the four hour incubation. Since the respiration rates from the five and six hour incubations do not follow this increase, it could be suggested that this point is an outlier and microbial growth had not occurred in this soil. The R^2 values for the remaining temperatures for soil with and without added glucose can be found in the appendices.

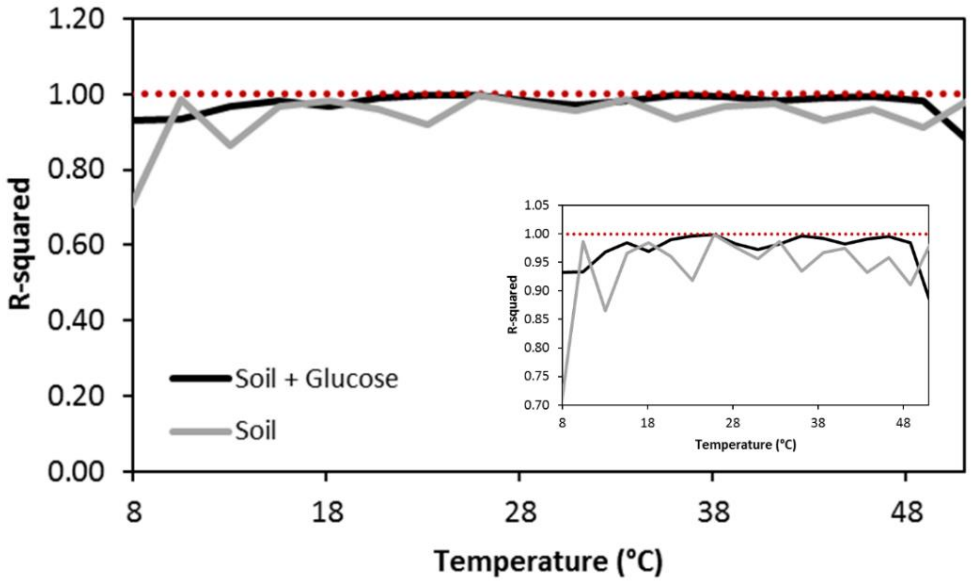

Figure 3.4 Plot of the R2 values of the CO2 concentration during six hour incubation for 18 different temperatures (8-51°C). Insert shows the same graph in greater detail with a rescaled y-axis

3.2.3 Determining the temperature block had a linear temperature gradient

The air temperature of the cells was measured using temperature data loggers (Maxim iButton® devices) (Figure 3.5). The loggers were set to record temperature every 1 minute in degrees Celsius. During incubations, four iButtons were spaced out across the range of cells and were placed at the bottom of their respective cell.

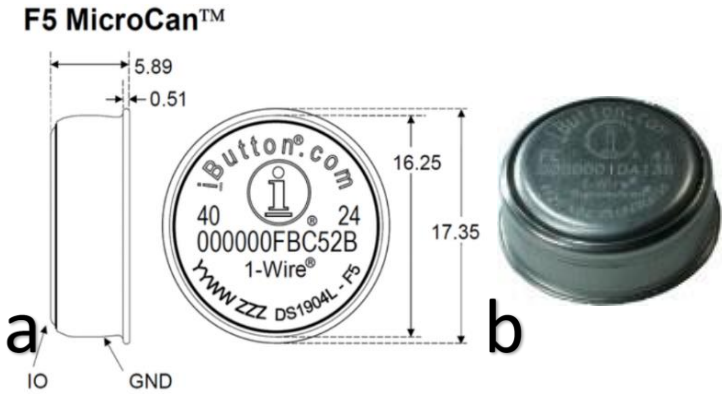

Figure 3.5 (a) Dimensions of the iButton, and (b) an image of a generic iButton (Hindman, 2006)

Preliminary work was undertaken to test how linear the temperature gradient of the block was during a typical experiment. The results presented (Figure 3.6) show that once all cells reach a stable temperature, the temperature gradient across the block was linear (R^2=0.99). Another preliminary study showed that these temperatures only varied ~1°C during the five hour incubation.

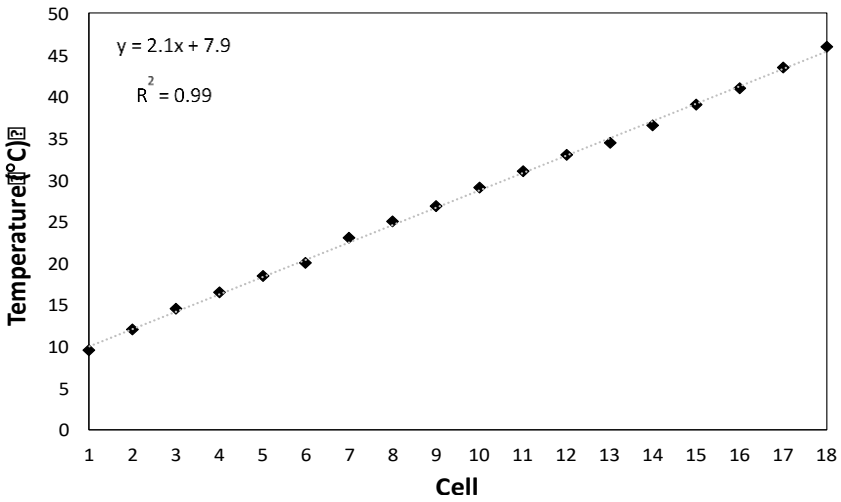

Figure 3.6 Stable temperatures of cells 1-18 fitted to a linear trendline

Chapter 4

Temperature Response of Soil Respiration from Labile and Stable C Pools

4.1 Abstract

Broadly, soil C can be compartmentalised into two distinct pools: labile C which comprises easy to degrade and highly available compounds, and stable C which comprises harder to degrade and physiochemically protected C (Von Lützow & Kögel-Knabner, 2009). Although the temperature response of these two pools has been explored, the literature presents contradicting conclusions with no consensus reached.

Here, I measured the temperature response from soil with added C (450 mM C) to represent the labile C pool, and soil without added C to represent the stable C pool (i.e. soil organic matter (SOM)). This theory was based on the theory that differences in the temperature response of these two pools is attributed to substrate availability. That is the labile C pool is highly available to microbes for decomposition, while the stable C pool is not. Both soils were incubated at 18 discrete temperatures (~8-52°C) and their CO_2 production was measured after five hours. A range of simple C compounds (glucose, glutamine, arginine, lysine, and maltose), yeast extract (wide variety of C compounds), and a more complex C compound (dextran) was added to soil. Respiration derived from the addition of these C compounds (and thus the labile C pool) was described as the difference between CO_2 produced from soil with and without added C. This difference would have also included respiration induced by priming. Additionally, I tested to see if these responses to temperature were dependant on soil properties by incubating three very different soil types (allophanic, gley, and organic) with and without added glucose. Data was fitted with macromolecular rate theory (MMRT) that allowed calculation of temperature optima (T_{opt}) and the temperature at which the rate of change was the greatest (T_{inf}).

Labile C was well represented by the decomposition of simple C compounds and yeast extract, which exhibited an average T_{opt} ~37°C and T_{inf} ~22°C. Dextran and SOM represented the stable C pool, which did not have a T_{opt} or T_{inf} point as their temperature-respiration curves increased exponentially (Arrhenius-like behaviour). Overall, the stable C pool had a higher relative temperature sensitivity and lower absolute temperature sensitivity than the labile C pool.

This study also found that the temperature response of these two pools did not vary with soil properties. The temperature parameters for glucose respiration rates (i.e. labile C pool) only differed by 3°C (i.e. T_{opt} = 35°C, T_{inf} = 18-21°C), and the decomposition of SOM (i.e. stable C pool) exhibited Arrhenius-like behaviour within the three soil types.

A preliminary experiment using isotopic analysis of soils incubated with and without ^{13}C labelled glucose suggested that the decomposition of the stable C pool induced by priming contributed a significant proportion (~30%) to the total soil respiration when exogenous C was added to soil. The temperature response of priming was also remarkedly similar to the labile C pool, indicating that the SOM decomposed through priming was highly available to microbes for decomposition.

Overall, this study concluded that the decomposition of the labile C pool was well described by MMRT, exhibiting both a T_{opt} and T_{inf} in all cases, which is typical of biological reactions. Whereas, the decomposition of the stable C pool exhibited an exponential, Arrhenius-like behaviour, typical for chemical reactions. As the temperature responses of these two pools did not significantly vary between the type of simple C compounds or soil properties, this study suggests that a two-pool soil C model may be adequate to describe soil C dynamics and, if reliable enough for a wider variety of C compounds/soil types could also be useful for larger ecosystem/global models.

4.2 Introduction

Understanding soil C dynamics is an important challenge for the development of reliable and accurate models of soil C cycling, scaling up to biogeochemical Earth system models (ESMs). Typically, the soil C cycle is modelled on C inputs through litter production (derived from photosynthesis) and C outputs through soil respiration (Luo *et al.*, 2016). Soil respiration is traditionally represented by first-order decay functions which are regulated by factors such as temperature, soil moisture, SOM quality and soil texture (Luo *et al.*, 2016). SOM is released back into the atmosphere as carbon dioxide (CO_2) via heterotrophic respiration (R_H) by microorganisms within the soil (Ryan & Law, 2005; Brown & Markewitz, 2018). Heterotrophic respiration releases both young labile C and older stable C (Ryan & Law, 2005) and is frequently also referred to as microbial respiration.

Not all organic C fractions in soil are decomposed at the same rate due to apparent chemical and physical protection, reducing their availability to the microbes. Therefore, SOM can be compartmentalised into different conceptual 'pools' which are based on their turnover times (Bol *et al.*, 2009). A multitude of conceptual soil C pool models have been proposed in the literature with multiple different C pools that have turnover times ranging on a scale of days to millennia (von Lützow *et al.*, 2008; Bol *et al.*, 2009). Broadly, these can be distinguished into a minimum of two C pools, (1) labile pool (readily available and easily degradable (soluble) C), and (2) stable pool (physiochemically protected and harder to degrade C) (Von Lützow & Kögel-Knabner, 2009; Robinson *et al.*, submitted). Carbon substrates of the labile C pool are defined as 'high quality' due to their low activation energies for degradation, simple molecular structure and not being bound to soil surfaces. The substrates of the stable C pool are defined as 'low quality' due to their high activation energies for degradation, complex molecular structure or physical protection by soil particles or aggregation, and that these molecules are typically bound to soil surfaces (Sierra, 2012). Although there is a general consensus on the existence of the labile and stable C pools in soil, their respective sensitivity to temperature is still a highly debated topic with multiple studies presenting contrasting results (Von Lützow & Kögel-Knabner, 2009). It is important to

determine how sensitive these pools are to changes in temperature, as this can play a significant role in controlling the magnitude of CO_2 returned to the atmosphere. Over 75% of the stored C in soil is characterised by the stable C pool (Sanchez *et al.*, 1989), consequently, if this pool is more sensitive to changes in temperature, then significant, sustained C losses are probable if temperatures increase as a consequence of climate change (Von Lützow & Kögel-Knabner, 2009).

One approach for assessing the temperature response of different C pools is to add simple C sources to soil and determine their degradation by subtracting respiration rates measured from soil where C was not added. For example, Fierer *et al.* (2005) added C compounds of varying chemical complexities to soil and measured their temperature response. This study found that the *relative* temperature sensitivity (i.e. Q_{10}, ratio of rates) of C decomposition increased with the complexity of the C compound (Fierer *et al.*, 2005). Recently, Robinson *et al.* (submitted) conducted a similar experiment by adding ^{13}C labelled plant litter to soil and partitioning the temperature response of litter and SOM. Robinson *et al.* (submitted) found that the decomposition of labile litter was overall less temperature sensitive in relative terms than the more recalcitrant SOM.

Despite these approaches (Fierer *et al.*, 2005; Robinson *et al.*, submitted) to separate the temperature response of multiple pools in soil, these studies have not fully acknowledged the potential soil priming effect following the addition of labile C. Guenet *et al.* (2018) describe the priming effect as "a modification of soil organic carbon decomposition by the addition of fresh organic carbon", that is the decomposition rate of native SOM is altered. This modification could be either negative (decreases decomposition rate already present) or positive (increases decomposition rate already present). A meta-analysis conducted by Sun *et al.* (2019) studied the effect of exogenous C inputs into soil on SOM decomposition. The authors reviewed over 90 incubation studies and found that SOM decomposition was accelerated in nearly 70 % of the studies by an average of 47.5% (Sun *et al.*, 2019). There are two potential mechanisms for the observed positive priming effect from these studies: (1) *co-metabolism:* which increases the decomposition rate of native SOM through increased

microbial activity when labile C is added to the soil, and (2) *stimulation*: carbon additions may improve soil conditions (pH, nutrients. oxygen, and moisture content) for microbes thereby increasing microbial activity (DeCiucies *et al.*, 2018). Negative priming is the inhibition of microbial activity in soil; however, the mechanisms behind this remain unclear (DeCiucies *et al.*, 2018).

Understanding positive priming mechanisms will be important as atmospheric CO_2 levels rise. Generally, increases in atmospheric CO_2 levels increase leaf/root production and root exudation, thus increasing inputs of simple C compounds into the soil (Sulman *et al.*, 2014). Currently, priming and its response to increasing temperatures are not represented in global Earth system models (ESMs) associated with soil C dynamics. As a result, the omission of priming mechanisms could result in an overestimation of the terrestrial C sink in current ESMs (Guenet *et al.*, 2018).

A further challenge in interpreting the temperature response of soil respiration is the way that the temperature sensitivity is reported in the literature. Two explicit terms are used to describe the temperature sensitivity of a reaction rate; the absolute and relative temperature sensitivities (Sierra, 2012). The *absolute* temperature sensitivity represents the absolute change in rate (decomposition rate k or respiration rate R_S) for a given unit change in temperature (Sierra, 2012). In absolute terms, the temperature sensitivity is given by the partial derivative of the rate (X) with respect to temperature: $\frac{\partial X}{\partial T}$ (Sierra, 2012). Essentially, the absolute temperature sensitivity of k or R_S is the 1st partial derivative (hereafter referred to as the 1st derivative of a flux rate). In contrast, the *relative* temperature sensitivity represents the absolute temperature sensitivity relative to the actual value of the rate (Sierra, 2012). The relative temperature sensitivity is given by dividing the absolute temperature sensitivity by k: $\frac{1}{k}\frac{\partial k}{\partial T}$ (see section 2.4.2) (Sierra, 2012). A common measure of the relative temperature sensitivity is Q_{10}, which is defined as the factor by which a response variable (k or R_S) changes for a 10°C shift in temperature (Conant *et al.*, 2011). Q_{10} remains the most frequently used metric of temperature sensitivity but has some important disadvantages. Different studies use different equations to calculate Q_{10}, which makes it

difficult to compare values between studies. Also, unlike the absolute temperature sensitivity, which takes into account the size of the decomposing C pool, Q_{10} does not depict what C pool respires more CO_2 to the atmosphere (Sierra, 2012).

Based on its mathematical expression, the absolute temperature sensitivity (1st derivative) predicts the labile C pool (higher quality substrates with lower activation energies) to be more sensitive to changes in temperature compared to the stable C pool (Sierra, 2012). On the other hand, the relative temperature sensitivity (Q_{10}) predicts the stable C pool (lower quality substrates with higher activation energies) to be more sensitive to changes in temperature compared to the labile C pool (Sierra, 2012). The latter mathematical prediction is currently the most supported conclusion in soil experiments that have measured Q_{10} of labile and stable C pools (Von Lützow & Kögel-Knabner, 2009). However, when describing their results, authors frequently do not differentiate between absolute and relative temperature sensitivities which can confuse the literature, as these two measures, inherently, produce contrasting results.

The objective of this thesis was to determine the temperature sensitivity of the decomposition of labile and stable C pools in soil by measuring respiration rates from these pools. This was undertaken by incubating five different simple carbon compounds (i.e. arginine, glucose, glutamine, lysine, and maltose) added to soil across a temperature gradient at 18 discrete temperatures (~8-51°C). A complex carbon compound (dextran) and yeast extract (a wide range of different C compounds frequently used to support microbial growth) were separately incubated to observe the temperature response of different varieties of C compounds. A single C compound (glucose) was also added to three contrasting soils to determine whether the temperature response of labile and stable C pools varied with soil properties. Soils (with and without added C) were incubated for five hours, and CO_2 was measured.

The difference between CO_2 produced from soil and soil + C substrate was calculated to determine CO_2 produced from the added substrate (and potential priming). Macromolecular rate theory was used to describe its temperature

response (Robinson *et al.*, 2017). Due to its perceived importance outlined above, a preliminary one-off experiment was also undertaken to determine the magnitude of the potential priming effect and measure its response to changes in temperature. Separating the priming effect required use of ^{13}C substrates and rapid measurement of emitted ^{13}C content of CO_2. These methods (Robinson *et al.*, submitted) have been under development and only became somewhat routine by the end of this thesis.

4.3 Methods

The main objective was to separate the temperature response of labile and stable C pools in soil, and therefore be able to calculate their respective temperature sensitivities. However, as previously mentioned, most incubation studies measuring R_S from soil amended with C compounds do not account for the potential priming effect. This means that the observed increase in respiration from the treatment soil is likely generated by the utilisation of the added C compound in addition to possible priming. Priming is likely due to increasing C availability of the stable pool (DeCiucies *et al.*, 2018) and so would represent an additional labile C pool for respiration. Two broad experiments are described here: section 4.3.1 describes the general methods used to measure the respiration rates from soil amended with C substrates, and section 4.3.2 will describe the methods for a preliminary experiment to separate microbial respiration of added substrate from priming. More detailed methodologies are provided in Chapter 3.

4.3.1 Separating the R_H from the labile and stable C pools in soil

There were two key questions to be addressed here: (1) was the temperature response of a simple carbon compound (in this case glucose) the same for different soil types and (2) did different highly available carbon compounds have the same temperature response in a single soil. Briefly, the temperature response of an added C compound was described as the difference in R_S measured from soil incubated with and without the added C substrate for five hours at 18 different temperatures.

To address question (1), glucose was added to three soil types, and three replicates were run for each soil. To address question (2), five simple carbon compounds (arginine, glucose, glutamine, lysine, and maltose; see Table 4.1 from the previous chapter for properties) were added to soil (Horotiu silt loam) and individually incubated for five hours at 18 different temperatures before respiration rates were measured. These five simple carbon compounds were chosen as they were all water-soluble (important characteristic of the labile C pool) and they commonly existed in soil already. A complex, long-chained glucan (dextran) and yeast extract were also incubated with soil to determine how the temperature response changed with a wider variety of carbon compounds. Each carbon compound was made as a solution in distilled water at a concentration equivalent to 450 mM C. Preliminary work showed that less than 15% of the added substrate was consumed during the incubation so that carbon supply was non-limiting (see section 3.2.1 in the previous chapter).

Soil collection

Soil was collected from a DairyNZ research farm in the Waikato region, New Zealand using a bucket sampler from the top 10 cm. Three soil types were collected: a poorly-drained gley Te Kowhai soil (Typic Ochraqualf, 5.2% C, 0.51% N, and soil pH of 6.2), a moderately well-drained organic Te Rapa soil (Typic Epiaquoll, 16.5% C, 0.97% N, and soil pH of 5.8), and a well-drained allophanic Horotiu soil (Typic Udivitrand, 6.2% C, 0.6% N, soil pH of 6.0, and $\delta^{13}C_{VPDB}$: -26.5 ‰). The majority of these soil properties were measured by Robinson *et al.* (2017). These soils were passed through a 2 mm sieve, wetted up to 60 % maximum water holding capacity (MWHC) (section 3.1.1.2 from the previous chapter; (Harding & Ross, 1964), and stored at room temperature in a plastic zip-lock bag plugged with cotton wool to allow for gas exchange.

Determining the temperature response of simple carbon compounds

A temperature gradient block (Figure 4.1) was used for the rapid measurement of soil respiration, with methods developed by Robinson *et al.* (2017). The block (168 cm × 13 cm × 23 cm) was constructed from aluminium and was insulated using a foam mat and polystyrene to ensure temperatures were stable during incubations. To achieve a linear temperature gradient, one end was cooled to ~8°C using an ice bath which circulates coolant into the

block, and the opposite end was heated to ~52°C using a heating element. There were 18 discrete temperatures across the block, depicted by the holes in Figure 4.2a (hereafter referred to as 'cells'). A preliminary experiment showed that temperatures stabilised after ~2.5 hours of the cooler and heater being switched on, so for general operation, the temperature block was switched on overnight before starting each experiment. Temperatures were measured using iButtons (Maxim iButton® devices) and were shown to have a highly linear and predictable trend along the length of the block.

Due to the volume of the cells (~230 cm^3) up to 54 soil samples could be incubated in Hungate tubes (15 ml; Figure 4.2b) at a time (three tubes in each cell; Figure 4.2c) along the length of the block. This means that typically, three treatments (or replicates) were incubated in one day's operation. C solutions (0.25 ml) were pipetted into 18 Hungate tubes (15 ml) for the two treatment soils, and distilled water (0.25 ml) was pipetted into another 18 tubes for the control soil to ensure the SMC was homogeneous across the treatments.

Soil (2 g $\pm$ 0.01) was then weighed into all 54 Hungate tubes, and the tubes were then sealed with a rubber septum and plastic screw cap, then vortex mixed (3-5 seconds) following methods described by Degens *et al.* (2001). The soil samples were incubated for five hours in the temperature block before 1 ml gas samples were taken using insulin syringe needles (Figure 4.2d) and injected into an infrared gas analyser (IRGA). Preliminary experiments demonstrated that CO_2 production was linear during the five hour incubation which indicated that microbial growth had not occurred during the incubation period (section 3.2.2 of the previous chapter).

Figure 4.1 Temperature gradient block with the ice bath cooling the left side to ~8°C and the heating element warming the right side to ~52°C

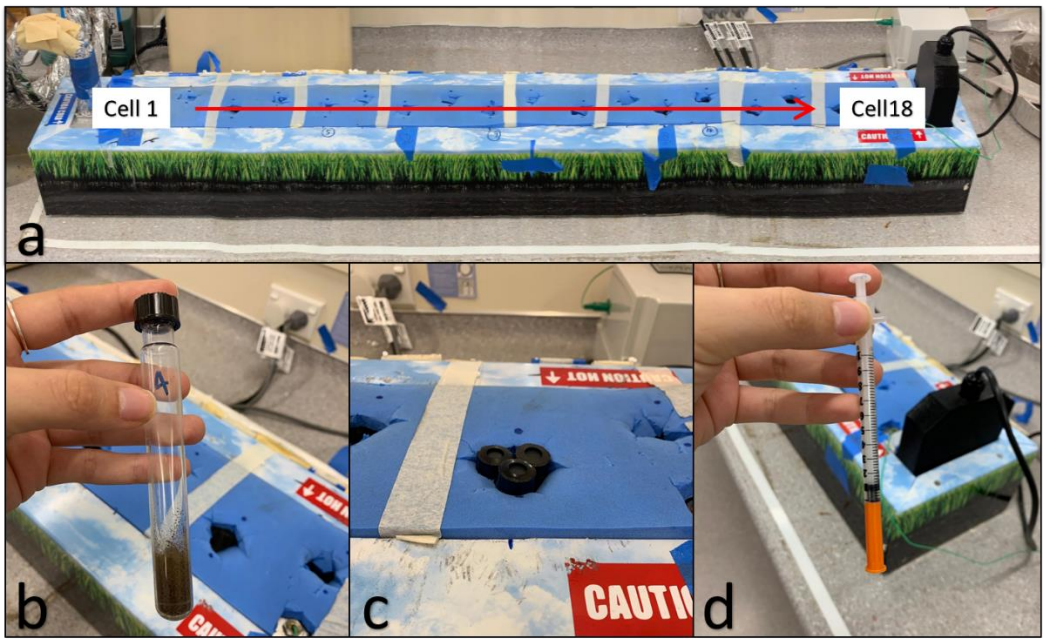

Figure 4.2 (a) The temperature block with the polystyrene lid open showing the 18 cells, (b) the 15 ml gas-tight tubes (Hungate tubes) which soils were incubated in, (c) demonstration of the Hungate tubes during incubation, and (d) the insulin syringe needles used to collect gas samples

The IRGA measured the amount of CO_2 produced during the five hour incubation period presented as a peak area for each sample. Substituting the peak area in equation (4-1), the respiration rate (μg C g^{-1} hr^{-1}) was calculated. A standard curve was also produced typically using up to 3% CO_2 for the highest standard and 0.04% CO_2 for the lowest. Occasionally the peak area of

the samples was higher than the 3% CO_2 standard; in this case, a 0.5 ml gas sample was taken and accounted for in equation 1.

$$R_S = \left[\left(\left(\frac{H_s/V_i}{H_{st}/V_i} \right) - \left(\frac{H_b/V_i}{H_{st}/V_i} \right) \right) \times S \times V \times 10^3 \right] \div (ODW \times t) \qquad (4\text{-}1)$$

Where R_S is respiration rate, H_s, H_{st}, and H_b are the peak area of the sample (mm^2), standard (1% CO_2, mm^2), and blank (mm^2), respectively. V_i is the injection volume (mL), S is the CO_2 concentration in the standard (1% = 0.01 µg CO_2 mL^{-1} gas), V is the headspace volume (mL), ODW is the oven-dry weight of the soil (g), and t is the incubation time (hr) (Robinson *et al.*, 2017).

The respiration rates for each treatment were fit to the natural log (In) of the macromolecular rate theory (MMRT):

$$\ln(R_S) = \ln\left(\frac{k_B}{h}\right) - \frac{\Delta H^{\ddagger}_{T_0} + \Delta C^{\ddagger}_P (T - T_0)}{RT}$$

$$+ \frac{\Delta S^{\ddagger}_{T_0} + \Delta C^{\ddagger}_P (\ln T - \ln T_0)}{R} \qquad (4\text{-}2)$$

Where k_B is Boltzmann's constant, T is temperature (K), h is Planck's constant, $\Delta H^{\ddagger}_{T_0}$ (J mol^{-1}) and $\Delta S^{\ddagger}_{T_0}$ (J mol^{-1} K^{-1}) are the changes in enthalpy and entropy, respectively between the enzyme-substrate complex and the enzyme bound to the transition state at a reference temperature T_0, $\Delta C^{\ddagger}_P$ (J mol^{-1} K^{-1}) is the change in heat capacity between the enzyme-substrate complex and the enzyme bound to the transition state, and R is the universal gas constant (Robinson *et al.*, 2017).

Three temperature-respiration rates curves were produced for each experimental run: (1) total-R_S (CO_2 derived from SOM, the added C compound, and potential priming measured directly from the treatment soil), (2) CO_2 derived from the bulk SOM (control soil with no added carbon), and (3) CO_2 derived from the added C compound (+priming) calculated from the difference between rates (1) and (2).

Using derived MMRT parameters, the temperature at which the reaction rate was maximal and the change in rate = 0 (temperature optima; T_{opt}), and the temperature at which the change in rate is the greatest (temperature inflection point; T_{inf}) was calculated (Robinson *et al.*, 2017; Schipper *et al.*, 2019) (Figure 4.3):

$$T_{opt} = \frac{\Delta H^{\ddagger}_{T_0} + \Delta C^{\ddagger}_{P} T_0}{-\Delta C^{\ddagger}_{P} - R} \tag{4-3}$$

$$T_{inf} = \frac{\Delta H^{\ddagger}_{T_0} + \Delta C^{\ddagger}_{P.} T_0}{-\Delta C^{\ddagger}_{P} \pm \sqrt{-\Delta C^{\ddagger}_{P.} R}} \tag{4-4}$$

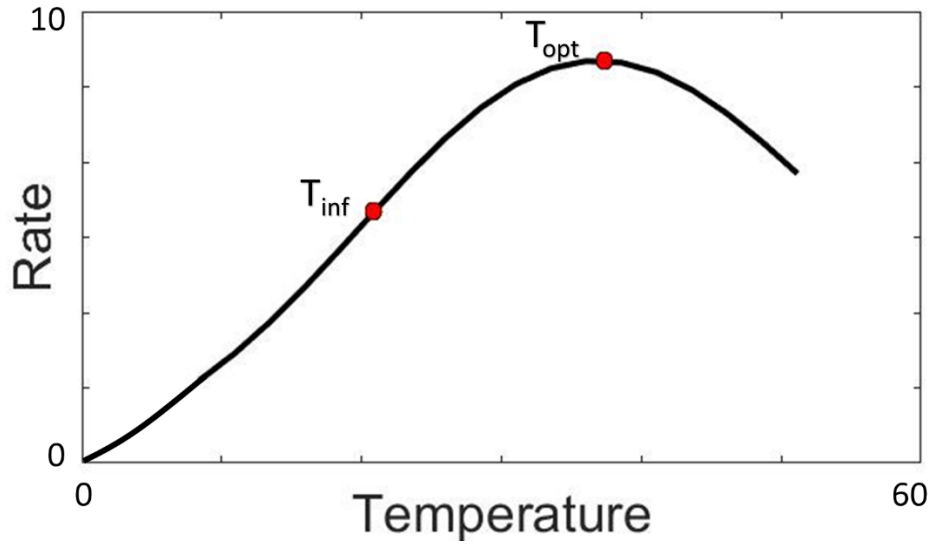

Figure 4.3 Example positions of T_{inf} and T_{opt} on an MMRT temperature-rate curve

The absolute temperature sensitivities for the three temperature-respiration curves were calculated using the 1st derivative of R_S with respect to temperature $\left(\frac{\partial R_S}{\partial T}\right)$. Their relative temperature sensitivities were calculated using Q_{10}, the factor by which the respiration rate changes as a consequence of a 10°C shift in temperature (Sierra, 2012). All calculations were conducted using Matlab (R2017b). Both temperature sensitivity measures was calculated at 10, 20, and 30°C.

In total, 60 independent temperature curves were determined through individual measurements of respiration rates at 18 discrete temperatures (i.e. experiment one: 3 $soils \times 2\ treatments$ $(with\ or\ without\ added\ glucose) \times 3\ replications = 18$, and experiment two: 7 $C\ compounds \times 2\ treatments \times 3\ replications = 42$)

Pair-wise comparisons between temperature response parameters T_{opt}, T_{inf}, $\Delta C_P^{\ddagger}$ were undertaken between glucose-R_S from the three soil types and C compound-R_S from the added C compound types (except dextran). Tukey's Honest Significant Difference (HSD) test was used to perform the pair-wise comparisons, which depicted where significant differences between the soil type and C compound groups originated. The critical value used for alpha was 0.05. These statistical differences are reported in the following chapter as different letters in the respective tables. Figures produced from Tukey's HSD test showing these differences can be found in the appendices.

4.3.2 Determining the magnitude and temperature response of soil priming

This section describes the methods used to separate the temperature response of CO_2 respiration directly from ^{13}C labelled glucose, the bulk SOM, and priming effect following the addition of glucose.

Section 4.3.2.1 discusses the pre-incubation methods and introduces a second temperature block that was used for this experiment. Section 4.3.2.2 covers the measurement of total respiration using a modified Los Gatos Research CO_2 Isotope analyser and an IRGA. Lastly, section 4.3.2.3 describes the data and statistical analysis. The methods outlined here were adapted from Robinson *et al.* (submitted) who developed a new laboratory approach for separating the temperature response of respiration from two C sources within one soil using isotopic techniques.

4.3.2.1 Pre-incubation and temperature block

A ^{13}C labelled glucose solution was made up to a concentration of 20.43 $\delta^{13}C$ using D-Glucose U-13C6, 99% powder (Cambridge Isotope Laboratories, CLM-

1396-PK) (0.01 g) and 27 g of D-Glucose powder (natural abundance) into a 2 L volumetric flask of distilled water.

The ^{13}C labelled glucose solution (0.75 ml) was pipetted into 40 different Hungate tubes (28 ml) before 3 g ± 0.01 of soil (Horotiu silt loam) was weighed in. 40 control soils without added glucose were also incubated to determine respiration from SOM. To prepare the control soil, distilled water (0.75 ml) was pipetted into another 40 Hungate tubes (28 ml) before adding 3 g ± 0.01 of soil (Horotiu silt loam) to each tube to ensure a homogeneous soil moisture content (SMC) for both soils. Each soil was wetted up to a SMC that, with the addition of the 0.75 ml ^{13}C-glucose solution/distilled, resulted in a final MWHC of 60%.

All 80 tubes were then sealed with a rubber septum, and aluminium caps were crimped on to ensure no gas leakage during incubation. Before incubation, each tube was mixed using a vortex mixer (3-5 seconds) to ensure the ^{13}C solution/distilled water was thoroughly mixed through the soil.

Sealed tubes were incubated at 40 discrete temperatures on an aluminium temperature gradient block, similar to the block used in section 4.3.1 (fully described in Robinson (2016). This block has a broader linear temperature gradient ranging from ~2-50°C increasing in ~1.2°C increments. Similar to the other block, one end is cooled by cycling anti-freeze through a water bath, while the opposite end is heated using a heating element. The block can incubate up to 132 Hungate tubes (28 ml) with three rows of 44 cells (Figure 4.4). The greater number of discrete temperatures than the previously described block was necessary to obtain accurate curve fitting. The block's dimensions were 140 cm × 13 cm × 19 cm and each cell had a diameter of 22 mm and a depth adequate to fit the 28 ml Hungate tubes. A clear Perspex lid covered the top of the block to maintain a stable temperature gradient during incubation. The temperature of seven cells spaced along the block were continuously (1 min intervals) measured for each incubation using thermistors coupled to a data logger. These temperature measurements demonstrated a stable and linear temperature gradient along the block (Robinson *et al.*, 2017).

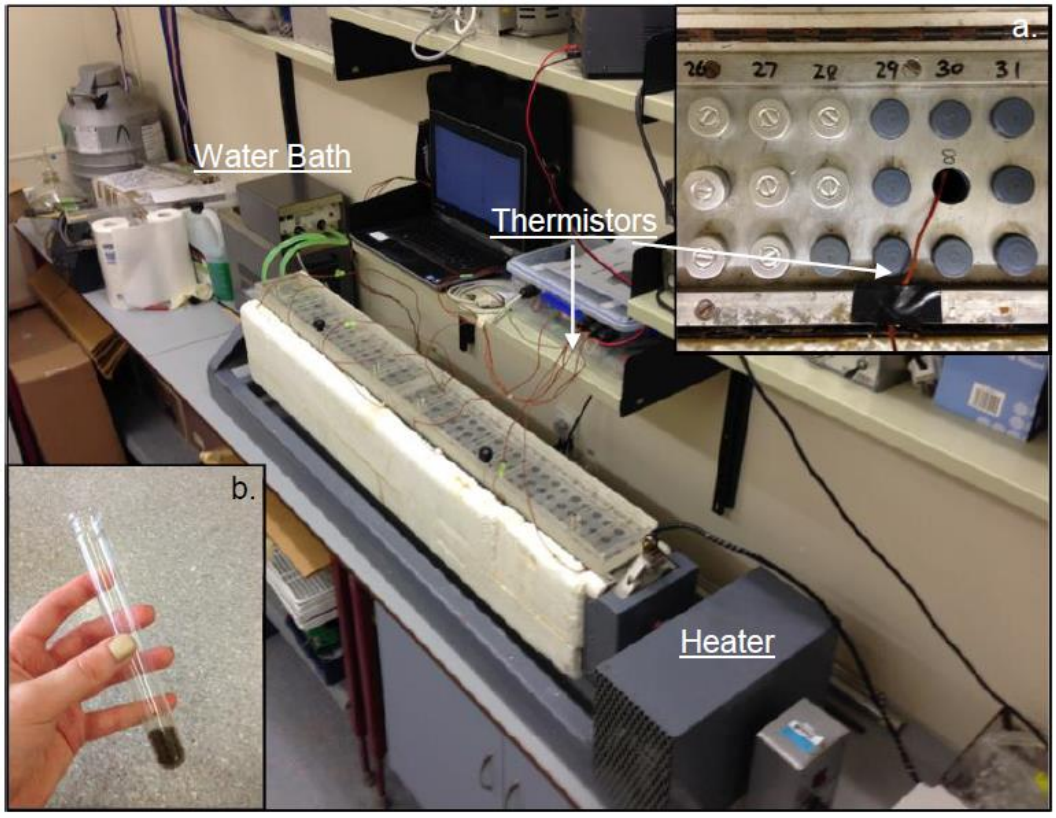

Figure 4.4 Temperature block used for section 4.3.2 with the water bath cooling one end and the heater warming the opposite end. (a) Top view of the block and the cells where the Hungate tubes are incubated in, and (b) Larger Hungate tubes (28 ml) used in this experiment (Robinson, 2016)

4.3.2.2 Total respiration measurement

Two treatments (soil with and without added ^{13}C glucose) were incubated in the temperature block at 40 different temperatures for five hours. Immediately following the five hour incubation (from when the first tube was inserted), the tubes were placed in ice to minimise any additional CO_2 production before analysis and then stored overnight at -20°C.

The next morning the Hungate tubes from the first treatment were taken from the freezer and placed in ice again. The CO_2 concentration was measured using insulin syringe needles and the IRGA as previously described (Section 3.1.4 from Chapter 3). The Hungate tubes were then transported to a modified off-axis integrated cavity output spectroscopy (OA-ICOS) which was used to determine the CO_2 derived from the ^{13}C-glucose by measuring $\delta^{13}C$. An inlet and outlet needle were inserted through the septum into the headspace of the Hungate tubes of the first treatment (one at a time). The inlet needle carried scrubbed nitrogen gas (N_2 carrier gas) and pumped it through the Hungate tube to mix with the CO_2 produced during incubation. The headspace gas was

then drawn into the outlet needle into the measurement cavity using the applied vacuum from the OA-ICOS instrument. The CO_2 concentration and isotopic analysis of each of the 40 tubes were continuously analysed and recorded as the sample gas passed through the analysis cavity. After treatment one was analysed, Hungate tubes from treatment two (i.e. control soil) were removed from the freezer and placed on ice. Respiration from treatment two was then analysed using both the IRGA and the OA-ICOS instrument as described above.

Five $\delta^{13}C$ standards (triplicates measured before, between, and after the samples) were also measured as well as blanks to ensure the accuracy of the $\delta^{13}C$ measurements. The standards used were: BDH (-24.95‰), WCS (-10.95‰), GNS (-2.04‰) and two bicarbonate solutions standardised using international reference carbonate standards (17‰ and 68‰). These standards cover a range of known "Vienna Pee Dee Belemnite" (VPDB) values and include both international standards (BDH and WCS) and internal carbonate standards (17 and 68 ‰). Drift correction was also determined every ten samples to ensure the accuracy of the measurements (Robinson *et al.*, submitted).

This experiment was repeated on three different days to determine variability. One limitation to this method was that the CO_2 produced from soil with added glucose for one replicate exceeded the upper limit of the OA-ICOS instrument at higher temperatures. Therefore, $\delta^{13}C$ was not measured at these temperatures.

4.3.2.3 Data and statistical analysis

In total, four temperature-respiration curves were produced for each replicate, and their relationship to each other is described in Figure 4.5:

1. Total-R_S which was directly measured from the treatment soil - soil with added ^{13}C labelled glucose. CO_2 measured from the treatment soil was derived from the bulk SOM, the added glucose, and the potential priming effect

2. Glucose-R_S was directly measured from the $\delta^{13}C$ values of CO_2 respired from the ^{13}C labelled glucose that was added to soil. A mixing model (Eq. (4-5)) separated the R_S derived from glucose

3. SOM-R_S was directly measured from the control soil – soil with no added glucose. R_S from SOM was also measured from the treatment soil and was separated using a mixing model (Eq. (4-5)). However, the R_S from SOM from the control soil (SOM_c) was used as it was hypothesised that the R_S from SOM from the treatment soil (SOM_t) also included priming

4. Priming-R_S was indirectly measured as the difference between the CO_2 fluxes from the SOM_t and SOM_c

A 2-pool mixing model (Eq. (4-5)) was used to separate SOM_t-R_S, and glucose-R_S from total-R_S.

$$f = \frac{(C_S - C_R)}{(C_G - C_R)} \quad (4\text{-}5)$$

Where C_S is the $\delta^{13}C_{VPDB}$ value of the soil, C_R is the $\delta^{13}C_{VPDB}$ value of the respired CO_2, and C_G is the $\delta^{13}C_{VPDB}$ value of the added glucose(Robinson *et al.*, submitted).

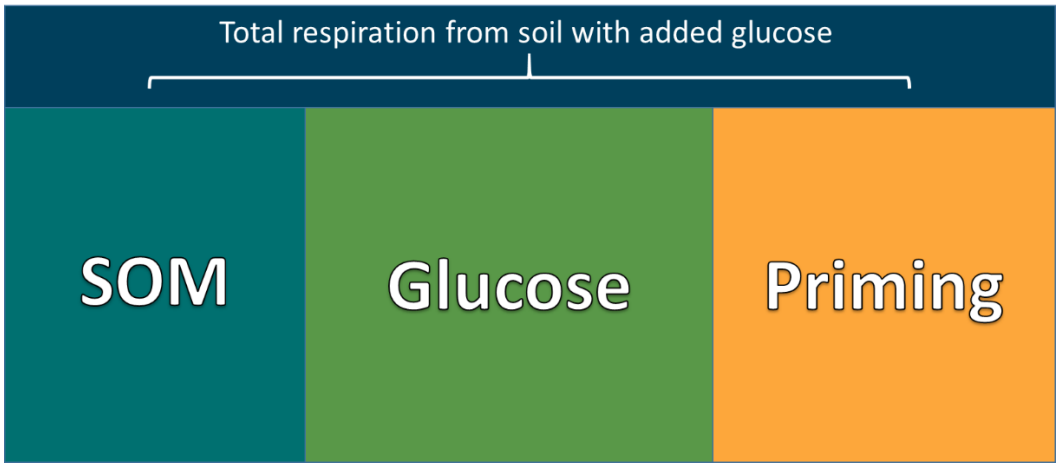

Figure 4.5 Simple schematic of the relationship between Total-R_S, SOM-R_S, Glucose-R_S, and Priming-R_S (see text above for descriptions)

Each of these four temperature responses were fitted with MMRT (equation (4-2)), and T_{opt} and T_{inf} were calculated using equations (4-3) and (4-4), respectively. Their absolute and relative temperature sensitivities were calculated at 10, 20, and 30°C using 1st derivatives and Q_{10}, respectively.

The temperature response parameters reported in the following chapter are given as the average of three replicates. These parameters for the replicates can be found in the appendices.

4.4 Results

Overall, respiration rates from soil with and without added carbon compounds generally increased with increasing temperatures. Respiration rates from soil with added simple carbon compounds increased with temperature up to a temperature optimum. Above the T_{opt}, respiration rates declined. After a typical experiment incubating soil with and without added carbon substrate, three sources of respiration were measured/calculated:

1. Total-R_S was directly measured from soil with added carbon compounds (treatment soil). The components of total-R_S include CO_2 derived from the added C compound (and any induced priming) and SOM

2. SOM-R_S directly measured from the control soil that was incubated simultaneously with the treatment soil, but with no added C

3. 'C compound'-R_S indirectly measured from the difference between CO_2 derived from the total R_S and the SOM-R_S (measured from the control soil). These respiration sources are referred to here as their C compound name (i.e. respiration from glucose). It is important to note that this respiration source may also include respiration from priming

A typical example of temperature-respiration curves (T-R_S) is shown in Figure 4.6 from a single experimental run, where the 'total-R_S' (a), 'SOM-R_S' (b), and 'C compound-R_S' (in this case glucose) (c) are presented.

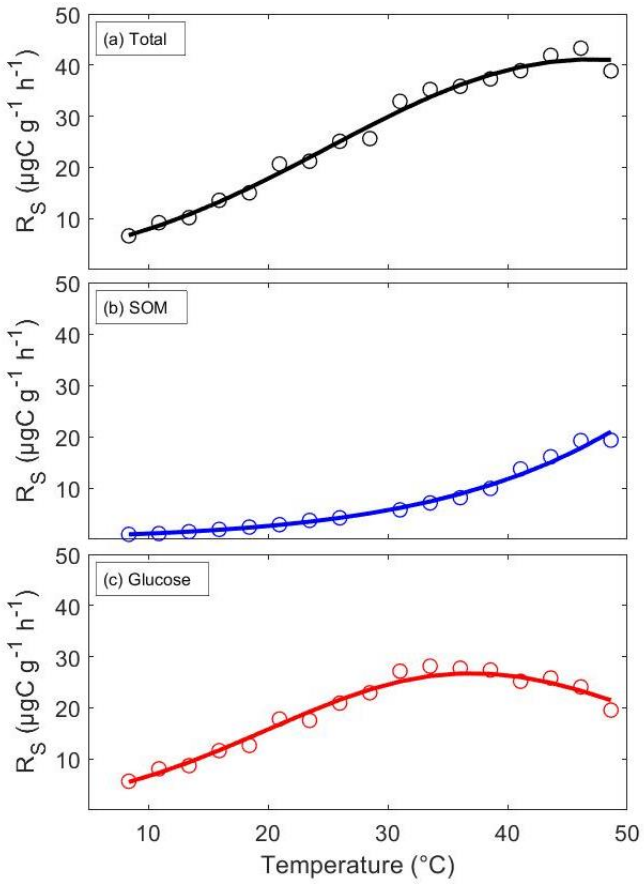

Figure 4.6 Typical example of temperature-respiration rates curves from one replicate of Horotiu soil with (a) added glucose (450 mM C) and (b) no added C incubated for 5 hours at 18 different temperatures (8-52°C). c) Is the difference between 'a' and 'b' representing respiration from added glucose and induced priming

When fitted to MMRT, the parameters T_{opt} and T_{inf} were derived for total-R_S and glucose-R_S, as their change in heat capacities were large negative values indicative of their curvature illustrated in Figure 4.6a and c. When fitted with MMRT, the temperature response of SOM-R_S collapsed to the Arrhenius equation as its change in heat capacity was not significantly different from zero, illustrated by its exponential curvature in Figure 4.6b. Since SOM-R_S was not fitted to MMRT, the parameters T_{opt} and T_{inf} could not be derived.

4.4.1 Temperature response of different soil types amended with glucose

The temperature response of soil with added glucose (450 mM C) was measured from three very different soil types: Te Kowhai, Horotiu, and Te Rapa soils (soil properties described in section 4.3.1).

The different soil types produced varying magnitudes of CO_2 from all three R_S sources. Horotiu produced the highest fluxes, and Te Kowhai produced the lowest (Figure 4.7a-c). Although the magnitudes of the respiration rates varied with soil type, the average temperature-respiration (T-R_s) curves for the three soil types generally produced the same curvature. Total and glucose R_S both exhibited curvature that was well fitted with MMRT and had changes in heat capacities $\left(\Delta C_P^{\ddagger}\right)$ that were nonzero (Table 4.1). Glucose-R_S from the three soils had an average T_{opt} of 35°C and T_{inf} of 19°C. These were consistently lower than the same parameters derived from total-R_S. A Tukey's HSD test showed that for glucose-R_S, generally, there were no significant differences between these parameters from the different soil types. An exception to this was the significant difference between the T_{inf} point for Te Rapa and Te Kowhai of 3°C (Table 4.1).

When fitted to MMRT, SOM-R_S for each soil type did not exhibit a T_{opt} or T_{inf} point as their change in heat capacities $\left(\Delta C_P^{\ddagger}\right)$ were too close to zero (Table 4.1), thus the MMRT equation to collapsed to the Arrhenius equation (Figure 4.7c).

Table 4.1 Calculated MMRT parameters (mean ± SD, n=3) derived from three soil types (Te Kowhai, Horotiu, and Te Rapa) with two treatments (1) soil amended with a glucose solution (450 mM C) and (2) soil with no added glucose. See text for respiration source definitions

Carbon source	Soil type	T_{inf} (°C)	T_{opt} (°C)	$\Delta C_P^{\ddagger}$ (J mol^{-1} K^{-1})	$\Delta H_{T_0}^{\ddagger}$ (J mol^{-1} K^{-1})	$\Delta S_{T_0}^{\ddagger}$ (J mol^{-1} K^{-1})
Total	Te Kowhai	24 ± 0.4	45 ± 1	-2055 ± 194	37536 ± 1025	-174 ± 4
	Horotiu	23 ± 2	46 ± 2	-1676 ± 338	38274 ± 14979	-180 ± 18
	Te Rapa	22 ± 1	44 ± 3	-1505 ± 204	28902 ± 1349	-192 ± 5
Glucose	Te Kowhai	21 ± 1^{ab}	35 ± 1^{a}	-3973 ± 365^{b}	36767 ± 1583	-179 ± 5
	Horotiu	19 ± 1^{a}	35 ± 2^{a}	-2929 ± 204^{a}	26691 ± 3740	-199 ± 10
	Te Rapa	18 ± 1^{ac}	35 ± 3^{a}	-2868 ± 525^{a}	25102 ± 2636	-206 ± 14
Soil	Te Kowhai	n/a	n/a	486 ± 435	36431 ± 3574	-189 ± 13
	Horotiu	n/a	n/a	325 ± 741	36529 ± 30635	-146 ± 46
	Te Rapa	n/a	n/a	745 ± 214	34938 ± 1986	-185 ± 6

n/a – not applicable as $\Delta C_P^{\ddagger}$ was not significantly different from zero and so T_{inf} and T_{opt} were not able to be calculated. Significant differences (<0.05) between soil types for the glucose C source are noted by different letters (i.e. a, b, c).

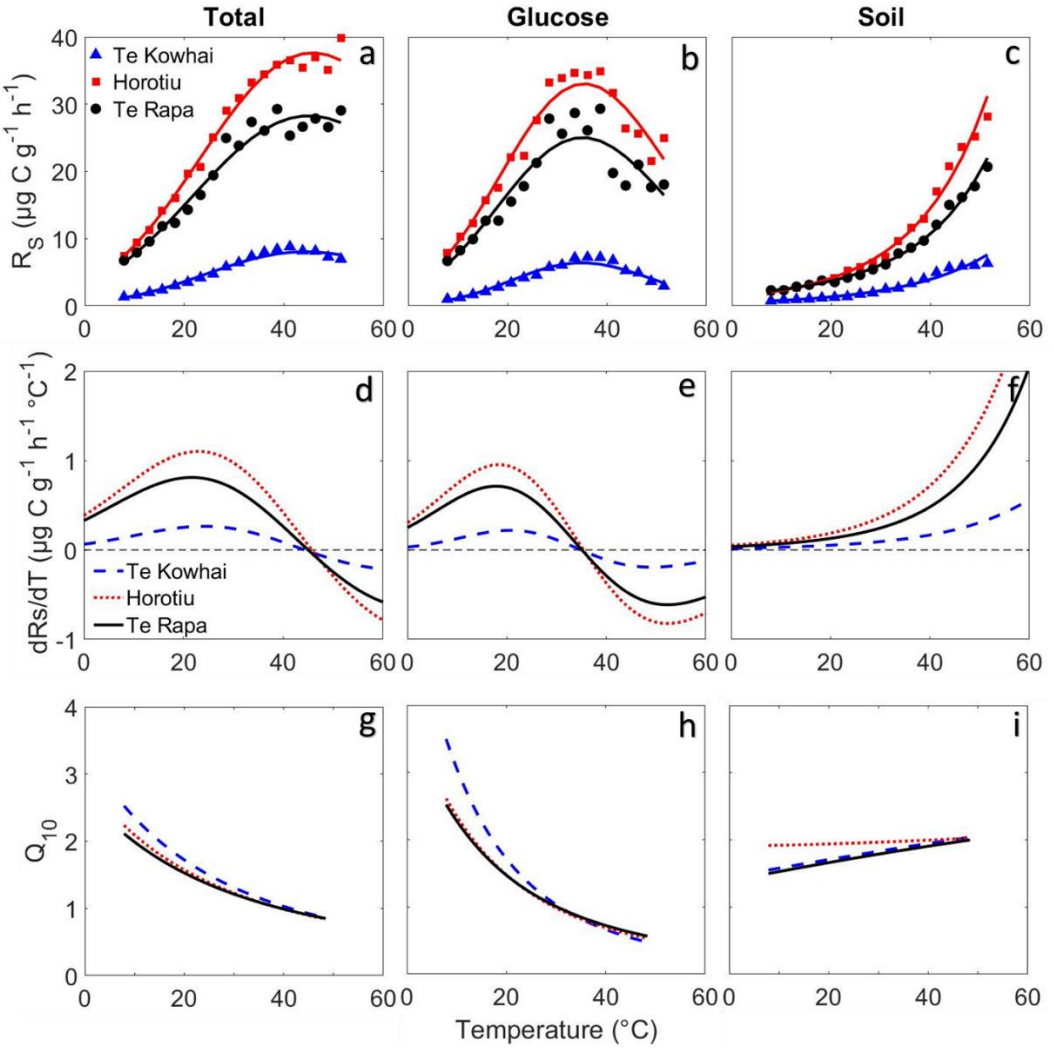

Figure 4.7 Average respiration rates (µg C g^{-1} soil h^{-1}) against temperature (°C) of three soil types (Te Kowhai –blue, Horotiu – red, and Te Rapa – black) and fitted with the MMRT equation derived from (a) total-R_S, (b) glucose-R_S, and (c) SOM-R_S. The average absolute temperature sensitivities (first derivative; µg C g^{-1} soil h^{-1} °C^{-1}) against temperature (°C) of the three soil types of (d) total-R_S, (e) glucose-R_S, and (f) SOM-R_S. The temperature inflection point is the temperature (x-axis) at which the absolute temperature sensitivity (y-axis) is the highest (maximum rate of change). The temperature optimum is the temperature at which the absolute temperature sensitivity reaches zero. The average relative temperature sensitivities (Q_{10}) for (g) total-R_S, (h) glucose-R_S, and (i) SOM-R_S. Measures of variability are not shown to maintain clarity, but see Table 4.1 and text for significant differences

In previous studies, others have been able to fit MMRT to the temperature response of SOM decomposition (e.g. Robinson *et al.* (2017); Schipper *et al.* (2019)), but these studies had a more extensive temperature range and more individual temperature measurements which improved fitting precision.

The average absolute (Figure 4.7d-f) and relative (Figure 4.7g-i) temperature sensitivities were derived for each soil. The absolute temperature sensitivity was measured as the rate of change calculated from $\frac{\partial R_S}{\partial T}$, and the relative temperature sensitivity was calculated as Q_{10}. These measures were also

calculated at 10, 20, and 30°C for glucose-R_S (Figure 4.7e; Table 4.2) to determine a clear trend with temperature. Te Kowhai soil had the lowest absolute temperature sensitivity, which was observed for all three R_S sources, while Horotiu consistently had the highest (Figure 4.7; Table 4.2). From the three temperatures calculated, the absolute temperature sensitivities for glucose-R_S was the highest at 20°C for all soils, where it then declined above their T_{inf} points (Figure 4.7e; Table 4.2). This trend was also produced by total-R_S, as illustrated in the curvature of their absolute temperature sensitivity curves in Figure 4.7d. The exception to this was the $\frac{\partial R_S}{\partial T}$ derived from SOM-R_S, which consistently increased with temperature for all soils, shown by their exponential curvature in Figure 4.7f.

Q_{10} was reasonably similar between soils for total, glucose, and SOM decomposition (Figure 4.7; Table 4.2). In all cases, Q_{10} *decreased* with temperature for total-R_S and glucose-R_S and slightly *increased* with temperature for SOM-R_S.

For the remainder of the experiments, Horotiu silt loam (allophanic) was selected as a model soil to test how different simple carbon compounds responded to temperature when added to soil.

Table 4.2 Calculated Q_{10} and $\frac{\partial R_S}{\partial T}$ (mean ± SD, n=3) derived from MMRT fits of glucose-R_S (see above text for description) for three soil types with added glucose (450 mM C) at three temperatures

Soil type	Temperature (°C)	Q_{10}	$\frac{\partial R_S}{\partial T}$ (μg C g^{-1} h^{-1} $°C^{-1}$)
Te Kowhai	10	3.06 ± 0.1	0.12 ± 0.01
	20	1.72 ± 0.02	0.21 ± 0.01
	30	1.03 ± 0.04	0.12 ± 0.01
Horotiu	10	2.35 ± 0.2	0.76 ± 0.2
	20	1.48 ± 0.1	0.99 ± 0.3
	30	0.98 ± 0.1	0.43 ± 0.2
Te Rapa	10	2.28 ± 0.1	0.58 ± 0.08
	20	1.47 ± 0.05	0.74 ± 0.1
	30	1.01 ± 0.1	0.35 ± 0.2

4.4.2 Temperature response of soil amended with different carbon compounds

Numerous incubations were undertaken to generate at least three replicates of T-R_S curves for each of the five simple C compounds, dextran, and yeast extract added to soil (Horotiu silt loam, see section 4.3.1 for soil properties). When fitted with the MMRT equation, respiration rates for soil alone collapsed to the Arrhenius equation as its $\Delta C_P^{\ddagger}$ was close enough to zero (Table 4.3). Therefore, no T_{opt} or T_{inf} points were derived.

The five simple carbon compounds chosen were glucose, glutamine, arginine, maltose, and lysine, and these all exhibited similar MMRT behaviour when added to soil. Their average T-R_S curves for C compound-R_S are shown in Figure 4.8a-e, which demonstrates their strong curvature when fitted to MMRT which allowed the calculation of T_{opt} and T_{inf} parameters that were lower than observed for total-R_S (Table 4.3). T_{inf} points ranged from 19°C for glutamine and glucose to 25°C for arginine, and the average T_{inf} was 21°C (SD=3) (Table 4.3). T_{opt} points ranged from 33°C for glutamine to 39°C for maltose, and the average T_{opt} was 37°C (SD=3) (Table 4.3).

Significantly lower R_S rates were observed from Lysine (Figure 4.8e) compared to the other simple C compounds, but it still exhibited MMRT behaviour due to its large negative $\Delta C_P^{\ddagger}$ value (Table 4.3). Interestingly, the average total R_S for soil with added Lysine collapsed to the Arrhenius equation when fitted to MMRT; thus, no T_{opt} or T_{inf} points could be derived (Table 4.3). This indicated that Lysine-R_S was small, and that it was difficult to separate from SOM-R_S.

Table 4.3 MMRT parameters (mean ± SD, n=3) of soil with added C compounds (total), calculated C compound data: total − SOM RS (carbon), and control soil (SOM). A is average SOM parameters from control soil incubated with glucose and maltose, B with glutamine and dextran, C with arginine and lysine, and D with yeast extract

Carbon source	Carbon compound/ Replicate	T_{inf} (°C)	T_{opt} (°C)	$\Delta C_P^{\ddagger}$ (J mol^{-1} K^{-1})	$\Delta H_{T_0}^{\ddagger}$ (J mol^{-1} K^{-1})	$\Delta S_{T_0}^{\ddagger}$ (J mol^{-1} K^{-1})
	Glucose	23 ± 1	46 ± 2	-1676 ± 276	38274 ± 12230	-180 ± 14
	Maltose	30 ± 2	51 ± 8	-1437 ± 196	39608 ± 3196	-157 ± 12
	Glutamine	24 ± 0.5	44 ± 2	-2104 ± 269	36712 ± 1368	-168 ± 5
Total	Dextran	n/a	n/a	631 ± 402	47905 ± 5968	-142 ± 19
(SOM+glucose)	Arginine	36 ± 2	59 ± 4	-1779 ± 224	56174 ± 920	-109 ± 3
	Lysine	n/a	n/a	-47 ± 288	48440 ± 1712	-139 ± 6
	Yeast	25 ± 1	42 ± 1	-2790 ± 327	44443 ± 4128	-134 ± 15
	Glucose	19 ± 1^{ab}	35 ± 2^{a}	-2929 ± 166^{a}	26691 ± 3053	-199 ± 12
	Maltose	22 ± 0.5^{a}	39 ± 3^{ab}	-2993 ± 893^{a}	37699 ± 3096	-166 ± 9
	Glutamine	19 ± 0.7^{ab}	33 ± 1^{ac}	-4213 ± 560^{a}	30004 ± 2938	-191 ± 10
Carbon	Dextran	n/a	n/a	n/a	n/a	n/a
	Arginine	25 ± 1^{ac}	37 ± 2^{a}	-4795 ± 872^{a}	60596 ± 10349	-98 ± 34
	Lysine	21 ± 3^{a}	38 ± 2^{a}	-2972 ± 1217^{a}	36908 ± 16252	-186 ± 54
	Yeast	$24 \pm {}^{ac}3$	39 ± 3^{a}	-3645 ± 256^{a}	48646 ± 9913	-121 ± 33
	A	n/a	n/a	325 ± 605	36529 ± 25014	-146 ± 37
SOM	B	n/a	n/a	452 ± 405	48454 ± 1768	-140 ± 6
	C	n/a	n/a	484 ± 240	52736 ± 4359	-128 ± 14
	D	n/a	n/a	422 ± 1136	34800 ± 15053	-185 ± 46

n/a – not applicable as $\Delta C_P^{\ddagger}$ was not significantly different from zero, and so T_{inf} and T_{opt} were not able to be calculated. Significant differences (<0.05) between C types for the C compound R_S are noted by different letters (i.e. a, b, c).

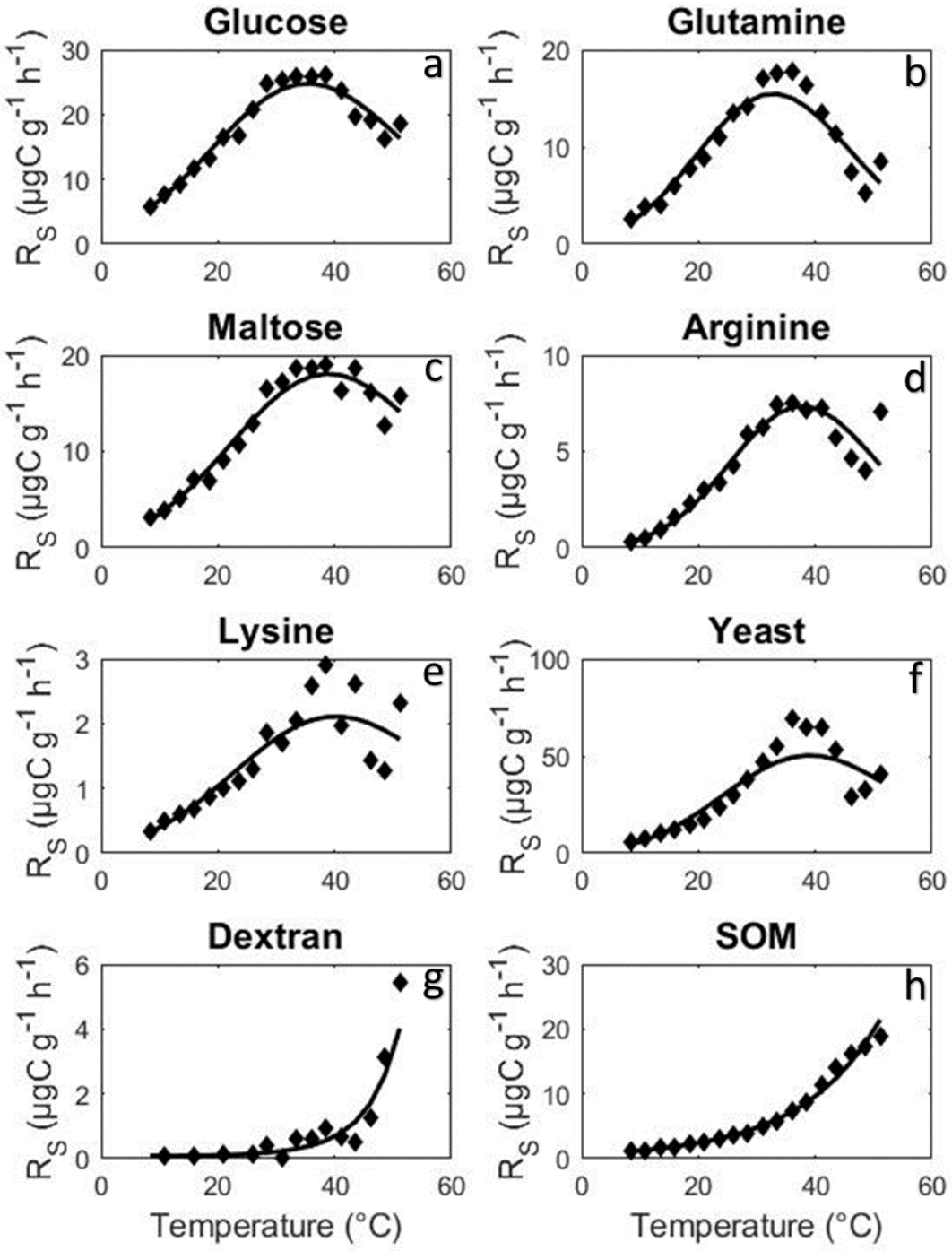

Figure 4.8 Average temperature response of respiration rates derived from different simple C compounds (a-e), a yeast extract consisting of a wide variety of carbon compounds (f), a complex, long-chained carbon compound – dextran (g), and the bulk SOM (h). Respiration rates a-g display the C compound-R_S. Note the changes in the y-axis scale to illustrate their curvatures. Averages are from three independent analysis but variation is not shown to maintain clarity. Variance and statistical differences are reported in Table 4.3

The absolute and relative temperature sensitivities for the decomposition of each of the simple C compounds were derived (Figure 4.9a and b, respectively). Of the simple C compounds, Lysine's absolute temperature sensitivity was consistently the lowest at 10, 20, and 30°C (Table 4.4). This was also depicted

by Lysine's weak absolute temperature sensitivity $\left(\frac{\partial R_S}{\partial T}\right)$ curvature shown in Figure 4.9a. For the calculated absolute temperature sensitivities for each simple C compound, the highest temperature sensitivity was observed at 20°C (Table 4.4). The curvature illustrates this trend in Figure 4.9a, where the absolute temperature sensitivity $\left(\frac{\partial R_S}{\partial T}\right)$ peaks at their T_{inf} point and decreases past this temperature.

Q_{10} for all simple C compounds decreased with temperature, with the highest relative temperature sensitivity calculated at 10°C for arginine, and the lowest at 30°C for glutamine (Table 4.4).

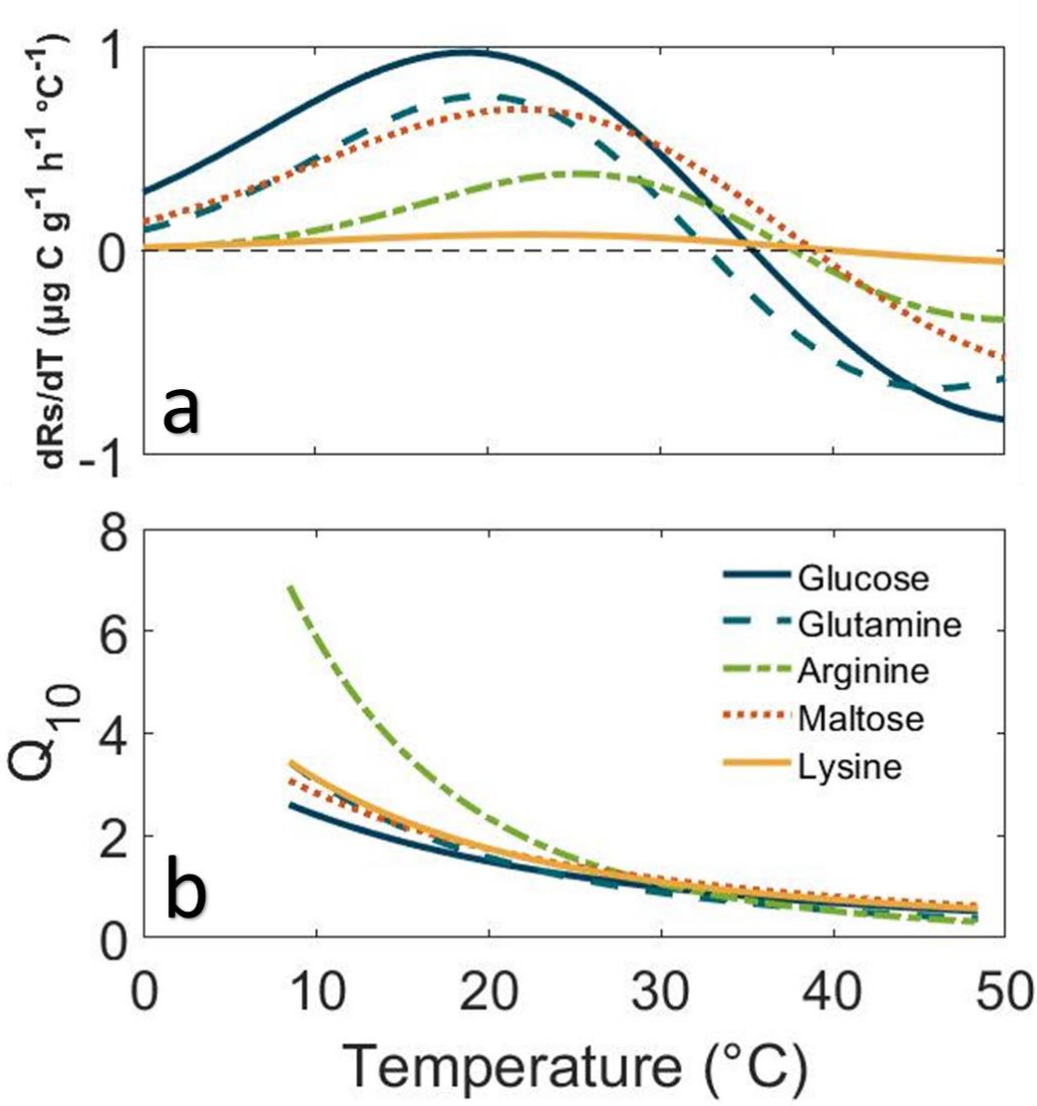

Figure 4.9 (a) Average absolute temperature sensitivity $\left(\frac{\partial R_S}{\partial T}\right)$ and (b) average relative temperature sensitivity (Q_{10}) against temperature (°C) for five simple carbon compounds

The addition of yeast extract to soil illustrated similar curvature to the previous simple carbon compounds (Figure 4.8f). While the magnitude of the CO_2 respired derived from yeast was much larger than that respired from the simple carbon compounds, its T_{opt} and T_{inf} points were very similar to the simple C compounds at an average of 39°C and 24°C, respectively (Table 4.3).

Dextran, which likely strongly binds to soil, behaved very similarly to the bulk SOM (from the control soil) compared to the other carbon compounds, illustrated by its exponential shaped T-R_S curve (Figure 4.8g). Dextran's average $\Delta C_P^{\ddagger}$ was close enough to zero to collapse from the MMRT to the Arrhenius equation; thus, like the SOM, $T_{opt,}$ and T_{inf} points could not be derived (Table 4.3).

The absolute and relative temperature sensitivities of yeast extract, dextran, and SOM were derived from their MMRT fits and presented in Figure 4.10a and b, respectively. Dextran had the lowest absolute temperature sensitivity and exhibited the same behaviour as SOM as both C source's $\frac{\partial R_S}{\partial T}$ values increased with temperature (Table 4.4; Figure 4.10a). Yeast extract had the highest absolute temperature sensitivity for all C compounds (Figure 4.10a), which peaked at its T_{inf} point before declining like the other simple compounds (Table 4.4; Figure 4.10).

Yeast extract was significantly more temperature sensitive (in relative terms) than dextran and SOM at lower temperatures, and its Q_{10} decreased with increasing temperatures. However, at higher temperatures, dextran and SOM had a higher relative temperature sensitivity as their Q_{10} increased with increasing temperatures.

Generally, there were no significant differences for the T_{opt} and T_{inf} points between the C compounds. The exceptions to this were that T_{opt} for glutamine was significantly different from maltose, and the T_{inf} points for arginine and yeast extract were significantly different from both glutamine and glucose (Table 4.3).

There were no significant differences for changes in heat capacity values between the carbon substrates. It is important to note that dextran was not included in these comparisons as its T_{opt} and T_{inf} points were non-existent, however, its $\Delta C_P^{\ddagger}$ would be significantly different from the other substrates as it was very close to zero due to its Arrhenius-like behaviour.

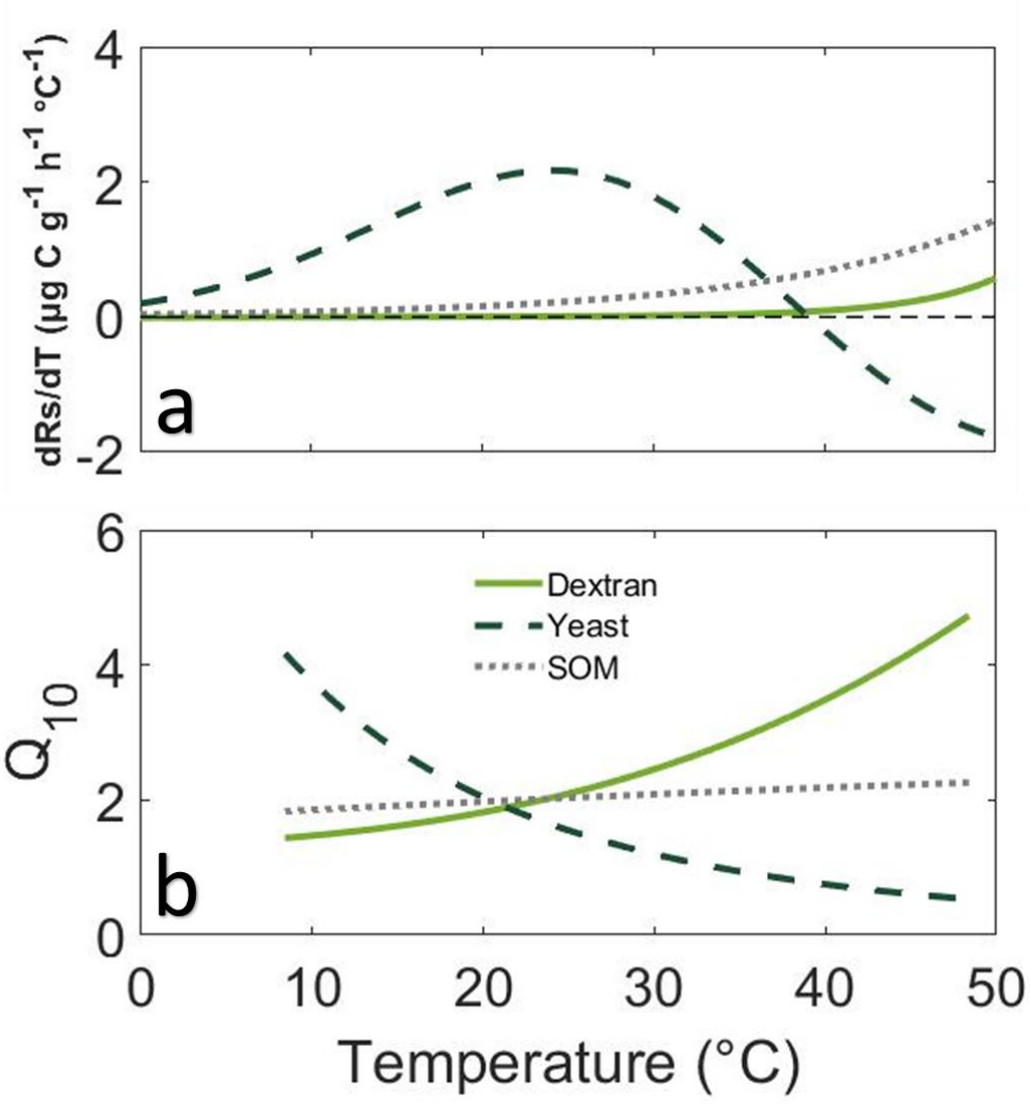

Figure 4.10 (a) Average absolute temperature sensitivity $\left(\frac{\partial R_S}{\partial T}\right)$ and (b) Average relative temperature sensitivity (Q_{10}) against temperature (°C) of a complex, long-chained C compound (dextran), yeast extract, and the bulk SOM (control soil).

Table 4.4 Calculated Q_{10} and $\frac{\partial R_S}{\partial T}$ (mean ± SD, n=3) derived from MMRT fits of C compound-R_S (see text for description) for five simple carbon compounds (arginine, glucose, glutamine, lysine, and maltose), a complex, long-chained carbon compound (dextran), a yeast extract, and SOM at three temperatures

Carbon compounds	Temperature (°C)	Q_{10}	$\frac{\partial R_S}{\partial T}$ (μg C g^{-1} h^{-1} °C^{-1})
Arginine	10	5.84 ± 1	0.11 ± 0.02
	20	2.33 ± 0.3	0.35 ± 0.02
	30	1.05 ± 0.1	0.27 ± 0.08
Dextran	10	1.47 ±0.7	0.02 ± 0.03
	20	1.89 ±0.3	0.03 ± 0.05
	30	2.48 ±1	0.05 ± 0.07
Glucose	10	2.40 ± 0.1	0.75 0.21
	20	1.49± 0.1	0.99 0.11
	30	0.98± 0.1	0.44 0.02
Glutamine	10	3.05 ± 1	0.45 ± 0.02
	20	1.57± 0.1	0.72 ± 0.18
	30	0.88 ± 0.1	0.24 ± 0.08
Lysine	10	3.09 ± 1	0.05 ± 0.01
	20	1.74 ± 0.4	0.08 ± 0.01
	30	1.09 ± 0.3	0.05 ± 0.04
Maltose	10	2.82 ± 1	0.42 ± 0.11
	20	1.73 ± 0.1	0.69 ± 0.11
	30	1.15 ± 0.1	0.54 ± 0.19
Yeast extract	10	3.36 ±1	0.92 ± 0.28
	20	2.12 ±0.2	2.04 ± 0.56
	30	1.53 ± 0.5	1.80 ± 0.71
SOM	10	1.86 ±0.4	0.08 ± 0.03
	20	1.98 ±0.3	0.16 ± 0.07
	30	2.08 ±0.6	0.33 ± 0.08

4.4.3 Priming

Three replicate incubations were completed for quantifying priming when adding a simple C compound (in this case ^{13}C labelled glucose) to soil. Unfortunately, due to technical error using the methods (section 4.3.2.2), only two of these replicates (A and C) had data that spanned nearly the full temperature range. With little replication, this data is presented without statistical analysis and is discussed as observational.

Each replicate produced four temperature-respiration curves, Total-R_S, Glucose-R_S, SOM-R_S, and Priming-R_S (see section 4.3.2.3 for their derivation).

As an average of the three replicates, the contribution of priming-R_S to the total R_S was about 30%, with glucose and SOM_C contributing 54% and 16%, respectively. The proportion of each of the contributions slightly varied with temperature; however, they were generally consistent (Figure 4.11).

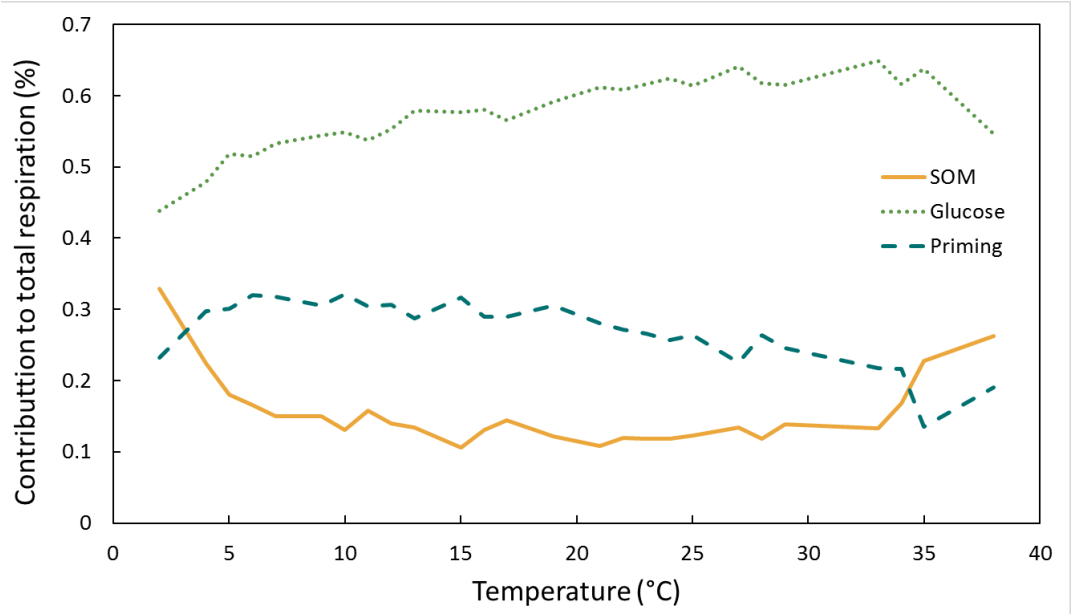

Figure 4.11 Example figure showing the contribution of SOM, glucose, and priming decomposition to the total respiration from replicate A across a temperature gradient

The T-R_S curves from the total, glucose, and priming R_S from replicate A and C (Figure 4.12a and c, respectively) exhibited MMRT behaviour along the temperature gradient, illustrated by their strong curvature. Priming had lower T_{inf} and T_{opt} points than both total-R_S and glucose-R_S, at an average from the two replicates of 15°C and 30°C, respectively.

Due to the shorter temperature range of the second replicate (~2-30°C compared to ~2-40°C or ~2-46°C) (Figure 4.12b), none of these four T-R_S

curves exhibited discernible MMRT-like behaviour. While there was slight curvature observed in the T-R_S curves for glucose and priming, their $\Delta C_P^{\ddagger}$ were still too close to zero and thus collapsed to the Arrhenius equation (Table 4.5). This was also illustrated in Figure 4.12e, where the absolute temperature sensitivity curves do not reach observable T_{opt} or T_{inf} points. Like the previous experiment, the SOM_c-R_S followed an Arrhenius-like behaviour for all three replicates (Figure 4.12).

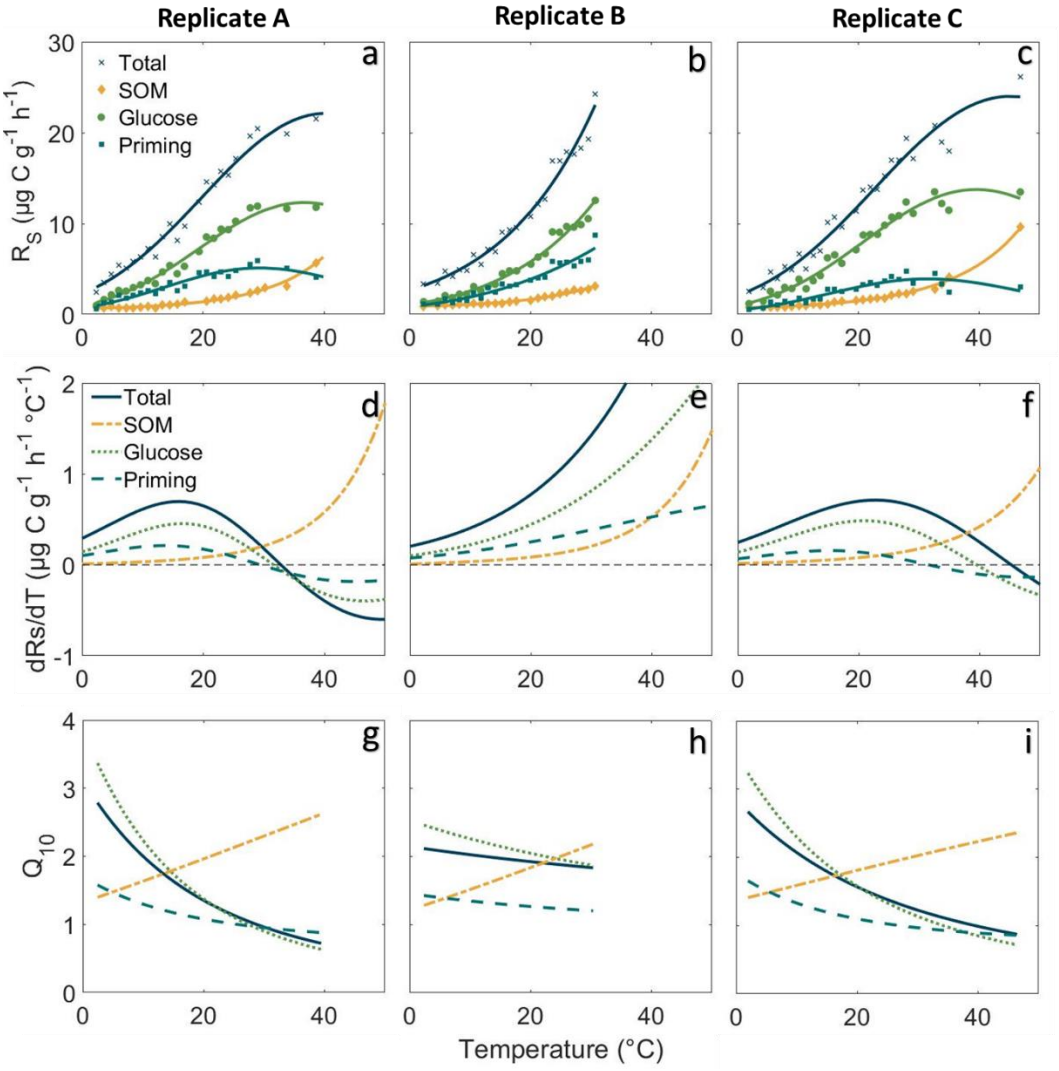

Figure 4.12 Respiration rates (µg C g^{-1} h^{-1}) against temperature (°C) of total, SOM_c, added glucose, and the calculated priming for three replicate incubations (a-c). Absolute temperature sensitivities $\left(\frac{\partial R_S}{\partial T}\right)$ (d-f) and average relative temperature sensitivities (Q_{10}) (g-i) against temperature (°C) for total, SOM_c, glucose, and priming-R_S for three replicate incubations.

The relative (Q_{10}) and absolute $\left(\frac{\partial R_S}{\partial T}\right)$ temperature sensitivities for the decomposition induced by priming were calculated at 10, 20, 30°C for all three replicates (Table 4.6). Q_{10} for all replicates decreased with temperature, which

was also observed for the decomposition of labile C compounds (Table 4.6). The absolute temperature sensitivity for replicates A and C depicted similar behaviour to the absolute temperature sensitivity of the labile C compounds where Figure 4.12d and f showed that these replicates increased up to their T_{inf} points, and then declined. However, for replicate B, the decomposition of priming showed similar behaviour to the decomposition of SOM as its absolute temperature sensitivity increased with temperature (Figure 4.12e and Table 4.6).

Table 4.5 MMRT parameters for three replicates of soil amended with 13C labelled glucose. Total = SOM + glucose + priming CO2 flux. SOM is respiration from control soil. Glucose is respiration directly measured from 13C-glucose. Priming is the difference between SOM (control soil) and SOM (treatment soil)

Carbon source	Replicate	T_{inf} (°C)	T_{opt} (°C)	$\Delta C_P^{\ddagger}$ (J mol^{-1} K^{-1})	$\Delta H_{T_0}^{\ddagger}$ (J mol^{-1} K^{-1})	$\Delta S_{T_0}^{\ddagger}$ (J mol^{-1} K^{-1})
Total	A	16	33	-2734	18759	-227
	B	n/a	n/a	54	46791	-133
	C	23	45	-1670	31119	-186
SOM	A	n/a	n/a	1636	47617	-148
	B	n/a	n/a	1528	41895	-166
	C	n/a	n/a	1227	41512	-168
Glucose	A	17	32	-3423	19961	-227
	B	n/a	n/a	-270	50973	-124
	C	21	40	-2352	31532	-188
Priming	A	14	29	-3098	11078	-263
	B	n/a	n/a	-483	41727	-159
	C	15	31	-2925	16379	-247

n/a – not applicable as $\Delta C_P^{\ddagger}$ was not significantly different from zero and so T_{inf} and T_{opt} were not able to be calculated

Table 4.6 Calculated Q_{10} and $\frac{\partial R_S}{\partial T}$ derived from MMRT fits of priming-R_S (see above text for description) for three replicates (A, B, and C) at three temperatures

Replicate	Temperature (°C)	Q_{10}	$\frac{\partial R_S}{\partial T}$ (µg C g^{-1} h^{-1} °C^{-1})
A	10	1.91	0.19
	20	1.28	0.19
	30	0.91	0.05
B	10	2.00	0.15
	20	1.53	0.25
	30	1.24	0.35
C	10	2.01	0.14
	20	1.28	0.15
	30	0.86	0.05

4.5 Discussion

4.5.1 Temperature response of different soil types with added glucose

The magnitude of respiration rates derived from SOM differed between the three soil types. The lowest magnitudes (from Te Kowhai soil) were associated with the lowest C content, but surprisingly Horotiu had higher respiration rates than the Te Rapa soil, which had the highest C content. As described in methods, the priming response is captured within the glucose-induced respiration and the extent and role of priming are discussed in section 4.5.3.

The temperature-respiration curves for SOM (for all three soil types) were best fit with the exponential Arrhenius equation (i.e. $\Delta C_P^{\ddagger} = 0$) and T_{opt} and T_{inf} could not be determined. Many other measurements of the temperature response of soil respiration are also best fitted by exponential or similar models. Chen *et al.* (2010) looked at the relationship between soil R_S and temperature by incubating different soil types between 1-12 hours at five discrete temperatures (7-35°C). The study found that the temperature-respiration relationship was well-fitted to an exponential function (similar to Arrhenius). A highly influential study by Lloyd and Taylor (1994) compared the goodness of fit of various mathematical functions to the soil. This study found that an exponential and Arrhenius function provided a biased estimate of respiration rates and that the Arrhenius equation underestimated respiration rates at low temperatures and overestimated R_S at high temperatures.

Here, the inability to fit SOM-R_S with MMRT was likely due to insufficient discrete temperatures. Robinson *et al.* (2017) and Schipper *et al.* (2019), using the same experimental setup but with 40 discrete temperatures (~2-50°C), were able to calculate T_{opt} and T_{inf} parameters for the decomposition of SOM which were T_{inf}=35°C T_{opt}=54°C and T_{inf}=45°C T_{opt}=65°C, respectively. On the other hand, when using only 30 discrete temperatures with the same experimental setup, Robinson *et al.* (submitted) failed to derive T_{opt} and T_{inf} parameters from SOM-R_S which highlights the need for more discrete

incubation temperatures for accurate curve fitting and potentially limits the use of MMRT when describing SOM cycling.

The magnitude of respiration rates derived from glucose followed the same order as respiration derived from SOM (i.e. highest R_S in Horotiu, then Te Rapa, and lastly Te Kowhai). This consistent order was likely reflective of the size of the active microbial biomass that was capable of decomposing both SOM and glucose. The temperature-respiration curves from glucose of all three soil types were all fitted to MMRT, with an average T_{opt} and T_{inf} point of 35°C and 19°C, respectively. Both T_{opt} and T_{inf} from glucose addition were slightly lower, but similar, than the same parameters derived from litter R_S calculated by Robinson *et al.* (submitted) (i.e. T_{opt} = 42°C and T_{inf} = 24°C). Robinson *et al.* (submitted) incubated soil with and without added ^{13}C labelled plant litter across a temperature gradient of ~2-50°C to determine the temperature sensitivity of the labile (litter) and stable (SOM) C pools in soil using isotopic analysis. The slightly lower temperature response from glucose-R_S than litter suggests that the litter was not as available to microbes for decomposition and thus relied more on solubilisation and diffusion processes that have an Arrhenius temperature response (Schipper *et al.*, 2019). Robinson *et al.* (submitted) argued that the difference between the temperature response of SOM-R_S and litter-R_S was due to differences in substrate availability, as previously suggested by Schipper *et al.* (2019). Schipper *et al.* (2019) determined the temperature response from irrigated (lower available C) and non-irrigated (higher available C) soils and found that in *relative* terms, irrigated soils (with lower available C) exhibited a higher temperature sensitivity (Q_{10}) than non-irrigated soil (higher available C).

Despite no MMRT parameters derived from SOM in the current study, the T_{opt} and T_{inf} parameters derived from glucose were much lower than for SOM measured from previous studies with more independent temperatures (Schipper *et al.*, 2014; Robinson *et al.*, 2017). As the soil with added glucose only respired less than a maximum of 15% of added glucose, respiration was not substrate limited, and thus its intrinsic temperature sensitivity was the observed temperature response (Davidson & Janssens, 2006). Also, despite the significant differences in the magnitudes of both total SOM and glucose

respiration rates from the different soil types, the derived MMRT parameters of glucose were very similar. The T_{opt} of the different soils was 35°C and the T_{inf} only differed by ~3°C (18-21°C). The similarity of the T_{opt} and T_{inf} between the three soils indicated that although these are very different soils with varying properties (i.e. carbon content and microbial biomass size), the microbial communities must be functionally similar even if the species composition differed significantly between soils. Robinson *et al.* (2017) also found no significant differences between all MMRT parameters derived from SOM-R_S for these three soils.

Currently, the amount of variation in the temperature response of respiration processes that occur across the globe is not well defined. A synthesis study conducted by Carey *et al.* (2016) used a large global dataset (>3,800 observations from 27 soil temperature response studies) from over 20 years of global warming to compare the temperature sensitivity of soil respiration of nine different biomes. This study concluded that the temperature sensitivity of soil respiration might vary with region, with particularly strong evidence that soil at higher altitudes will be more responsive to temperature changes (Carey *et al.*, 2016). Soils in the current study were collected in close proximity to each other (Newstead, Waikato), and it remains unclear whether T_{opt}/T_{inf} for added glucose and SOM differs in soils from a broader range of climates and ecosystems, including variations in average annual temperature, land management, and elevation.

4.5.2 Temperature response of simple C compounds added to soil

The magnitude of respiration rates also varied for the simple C compounds added to soil. Glucose had the highest R_S rates, followed by glutamine and maltose, which had similar magnitudes, then arginine, and lastly respiration rates from lysine were significantly lower. When yeast extract, which is a combination of a wide variety of carbon compounds, was added to soil the magnitude of respiration rates were much higher than any of the individual substrates. This behaviour might be expected as not all active microbes can utilise glucose or one of the other added C compounds, so when yeast extract was added a wide range of compounds were supplied to different microbes in soil.

The respiration rates derived from the added C sources also comprise priming-R_S based on evidence from a preliminary experiment conducted for this thesis. It is also important to note that a sufficient concentration of the C compounds was added to soil; thus C was not limiting (even at higher temperatures), as a preliminary experiment found that less than 15% of the added C was respired. This relatively low percentage of C respired (sourced from the added C) provided confidence that the highly available labile C pool could be represented by the addition of simple C compounds to soil.

The simple C compounds and the yeast extract respiration rates were fitted to MMRT and produced clear T_{opt} and T_{inf} points that averaged 37°C and 22°C, respectively. Interestingly, a wide range of enzymes also have a temperature optimum around 37°C or slightly higher (Schipper *et al.*, 2014; Arcus *et al.*, 2016). Despite differences in magnitudes of respiration rates, there were largely no significant differences between the MMRT parameters for the simple C compounds and the mix of C compounds in yeast extract. The average T_{opt} and T_{inf} for the added simple C compounds and yeast extract were slightly lower, but similar to the same parameters derived from litter-R_S by Robinson *et al.* (submitted) at 42°C and 24°C, respectively. Robinson *et al.* (2017) calculated the T_{opt} and T_{inf} of SOM-R_S which ranged from 64-75°C and 43-49°C, respectively (for soil collected in autumn, i.e. the same collection period for this study). T_{opt} and T_{inf} for SOM measured from irrigated and non-irrigated soils by Schipper *et al.* (2019) ranged 54-70°C and 33-46°C, respectively. Compared to the T_{opt} and T_{inf} derived from SOM reported by Robinson *et al.* (2017) and Schipper *et al.* (2019), the T_{opt} and T_{inf} for the simple C compounds and yeast extract were significantly lower (37°C and 22°C, respectively). While only simple C compounds were tested, a preliminary conclusion could be that the temperature optimum of labile C when not substrate limited (i.e. intrinsic temperature response) was around 37°C and much less than the stable organic matter.

Generally, respiration derived from C compounds had higher respiration rates than their respective SOM R_S. An exception to this was lysine, which had significantly lower respiration rates than its corresponding SOM-R_S. The utilisation of soil C by microbes depends on two factors, (1) the chemical

structure of the compound, and (2) the availability of the C to the microbes for decomposition (Bartlett & Doner, 1988). Lysine has a simple molecular structure, and with the concentration (450 mM C) of the substrate added, the compound should have been highly available to the microbes. Therefore, the lower R_S rates derived from lysine were likely due to its metabolic incompatibility with the microbes present at the time of collection (Bartlett & Doner, 1988).

In contrast to the simple C compounds, respiration rates from dextran, (a complex, long-chained glucan) resulted in a very similar temperature response to SOM. The measured temperature-respiration curve derived from dextran could not be fitted to MMRT with no calculable T_{opt} or T_{inf}. This behaviour was likely due to the large size and high complexity of this carbon compound that made it more difficult to degrade. Additionally, the large molecular size of dextran would likely have increased its sorption onto soil surfaces and resulting in lower concentrations in the soil solution available for microbes to decompose.

Temperature sensitivity:

The temperature sensitivity for the decomposition of the carbon compounds and SOM was assessed using two measures, relative (Q_{10}) and absolute $\left(\frac{\partial R_S}{\partial T}\right)$ temperature sensitivities. In the literature, Q_{10} is the most commonly used measure of temperature sensitivity. However, issues can arise when using this measure as it is challenging to compare Q_{10} values between studies when different equations are fitted to different datasets, producing varying Q_{10} values (Fierer *et al.*, 2005). Here, both the Q_{10} and $\frac{\partial R_S}{\partial T}$ values were calculated at 10, 20, and 30°C to compare how this measure changed with temperature.

For all the simple C compounds reported here and the yeast extract, Q_{10} decreased with increasing temperature. This trend was also reported in Robinson *et al.* (submitted) for litter-R_S, as well as in Schipper *et al.* (2014); Robinson *et al.* (2017); Schipper *et al.* (2019) for SOM-R_S. The simple C compounds and yeast all had very similar magnitudes of Q_{10} across the temperature gradient, with an exception to Arginine, which had a significantly higher relative temperature sensitivity at lower temperatures. Dextran

behaved similarly to SOM as the Q_{10} for both of these carbon sources increased with temperature, which is not typically reported in the literature.

For all simple C compounds and the yeast extract, the absolute temperature sensitivity peaked at their T_{inf} (maximum rate of change). The magnitudes of their absolute temperature sensitivities were of the same order for their respiration rates (from highest to lowest: yeast, glucose, glutamine, maltose, arginine, and then lysine). Again, dextran and SOM behaved very similarly as their $\frac{\partial R_S}{\partial T}$ values increased with temperature. The relative temperature sensitivity of SOM was higher at 20 and 30°C than the added labile C substrates. However, at 10°C, the opposite was observed. This behaviour was also observed by Robinson *et al.* (submitted), where at 10°C SOM had a lower temperature sensitivity (Q_{10}) than the added labile litter. Conant *et al.* (2011) suggested that the temperature sensitivity of soil is likely caused by three temperature sensitive processes, depolymerisation of C, the rate of enzyme production, and substrate availability. Robinson *et al.* (submitted) suggested that at low temperatures, the prior two processes dominated over substrate availability, which was likely the case in this study too.

A frequently cited paper by Fierer *et al.* (2005) studied the temperature sensitivity of a range of carbon compounds with varying chemical complexities added to soil. This study concluded that the relative temperature sensitivity (measured by Q_{10}) for the decomposition of the carbon substrates decreased with increasing organic C quality (high-quality organic compounds are simple in chemical structure and have low activation energies, i.e. labile C pool). From this, it would be expected that the relative temperature sensitivity of dextran would be higher than glucose and other simple C compounds; however, this was not the case here as although they had opposing behaviours they generally had similar Q_{10} values overall. In absolute terms $\left(\frac{\partial R_S}{\partial T}\right)$ the temperature sensitivity of dextran was significantly lower than the simple carbon compounds and yeast extract, which agreed with the mathematical expression of the absolute temperature sensitivity which inherently states that higher quality substrates have a higher temperature sensitivity than lower quality substrates (Sierra, 2012).

In this study, the temperature response of the labile C pool was represented by the respiration rates from the simple C compounds and yeast extract, as these compounds were highly soluble and available to microbes. The respiration rates from SOM represented the stable C pool in soil. As mentioned previously, the temperature sensitivity of these two pools are highly debated in the literature, and a consensus has yet to be reached. Currently, the most supported conclusion is that in relative terms, the stable C pool has a higher temperature sensitivity than the labile C pool (Von Lützow & Kögel-Knabner, 2009). The current study also observed this, where SOM had a higher Q_{10} than the simple C compounds at higher temperatures but had lower Q_{10} values at low temperatures. However, SOM consistently had lower $\frac{\partial R_S}{\partial T}$ values than the simple C compounds and yeast (except for lysine) across all temperatures, suggesting that SOM (stable C pool) had a lower temperature sensitivity (in absolute terms) than the simple C compounds (labile C pool). These conflicting temperature sensitivities highlight the importance of making the distinction between the relative and absolute temperature sensitivities in the literature as these measures produce contradictory results.

4.5.3 Determining the magnitude and temperature response of priming in soil

A preliminary experiment was conducted to determine the magnitude of the potential priming effect when labile, exogenous carbon was added to soil. The results presented here showed that while respiration rates from priming were lower than derived from glucose-R_S, they do suggest that the temperature dependence of priming should be accounted for when determining the response of added C compounds in the future. On average, priming contributed ~30% of the total respiration from soil with added glucose, and the percentage of this contribution only varied slightly with temperature. This contribution of priming to total soil respiration was within the range (26-60%) of the priming-induced increase in SOM decomposition reported by Sun *et al.* (2019), who conducted a meta-analysis reviewing over 90 incubation studies.

Few studies have examined the temperature sensitivity of priming from soil, and these tend only to include a shorter temperature range with few different temperatures. For example, Thiessen *et al.* (2013) conducted a laboratory

incubation of soil with and without ^{13}C labelled plant litter at two diurnal temperatures (5-15°C and 15-25°C), and Zhu and Cheng (2011) studied the effect rhizosphere priming had on the overall temperature sensitivity of SOM decomposition by continuously labelling plants in a closed chamber with depleted ^{13}C labelled CO_2 at three different temperatures (2.7, 4.5, and 5°C above ambient temperature). While both studies derived priming-R_S using the same calculation used in this current study (i.e. the difference between SOM-R_S from soil with added C and SOM-R_S from soil with no added C), the temperature response of priming was only derived from 2-3 temperatures. Neither of these studies described the relationship between priming-R_S and temperature; however curve fitting to these datasets would not have produced reliable fits due to insufficient incubation temperatures (Robinson *et al.*, 2017). Whereas, due to the broader incubation range (2-52°C) and more discrete temperatures, this study was able to fit priming-R_S with MMRT.

Like respiration from added C sources, priming R_S exhibited MMRT behaviour, and clear T_{opt} and T_{inf} points were derived for two replicate incubations (A and C) averaged 30°C (SD=1) and 15°C (SD=0.7), respectively. These MMRT parameters were significantly lower than the average T_{opt} and T_{inf} points for the simple C compounds and yeast extract, but given that only two replicates were successful certainty about this difference in T_{opt} and T_{inf} was low. Since priming-R_S was well described by MMRT, this indicated that priming might have been due to increased access of C from SOM to soil microbes.

The relative and absolute temperature sensitivity of the three priming R_S replicates were calculated at 10, 20, and 30°C. For all replicates, Q_{10} decreased with temperature following the same trend as the simple C compounds and yeast extract and matching generally observed patterns of Q_{10} with temperature (Schipper *et al.*, 2014; Robinson *et al.*, 2017; Schipper *et al.*, 2019). Only replicates A and C could be fitted to MMRT due to the excess loss of data points for replicate B. The absolute temperature sensitivity for replicates where MMRT was fitted was the highest at 20°C. Generally, priming's absolute temperature sensitivity was lower than for simple C compounds, yeast, and SOM at high temperatures; however, it was higher than SOM at lower temperatures. On the other hand, priming was less temperature sensitive in

relative terms (Q_{10}) overall compared to simple C compounds, yeast, and SOM. These results suggested that the C made available to microbes through priming must be more labile and physically accessible for decomposition than the added C substrates and SOM.

Although the results presented here are preliminary, they do suggest that this newly developed methodology adapted from Robinson *et al.* (submitted) will be useful for separating the temperature response from two different C pools in soil, as well as determining the response of priming in soil. To date, the temperature response of priming has not been well explored. It is essential to determine priming's response to temperature changes (particularly increased temperatures) to gain a fuller understanding of potential global warming feedbacks (i.e. positive feedback loop).

Separating the temperature response of labile and stable C pools through the addition of simple carbon compounds to soil is not well documented in the literature. Fierer *et al.* (2005) measured the temperature response of a variety of carbon compounds with varying complexities by incubating soils at five different temperatures (10, 15, 20, 25, and 30°C). This study assumed that the rate of mineralisation from the added carbon compounds was the difference between total-R_S and SOM-R_S and did not account for the potential priming effect that would occur through the addition of these carbon compounds. Although (Fierer *et al.*, 2005) acknowledged priming in their discussion, they deemed it insignificant as their study focused on the relationship between carbon quality and temperature sensitivity. Based on the meta-analysis conducted by Sun *et al.* (2019) combined with the preliminary results presented here, priming is a significant source of soil respiration when exogenous carbon is added to soil. As previously mentioned, priming is the enhancement of the decomposition of stable C pool (or SOM), which is concerning as this pool makes up to 75% of the total soil C pool (Sanchez *et al.*, 1989).

This priming experiment needs to be repeated using smaller CO_2 subsamples to overcome the limitation that CO_2 was too high at specific temperatures for accurate measurements. A wide variety of soil types and ^{13}C labelled compounds should also be incubated and analysed to observe if the

temperature response of priming varies within different regions. If the priming effect does persist past the preliminary experiment described here, then future incubations of soil with exogenous carbon should be either conducted using isotopic analysis or the presence of priming should be explicitly acknowledged.

4.6 Conclusion

The addition of glucose and other simple C compounds to soil had a clear, definable, and relatively consistent temperature optimum and temperature inflection. These two temperature parameters were not observed from SOM respiration but have been previously observed from added plant litter (Robinson *et al.*, submitted). This study suggests that decomposition of labile C in soil has a temperature optimum of around 33-39°C. Additionally, T_{opt} and T_{inf} derived from glucose respiration were not different for three very contrasting soils. While Robinson *et al.* (2017) compared the temperature response of SOM from the same three soils, to date, the response from glucose itself from different soil types has not been reported.

Overall, the simple C compounds and yeast extract had a lower relative temperature sensitivity than SOM decomposition. As expected, the opposite was observed for their absolute temperature sensitivities. Generally, respiration rates from the added C compounds were higher than from SOM and were able to be fitted to MMRT, while SOM exhibited typical Arrhenius behaviour. These differences between the added C compounds and SOM were likely attributed to differences in substrate availability. That is the added C compounds were highly for biological degradation by microbes (which typically exhibits MMRT-like behaviour), while the SOM was physiochemically protected. Before SOM is decomposed by soil microbes, chemical processes (i.e. diffusion and sorption/desorption) need to transport the protected C to the microbes. The Arrhenius function well describes these chemical processes.

The temperature response of glucose added to three very different soil types was also measured, and the results suggested that despite the varying properties and likely microbial biomass size between the soils, their temperature response parameters (T_{opt} and T_{inf}) derived from glucose (and priming) were not significantly different. The similar temperature responses

of these different soil types indicated that the microbial communities were functionally the same even if made up of different species, which was expected since these soils were collected from the same location.

A newly developed method demonstrated that priming contributed a significant proportion of the total-R_S from soil with added glucose. The respiration rates from priming exhibited typical MMRT behaviour and had lower T_{opt} and T_{inf} points than C compounds. Generally, priming was less temperature sensitive than C compounds and SOM in both relative and absolute terms. While these results were preliminary, future studies separating the temperature response of the labile and stable C pools in soil should consider the use of isotopic techniques to determine the actual temperature sensitivity of the labile pool by separating priming.

In all cases, the temperature response of the added simple C compounds representing the labile C pool, and SOM representing the stable C pool were significantly different. This remained true even within three very different soil types and six different labile C substrates (simple C compounds and yeast extract). Therefore, when modelling soil C dynamics, a simple two-pool soil C model might potentially be sufficient enough to provide reliable and accurate predictions. To test the appropriateness of this simple C model, further research should determine if there are significant variations in the temperature response of labile and stable C decomposition from different locations (i.e. elevation, climate, vegetation type, and land management). If results show that there is a significant difference within the temperature response of the individual pools from different locations, these variations should be incorporated into soil C models. If there is not a significant difference, this could provide confidence in using a two-pool model (i.e. labile and stable C) to describe soil C dynamics.

Chapter 5

Conclusion

5.1 Conclusions

The temperature response of the decomposition of different carbon pools in soil remains unclear in the literature. Here, I have broadly separated soil carbon into two distinct pools: labile and stable C pools. The labile pool consists of readily available and easily degradable C, and the stable pool consists of physiochemically protected and consequently hard to degrade C (Von Lützow & Kögel-Knabner, 2009). The temperature sensitivity of these two pools has not yet reached a consensus, with many studies producing contradicting results (Von Lützow & Kögel-Knabner, 2009). Currently, the most supported conclusion is that in *relative* terms (i.e. Q_{10}) the stable C pool is more temperature sensitive than the labile C pool (Von Lützow & Kögel-Knabner, 2009). The stable C pool makes up ~75% of total soil C, which itself is the largest terrestrial C pool (Sanchez *et al.*, 1989). Therefore, this conclusion is a cause for concern under a global warming scenario. As temperatures increase, large CO_2 outputs from the stable C pool would result if the stable C pool is moderately to highly sensitive to changes in temperature.

Here, I determined the temperature response of two model carbon pools (nominally designated labile and stable), by incubating soil with and without added simple C compounds at 18 discrete temperatures (~8-52°C) for five hours. The labile C pool was represented by the decomposition of simple C compounds, which included the priming effect that is enhanced by the addition of these simple C compounds. Past experiments examining the temperature response of labile C have not considered priming as important (Fierer *et al.*, 2005; Chen *et al.*, 2010). The stable C pool was represented as the respiration rates derived from soil organic matter (SOM), measured directly from the soil incubated without added C. Generally, the differences in temperature sensitivity of the labile and stable C pools in soil are indicative of their varying substrate availabilities (Schipper *et al.*, 2019). A preliminary experiment was undertaken to determine an appropriate concentration for the C compounds

to ensure a significant difference in substrate availability between the two pools (i.e. labile C is highly available, and stable C is not). All added carbon compounds were made up to a concentration of 450 mM C which, after a six-hour incubation period, less than 15% of the carbon added was respired. This experiment suggested that the soil with added C was not C limited, and was, therefore, a fair representation of the labile pool with high substrate availability.

The temperature response of a typical simple C compound (glucose) in soil was measured from three different soil types (allophanic, gley, and organic soil). This experiment revealed that although these soils had very different properties, the temperature response of added glucose decomposition was remarkedly similar (T_{opt} = 35°C, T_{inf} = 18-21°C). This similarity was attributed to the soils consisting of microbial communities that were functionally similar, although potentially of different species composition. This similarity in microbial communities was attributed to these soils being collected in very close proximity to each other (Newstead, Waikato), which might explain their temperature response.

I also demonstrated that the temperature response of five different simple carbon compounds and yeast extract (wide variety of carbon compounds) added to one soil (allophanic) was also very similar when their temperature parameters (Topt = 33-39°C, Tinf = 19-25°C) were compared. In all cases, the simple C compounds and yeast extract were well described by MMRT, and their T_{opt} and T_{inf} points could be derived. However, respiration rates from SOM (stable C pool) could not be fitted to MMRT and exhibited typical Arrhenius behaviour with no $T_{opt,}$ or T_{inf} points producible. Robinson *et al.* (submitted) added ^{13}C labelled plant litter to soil which was incubated at 30 temperatures (~2-50°C) and also found that SOM-R_S could not fit MMRT. However, two other similar studies Robinson *et al.* (2017); Schipper *et al.* (2019), incubated soil at 40 discrete temperatures (2-50°C) and were able to fit MMRT to SOM, which highlights the importance of sufficient incubation temperatures for accurate curve fitting. The temperature response parameters of litter added to soil (T_{opt} = 42°C, and T_{inf} = 24°C) reported in Robinson *et al.* (submitted) were slightly higher, but comparable, with the

parameters for the added C compounds in this study. The slightly lower T_{opt} and T_{inf} points for added C substrates indicated that these compounds were more available to the microbes for decomposition compared to the added plant litter.

Two types of processes lead to the decomposition of soil C: biological degradation of C by microbes and the physiochemical processes of diffusion and sorption/desorption that allow protected C to be utilised by soil microbes. When substrate availability is high, biological degradation will dominate the temperature response of soil respiration (i.e. MMRT). However, when substrate availability is low (likely due to physical and chemical protection), the physiochemical processes increasing C availability to the microbes will dominate the temperature response (i.e. Arrhenius) (Figure 5.1). Natural soil respiration will be a mixture of these two processes and will shift towards a more MMRT-like or Arrhenius-like function depending on the soil's substrate availability. This theory indicates that labile C compounds (simple C and yeast extract) were more available to the microbes than plant litter was, reported by Robinson *et al.* (submitted), as the labile compounds had lower temperature parameters (T_{opt} and T_{inf}), indicating a more dominant MMRT response (Figure 5.1).

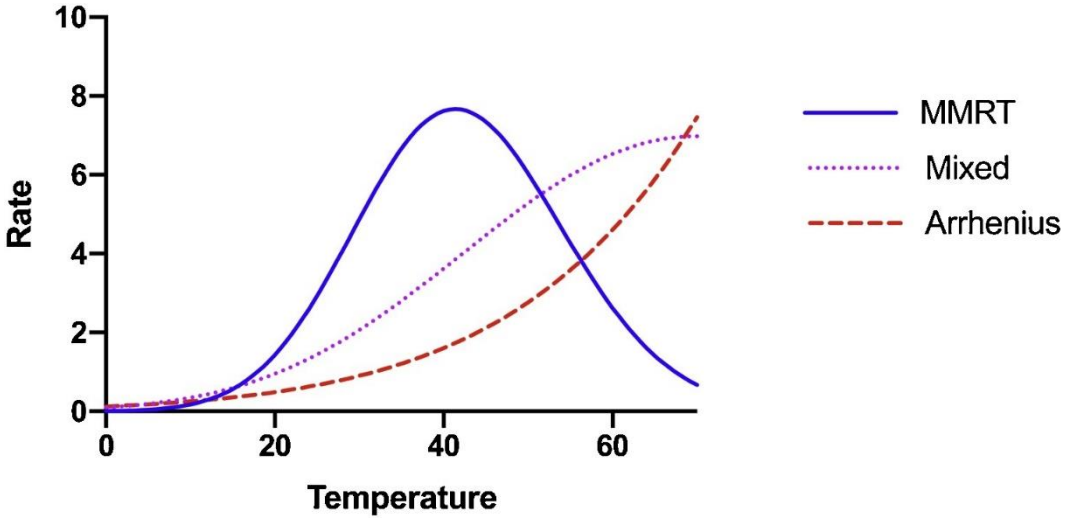

Figure 5.1 Conceptual figure by Schipper *et al.* (2019) showing a typical reaction rate response from biological processes where $\Delta C_P^{\ddagger} \neq 0$ (macromolecular rate theory, blue line) and from physical chemistry processes where $\Delta C_P^{\ddagger} = 0$ (Arrhenius, dashed red line) with increasing temperature. The dotted pink curve represents a 'mixture' of these two temperature responses. This 'mixture' curve will shift towards a more MMRT or Arrhenius curvature depending on the availability of C in the soil (i.e. MMRT is typically observed in soils with high available C, and Arrhenius is observed in soils with low availability of C (Schipper *et al.*, 2019)

Obvious temperature optima and inflection points for respiration (either labile or stable) are not frequently reported in the literature. While the temperature optima measured here were quite high for typically observed soil temperatures (37-39°C), the inflection temperature's (the temperature at which rates of change were greatest) were well within the range observed in soil. Two likely reasons for few observations of T_{opt} and T_{inf} are related to incubation methodologies. Most incubation studies attempt curve fitting for only 2-5 temperatures (Robinson *et al.*, 2017), with the warmest temperature usually set below 35°C. Robinson *et al.* (2017) reported that the reliability of curve fits begin to decline below 20 individual incubation temperatures. A further limitation of the methodologies described in the literature is the length of the incubation period. A preliminary experiment undertaken in this study found that after a six hour incubation CO_2 production was linear for soils both with and without added glucose. The linear production of CO_2 with time indicated that microbial growth had not occurred, thus an accurate representation of the temperature response from the microbial population at the time of collection was observed. However, most incubation studies incubate soils between 1-200+ days (e.g. Bekku *et al.* (2003)= 12-18 hours Fierer *et al.* (2005)= 53 days, Thiessen *et al.* (2013)= 199 days). These incubation lengths likely led to microbial adaptation resulting in an obscured 'in-situ' temperature response.

In this study, the temperature sensitivity of the labile (simple C compounds and yeast) and stable (SOM) C pool was calculated in relative and absolute terms. Overall, the labile C pool was less temperature sensitive in *relative* terms but more temperature sensitive in *absolute* terms (more so at lower temperatures) than the stable C pool as predicted by Sierra (2012). The opposing behaviours of these two measures of temperature sensitivity highlight the need for the explicit distinction between these two terms in the literature. Briefly, the absolute temperature sensitivity is the absolute change in rate for a given unit change in temperature $\left(\frac{\partial R_S}{\partial T}\right)$, and the relative temperature sensitivity is the absolute temperature relative to the actual value of the rate $\left(\frac{1}{R_S}\frac{\partial R_S}{\partial T}\right)$ (Sierra, 2012). The relative temperature sensitivity is the most commonly used measure within the literature, which is calculated using

Q_{10}. Q_{10} is the factor by which a reaction changes for a 10°C shift in temperature (Conant *et al.*, 2011). Despite the prevalent use of Q_{10} in the literature, it is difficult to compare these values between studies as there are several different equations used to calculate this coefficient (Sierra, 2012). Depending on the equation used, the interpretation and comparison of Q_{10} values should be executed with care, and in general, it should be noted that this coefficient does not provide information on what pools are releasing more C to the atmosphere (Sierra, 2012). According to Sierra (2012), only the absolute temperature sensitivity takes into account the size of the decomposing C pool; therefore, this measure of sensitivity also depicts the amount of C respired.

There have been very few studies examining the *reasons* for differences in temperature sensitivities of labile and stable C pools. Previously, differences in the temperature sensitivities in soil have been attributed to the chemical complexity of the decomposing carbon molecule. Fierer *et al.* (2005) looked at the temperature sensitivity of different C compounds of varying qualities (i.e. high-quality substrates comprise simple carbon compounds, and low-quality substrates comprise more complex carbon compounds). The study found that the relative temperature sensitivity (measured using Q_{10}) of the higher quality substrates (i.e. labile C pool) was lower than for the lower quality substrates (i.e. stable C pool). Sierra (2012) states that although this agrees with the most common conclusion in the literature, this relationship could have been produced using randomly generated numbers. Sierra (2012) suggested that the term *B*, an exponential fit parameter, was the reason for this behaviour, as *B* was used to calculate Q_{10} (i.e. $Q_{10} = exp\left(\frac{10}{T}\ln\left(\frac{R}{B}\right)\right)$ and also the organic C quality index (*B*). Therefore, this relationship is likely a mathematical artefact rather than a true representation of the temperature sensitivities of different C qualities. The general consensus in the literature now is that the difference in temperature sensitivities between two C pools is attributed to differences in substrate availability (Davidson & Janssens, 2006; Gershenson *et al.*, 2009; Schipper *et al.*, 2019).

As the methodologies of this study required the addition of exogenous labile C to soil, I also conducted a preliminary study to determine the magnitude and temperature response of the soil priming effect, where additions of C to soil

induce native SOM decomposition. I observed that priming contributed a significant proportion (~30%) to the total soil respiration when labile C was added to soil. The respiration rates derived from priming was well fitted with MMRT and shared a very similar response to temperature with the added labile C substrates. The T_{opt} and T_{inf} points for priming-R_S (30°C and 15°C, respectively) were slightly lower than for the added C substrates indicating that the C decomposed due to priming was more readily available (Figure 5.1). As discussed in the previous chapter, there were limitations of the novel method used to measure the temperature response of priming. Therefore, these results should be taken as observations only, clearly indicating the need for additional measurements.

Overall, this study determined that the temperature responses of the labile and stable C pools are distinctly different. I found that the relationship between temperature and the decomposition of the labile C pool was well described by MMRT, while an Arrhenius-like function better described the decomposition of the stable C pool (i.e. SOM). The difference in the temperature response of these two pools was attributed to their substrate availability (Schipper *et al.*, 2019). The labile C pool consists of C that is readily available to the microbes; therefore, the biological degradation process dominates the temperature response, which is well described by MMRT (Figure 5.1). On the other hand, the stable C pool consists of C that is protected from microbes; therefore, the physiochemical processes of diffusion and sorption/desorption will dominate which exerts an Arrhenius-like temperature response (Figure 5.1). This study found that the temperature response of these two pools did not significantly vary between the different simple C compounds or soil types. These results suggest that a simple two pool soil C model (i.e. labile and stable C) might be sufficient for describing soil C dynamics, which could also be extrapolated to larger ecosystem models. However, further research is needed to determine if these temperature response vary globally.

5.2 Future work

Currently, priming is not incorporated into most ecosystem models, which could potentially lead to an overestimation of soil C storage. Although this study provides some preliminary results indicating that priming is a significant source of total soil respiration, it is now important to determine if the priming effect varies with region (i.e. soil type, elevation, climate, C inputs). This could be determined by adding a range of ^{13}C labelled compounds to a wide variety of soils from different locations. By incubating these soils and using isotopic analysis, a more reliable temperature response of priming could be measured.

While this study showed a similar temperature response of three contrasting soils, how conserved are these responses for a much wider distribution of soils? An experiment should be conducted to determine whether soil from a wide range of locations has the same temperature response when incubated along a temperature gradient. This experiment could conclude what the influence of climate, elevation, vegetation, land management and more is on the temperature sensitivity of not only soil respiration but, more specifically, the different pools of C in soil. A large synthesis study by Carey *et al.* (2016) found that the temperature sensitivity of soil respiration potentially varies with region. Therefore, a study should be undertaken to confirm if this is accurate. To determine this, soils from a wide range of locations should be collected and incubated at >18 discrete temperatures with and without added labile C substrate for a period that ensures no microbial adaption/growth occurs.

It would also be interesting to measure the temperature response of soils from a natural temperature gradient (e.g. geothermal gradient) to determine if the enzymes that were adapted to their specific soil's temperature exhibited the same temperature response when all other variables are held constant (i.e. vegetation, climate, and land management). This study would also help determine if the temperature response of soil respiration varied with regions based on their annual average soil temperatures.

These future studies will be vital to the development of Earth system and smaller C cycling models, to ensure their accuracy and reliability, particularly for climate projection models.

5.3 Recommendations

While the suggestion of future work above are focused on testing hypotheses and improving our understanding of temperature responses, the following recommendations have been made to derive an accurate temperature response from soil, or more specifically to separate the temperature response of the labile and stable C pools in soil:

- Soils should be incubated at more than 18 different temperatures to produce accurate curve fits.
- Shorter incubation periods (<24 hours) should be undertaken to ensure microbial adaptation/growth does not occur. Determination of adaption requires a different set of experiments, and a preliminary experiment should be completed to confirm the occurrence/absence of adaption/growth.
- Clearly define what measure of temperature sensitivity is being reported, as relative and absolute temperature sensitivities can have opposing behaviours. Also, take note of the equations used to derive Q_{10} when interpreting or comparing values between different studies.
- The soil priming effect should be recognised when incubating soils with added C compounds. If an isotopic analysis is not conducted on ^{13}C labelled carbon, then the study should acknowledge that priming is likely occurring.
- When incubated soils are not substrate limited, the biological decomposition of the substrates dominates over diffusion/sorption processes. This study demonstrated that MMRT was a useful model to represent this biological process, whereas the Arrhenius model was useful when soil was limited by substrate availability and physiochemical processes dominated the system. Therefore, when describing the temperature response of soil respiration, both MMRT and Arrhenius should be considered, especially when describing the temperature response of two C pools.

References

Arcus, V. L., Prentice, E. J., Hobbs, J. K., Mulholland, A. J., Van Der Kamp, M. W., Pudney, C. R., Parker, E. J., & Schipper, L. A. (2016). On the Temperature Dependence of Enzyme-Catalyzed Rates. *Biochemistry, 55*(12), 1681-1688. 10.1021/acs.biochem.5b01094.

Arrhenius, S. (1896). Nature's heat usage. *Nordisk Tidskrift, 14*, 121-130.

Bartlett, J. R., & Doner, H. E. (1988). Decomposition of lysine and leucine in soil aggregates: Adsorption and compartmentalization. *Soil Biology and Biochemistry, 20*(5), 755-759. https://doi.org/10.1016/0038-0717(88)90163-0.

Bekku, Y. S., Nakatsubo, T., Kume, A., Adachi, M., & Koizumi, H. (2003). Effect of warming on the temperature dependence of soil respiration rate in arctic, temperate and tropical soils. *Applied soil ecology, 22*(3), 205-210.

Blankinship, J. C., Berhe, A. A., Crow, S. E., Druhan, J. L., Heckman, K. A., Keiluweit, M., Lawrence, C. R., Marín-Spiotta, E., Plante, A. F., Rasmussen, C., Schädel, C., Schimel, J. P., Sierra, C. A., Thompson, A., Wagai, R., & Wieder, W. R. (2018). Improving understanding of soil organic matter dynamics by triangulating theories, measurements, and models. *Biogeochemistry, 140*(1), 1-13. 10.1007/s10533-018-0478-2.

Bleam, W. F. (2011). *Soil and Environmental Chemistry*. Elsevier Science.

Bol, R., Poirier, N., Balesdent, J., & Gleixner, G. (2009). Molecular turnover time of soil organic matter in particle-size fractions of an arable soil. *Rapid Communications in Mass Spectrometry, 23*(16), 2551-2558. 10.1002/rcm.4124.

Bolin, B. (1970). The Carbon Cycle. *Scientific American, 223*(3), 124-135.

Bond-Lamberty, B., Bailey, V. L., Chen, M., Gough, C. M., & Vargas, R. (2018). Globally rising soil heterotrophic respiration over recent decades. *Nature, 560*(7716), 80-83. 10.1038/s41586-018-0358-x.

Brown, R., & Markewitz, D. (2018). Soil heterotrophic respiration: Measuring and modeling seasonal variation and silvicultural impacts. *Forest Ecology and Management, 430*, 594-608. 10.1016/j.foreco.2018.08.018.

Carey, J. C., Tang, J., Templer, P. H., Kroeger, K. D., Crowther, T. W., Burton, A. J., Dukes, J. S., Emmett, B., Frey, S. D., Heskel, M. A., Jiang, L., Machmuller, M. B., Mohan, J., Panetta, A. M., Reich, P. B., Reinsch, S., Wang, X., Allison, S. D., Bamminger, C., Bridgham, S., Collins, S. L., de Dato, G., Eddy, W. C., Enquist, B. J., Estiarte, M., Harte, J., Henderson, A., Johnson, B. R., Larsen, K. S., Luo, Y., Marhan, S., Melillo, J. M., Peñuelas, J., Pfeifer-Meister, L., Poll, C., Rastetter, E., Reinmann, A. B., Reynolds, L. L., Schmidt, I. K., Shaver, G. R., Strong, A. L., Suseela, V., & Tietema, A. (2016). Temperature response of soil respiration largely unaltered with experimental warming.

Proceedings of the National Academy of Sciences, 113(48), 13797. 10.1073/pnas.1605365113.

Chen, X., Tang, J., Jiang, L., Li, B., Chen, J., & Fang, C. (2010). Evaluating the impacts of incubation procedures on estimated Q10 values of soil respiration. *Soil Biology and Biochemistry, 42*(12), 2282-2288.

Conant, R. T., Ryan, M. G., Ågren, G. I., Birge, H. E., Davidson, E. A., Eliasson, P. E., Evans, S. E., Frey, S. D., Giardina, C. P., Hopkins, F. M., Hyvönen, R., Kirschbaum, M. U. F., Lavallee, J. M., Leifeld, J., Parton, W. J., Megan Steinweg, J., Wallenstein, M. D., Martin Wetterstedt, J. Å., & Bradford, M. A. (2011). Temperature and soil organic matter decomposition rates - synthesis of current knowledge and a way forward. *Global Change Biology, 17*(11), 3392-3404. 10.1111/j.1365-2486.2011.02496.x.

Cook, F. J., & Orchard, V. A. (2008). Relationships between soil respiration and soil moisture. *Soil Biology and Biochemistry, 40*(5), 1013-1018. 10.1016/j.soilbio.2007.12.012.

Dash, P. K., Bhattacharyya, P., Roy, K. S., Neogi, S., & Nayak, A. K. (2019). Environmental constraints' sensitivity of soil organic carbon decomposition to temperature, management practices and climate change. *Ecological Indicators, 107*, 105644. 10.1016/j.ecolind.2019.105644.

Davidson, E. A., & Janssens, I. A. (2006). Temperature sensitivity of soil carbon decomposition and feedbacks to climate change. *Nature, 440*(7081), 165-173. 10.1038/nature04514.

DeCiucies, S., Whitman, T., Woolf, D., Enders, A., & Lehmann, J. (2018). Priming mechanisms with additions of pyrogenic organic matter to soil. *Geochimica et Cosmochimica Acta, 238*, 329-342.

Degens, B. P., Schipper, L. A., Sparling, G. P., & Duncan, L. C. (2001). Is the microbial community in a soil with reduced catabolic diversity less resistant to stress or disturbance? *Soil Biology and Biochemistry, 33*(9), 1143-1153. https://doi.org/10.1016/S0038-0717(01)00018-9.

Dettmann, E. H. (2013). Turnover Time☆. In B. Fath (Ed.), *Encyclopedia of Ecology (Second Edition)* (pp. 503-508). Oxford: Elsevier. http://www.sciencedirect.com/science/article/pii/B9780124095489006096.

Falkowski, P., Scholes, R. J., Boyle, E., Canadell, J., Canfield, D., Elser, J., Gruber, N., Hibbard, K., Högberg, P., Linder, S., Mackenzie, F. T., Moore Iii, B., Pedersen, T., Rosenthal, Y., Seitzinger, S., Smetacek, V., & Steffen, W. (2000). The Global Carbon Cycle: A Test of Our Knowledge of Earth as a System. *Science, 290*(5490), 291.

Fang, C., & Moncrieff, J. B. (2001). The dependence of soil CO2 efflux on temperature. *Soil Biology & Biochemistry, 33*(2), 155-165. Doi 10.1016/S0038-0717(00)00125-5.

Fierer, N., Craine, J. M., McLauchlan, K., & Schimel, J. P. (2005). Litter quality and the temperature sensitivity of decomposition. *Ecology, 86*(2), 320-326. Doi 10.1890/04-1254.

Freeman, C., Ostle, N., & Kang, H. (2001). An enzymic 'latch' on a global carbon store. *Nature, 409*(6817), 149-149. 10.1038/35051650.

Gershenson, A., Bader, N. E., & Cheng, W. (2009). Effects of substrate availability on the temperature sensitivity of soil organic matter decomposition. *Global Change Biology, 15*(1), 176-183. 10.1111/j.1365-2486.2008.01827.x.

Girkin, N. T., Dhandapani, S., Evers, S., Ostle, N., Turner, B. L., & Sjögersten, S. (2020). Interactions between labile carbon, temperature and land use regulate carbon dioxide and methane production in tropical peat. *Biogeochemistry, 147*(1), 87-97. 10.1007/s10533-019-00632-y.

Gougoulias, C., Clark, J. M., & Shaw, L. J. (2014). The role of soil microbes in the global carbon cycle: tracking the below-ground microbial processing of plant-derived carbon for manipulating carbon dynamics in agricultural systems. *Journal of the Science of Food and Agriculture, 94*(12), 2362-2371. doi:10.1002/jsfa.6577.

Guenet, B., Camino-Serrano, M., Ciais, P., Tifafi, M., Maignan, F., Soong, J. L., & Janssens, I. A. (2018). Impact of priming on global soil carbon stocks. *Global Change Biology, 24*(5), 1873-1883. 10.1111/gcb.14069.

Harding, D. E., & Ross, D. J. (1964). Some factors in low-temperature storage influencing the mineralisable-nitrogen of soils. *Journal of the Science of Food and Agriculture, 15*(12), 829-834. 10.1002/jsfa.2740151203.

Hindman, B. (2006). *What is an iButton device?* Technical Documents. Retrieved 10th January 2020, from https://www.maximintegrated.com/en/design/technical-documents/app-notes/3/3808.html.

Hobbs, J. K., Jiao, W., Easter, A. D., Parker, E. J., Schipper, L. A., & Arcus, V. L. (2013). Change in Heat Capacity for Enzyme Catalysis Determines Temperature Dependence of Enzyme Catalyzed Rates. *ACS Chemical Biology, 8*(11), 2388-2393. 10.1021/cb4005029.

Hopkins, D. (2006). Microorganisms in Soils: Roles in Genesis and Functions - Edited by A. Buscot & A. Varma. *European Journal of Soil Science - EUR J SOIL SCI, 57*, 925-926. 10.1111/j.1365-2389.2006.00860_4.x.

Horwath, W. (2015). Chapter 12 - Carbon Cycling: The Dynamics and Formation of Organic Matter. In E. A. Paul (Ed.), *Soil Microbiology, Ecology and Biochemistry (Fourth Edition)* (pp. 339-382). Boston: Academic Press.

Janzen, H. H. (2004). Carbon cycling in earth systems - a soil science perspective. *Agriculture Ecosystems & Environment, 104*(3), 399-417. 10.1016/j.agee.2004.01.040.

Jenkinson, D. S., Andrew, S. P. S., Lynch, J. M., Goss, M. J., & Tinker, P. B. (1990). The Turnover of Organic Carbon and Nitrogen in Soil [and Discussion]. *Philosophical Transactions: Biological Sciences, 329*(1255), 361-368.

Jin, Q., & Kirk, M. F. (2018). pH as a Primary Control in Environmental Microbiology: 1. Thermodynamic Perspective. *Frontiers in Environmental Science, 6*. 10.3389/fenvs.2018.00021.

Kandeler, E., Stemmer, M., & Gerzabek, M. H. (2005). Role of microorganisms in carbon cycling in soils. In *Microorganisms in soils: roles in genesis and functions* (pp. 139-157). Springer.

Lal, R. (2014). Societal value of soil carbon. *Journal of Soil and Water Conservation, 69*(6), 186a-192a. 10.2489/jswc.69.6.186A.

Leifeld, J., & von Lützow, M. (2014). Chemical and microbial activation energies of soil organic matter decomposition. *Biology and Fertility of Soils, 50*(1), 147-153. 10.1007/s00374-013-0822-6.

Li, C., Peng, Y., Nie, X., Yang, Y., Yang, L., Li, F., Fang, K., Xiao, Y., & Zhou, G. (2018). Differential responses of heterotrophic and autotrophic respiration to nitrogen addition and precipitation changes in a Tibetan alpine steppe. *Scientific reports, 8*(1), 16546.

Li, J. Q., Pei, J. M., Cui, J., Chen, X. P., Li, B., Nie, M., & Fang, C. M. (2017). Carbon quality mediates the temperature sensitivity of soil organic carbon decomposition in managed ecosystems. *Agriculture Ecosystems & Environment, 250*, 44-50. 10.1016/j.agee.2017.09.001.

Liu, Y., He, N. P., Wen, X. F., Xu, L., Sun, X. M., Yu, G. R., Liang, L. Y., & Schipper, L. A. (2018). The optimum temperature of soil microbial respiration: Patterns and controls. *Soil Biology & Biochemistry, 121*, 35-42. 10.1016/j.soilbio.2018.02.019.

Lloyd, J., & Taylor, J. A. (1994). On the Temperature-Dependence of Soil Respiration. *Functional Ecology, 8*(3), 315-323. Doi 10.2307/2389824.

Luo, Y., Ahlström, A., Allison, S. D., Batjes, N. H., Brovkin, V., Carvalhais, N., Chappell, A., Ciais, P., Davidson, E. A., Finzi, A., Georgiou, K., Guenet, B., Hararuk, O., Harden, J. W., He, Y., Hopkins, F., Jiang, L., Koven, C., Jackson, R. B., Jones, C. D., Lara, M. J., Liang, J., McGuire, A. D., Parton, W., Peng, C., Randerson, J. T., Salazar, A., Sierra, C. A., Smith, M. J., Tian, H., Todd-Brown, K. E. O., Torn, M., Van Groenigen, K. J., Wang, Y. P., West, T. O., Wei, Y., Wieder, W. R., Xia, J., Xu, X., Xu, X., & Zhou, T. (2016). Toward more realistic projections of soil carbon dynamics by Earth system models. *Global Biogeochemical Cycles, 30*(1), 40-56. 10.1002/2015gb005239.

Malik, A. A., Puissant, J., Buckeridge, K. M., Goodall, T., Jehmlich, N., Chowdhury, S., Gweon, H. S., Peyton, J. M., Mason, K. E., Van Agtmaal, M., Blaud, A., Clark, I. M., Whitaker, J., Pywell, R. F., Ostle, N., Gleixner, G., & Griffiths, R. I. (2018). Land use driven change in soil pH affects microbial carbon

cycling processes. *Nature Communications, 9*(1). 10.1038/s41467-018-05980-1.

Moyano, F. E., Manzoni, S., & Chenu, C. (2013). Responses of soil heterotrophic respiration to moisture availability: An exploration of processes and models. *Soil Biology and Biochemistry, 59*, 72-85. 10.1016/j.soilbio.2013.01.002.

Moyano, F. E., Vasilyeva, N., Bouckaert, L., Cook, F., Craine, J., Curiel Yuste, J., Don, A., Epron, D., Formanek, P., Franzluebbers, A., Ilstedt, U., Kätterer, T., Orchard, V., Reichstein, M., Rey, A., Ruamps, L., Subke, J. A., Thomsen, I. K., & Chenu, C. (2012). The moisture response of soil heterotrophic respiration: interaction with soil properties. *Biogeosciences, 9*(3), 1173-1182. 10.5194/bg-9-1173-2012.

NASA. (2019). *Carbon Dioxide*. Vital Signs. Retrieved 21 November, 2019, from https://climate.nasa.gov/vital-signs/carbon-dioxide/.

NASA/GISS. (2019). *Global Temperature*. Vital Signs. Retrieved 21 November, 2019, from https://climate.nasa.gov/vital-signs/global-temperature/.

NOAA. (2019). *Monthly Average Mauna Loa CO2*. Retrieved 21 November, 2019, from https://www.esrl.noaa.gov/gmd/ccgg/trends/.

Parton, W. J., Schimel, D. S., Cole, C. V., & Ojima, D. S. (1987). Analysis of Factors Controlling Soil Organic Matter Levels in Great Plains Grasslands1. *Soil Science Society of America Journal, 51*, 1173-1179. 10.2136/sssaj1987.03615995005100050015x.

Redasani, V. K., & Bari, S. B. (2015). *Prodrug Design : Perspectives, Approaches and Applications in Medicinal Chemistry*. San Diego, UNITED STATES: Elsevier Science & Technology.

Robinson, J. M. (2016). *Temperature Sensitivity of Soil Respiration.* Masters thesis, University of Waikato, Hamilton, New Zealand.

Robinson, J. M., Barker, S., Arcus, V. L., McNally, S., & Schipper, L. A. (submitted). Measuring the temperature response of soil respiration from two distinct carbon pools. *Biogeochemistry*.

Robinson, J. M., O'Neill, T. A., Ryburn, J., Liang, L. L., Arcus, V. L., & Schipper, L. A. (2017). Rapid laboratory measurement of the temperature dependence of soil respiration and application to changes in three diverse soils through the year. *Biogeochemistry, 133*(1), 101-112. 10.1007/s10533-017-0314-0.

Rodhe, H., Charlson, R., & Crawford, E. (1997). Svante Arrhenius and the greenhouse effect. *Ambio*, 2-5.

Ryan, M. G., & Law, B. E. (2005). Interpreting, measuring, and modeling soil respiration. *Biogeochemistry, 73*(1), 3-27.

Sanchez, P., Palm, C., Szott, L., Cuevas, E., & Lal, R. (1989). Organic input management in tropical agroecosystems. *Dynamics of soil organic matter in tropical ecosystems, 25*, 152.

Schipper, L. A., Hobbs, J. K., Rutledge, S., & Arcus, V. L. (2014). Thermodynamic theory explains the temperature optima of soil microbial processes and high Q(10) values at low temperatures. *Global Change Biology, 20*(11), 3578-3586. 10.1111/gcb.12596.

Schipper, L. A., Petrie, O. J., O'Neill, T. A., Mudge, P. L., Liáng, L. L., Robinson, J. M., & Arcus, V. L. (2019). Shifts in temperature response of soil respiration between adjacent irrigated and non-irrigated grazed pastures. *Agriculture, Ecosystems & Environment, 285*, 106620. 10.1016/j.agee.2019.106620.

Schlesinger, W. H. (1995). *Soil respiration and changes in soil carbon stocks*. Biotic Feedbacks in the Global Climatic System: Will the Warming Feed the Warming? Oxford University Press.

Sierra, C. A. (2012). Temperature sensitivity of organic matter decomposition in the Arrhenius equation: some theoretical considerations. *Biogeochemistry, 108*(1/3), 1-15.

Sollins, P., Homann, P., & Caldwell, B. A. (1996). Stabilization and destabilization of soil organic matter: mechanisms and controls. *Geoderma, 74*(1-2), 65-105.

Sulman, B. N., Phillips, R. P., Oishi, A. C., Shevliakova, E., & Pacala, S. W. (2014). Microbe-driven turnover offsets mineral-mediated storage of soil carbon under elevated CO 2. *Nature Climate Change, 4*(12), 1099-1102.

Sun, Z., Liu, S., Zhang, T., Zhao, X., Chen, S., & Wang, Q. (2019). Priming of soil organic carbon decomposition induced by exogenous organic carbon input: a meta-analysis. *Plant and Soil, 443*(1-2), 463-471.

Thiessen, S., Gleixner, G., Wutzler, T., & Reichstein, M. (2013). Both priming and temperature sensitivity of soil organic matter decomposition depend on microbial biomass – An incubation study. *Soil Biology and Biochemistry, 57*, 739-748. 10.1016/j.soilbio.2012.10.029.

van't Hoff, J. H. (1884). *Etudes de dynamique chimique*. (Vol. 1). Muller.

Von Lützow, M., & Kögel-Knabner, I. (2009). Temperature sensitivity of soil organic matter decomposition—what do we know? *Biology and Fertility of Soils, 46*(1), 1-15. 10.1007/s00374-009-0413-8.

von Lutzow, M., Kogel-Knabner, I., Ekschmittb, K., Flessa, H., Guggenberger, G., Matzner, E., & Marschner, B. (2007). SOM fractionation methods: Relevance to functional pools and to stabilization mechanisms. *Soil Biology & Biochemistry, 39*(9), 2183-2207. 10.1016/j.soilbio.2007.03.007.

von Lützow, M., Kögel-Knabner, I., Ludwig, B., Matzner, E., Flessa, H., Ekschmitt, K., Guggenberger, G., Marschner, B., & Kalbitz, K. (2008). Stabilization mechanisms of organic matter in four temperate soils: Development and application of a conceptual model. *Journal of Plant Nutrition and Soil Science, 171*(1), 111-124. doi:10.1002/jpln.200700047.

Yang, Z., Wullschleger, S. D., Liang, L., Graham, D. E., & Gu, B. (2016). Effects of warming on the degradation and production of low-molecular-weight labile organic carbon in an Arctic tundra soil. *Soil Biology and Biochemistry, 95*, 202-211. 10.1016/j.soilbio.2015.12.022.

Zhu, B., & Cheng, W. (2011). Rhizosphere priming effect increases the temperature sensitivity of soil organic matter decomposition. *Global Change Biology, 17*(6), 2172-2183. 10.1111/j.1365-2486.2010.02354.x.

Appendix

Table A.1 R^2 values depicting the linearity of CO_2 production at 18 incubation temperatures (8-52°C) over six hours from soil (Horotiu, allophanic) with and without added glucose (450 mM C)

Soil with no added glucose (control)		Soil with added glucose (amended)	
Temperature (°C)	R^2	Temperature (°C)	R^2
8	0.70	8	0.93
10	0.99	10	0.93
13	0.87	13	0.97
16	0.97	16	0.98
18	0.98	18	0.97
21	0.96	21	0.99
23	0.92	23	1.00
26	1.00	26	1.00
28	0.98	28	0.98
31	0.96	31	0.97
33	0.99	33	0.98
36	0.93	36	1.00
39	0.97	39	0.99
41	0.97	41	0.98
44	0.93	44	0.99
46	0.96	46	1.00
49	0.91	49	0.98
52	0.99	52	0.87

Table A.2 Temperature response parameters for total soil respiration (soil with added glucose) derived from the macromolecular rate theory equation for three replicates of each soil type

Soil Type	Replicates	T_{inf} (°C)	T_{opt} (°C)	$\Delta C_P^{\ddagger}$ (J mol^{-1} K^{-1})	$\Delta H_{T_0}^{\ddagger}$ (J mol^{-1} K^{-1})	$\Delta S_{T_0}^{\ddagger}$ (J mol^{-1} K^{-1})
Te Kowhai	A	24	43	-2224	38221	-171
	B	25	46	-1843	36358	-178
	C	24	44	-2099	38029	-173
Horotiu	A	22	47	-1310	26481	-200
	B	23	43	-1976	33214	-174
	C	25	47	-1743	55127	-166
Te Rapa	A	22	44	-1696	30304	-186
	B	21	44	-1651	27966	-195
	C	23	49	-1263	27551	-197

Table A.3 Temperature response parameters for glucose respiration (difference between total-R_S and SOM-R_S) derived from the macromolecular rate theory equation for three replicates of each soil type

Soil Type	Replicates	T_{inf} (°C)	T_{opt} (°C)	$\Delta C_P^{\ddagger}$ (J mol^{-1} K^{-1})	$\Delta H_{T_0}^{\ddagger}$ (J mol^{-1} K^{-1})	$\Delta S_{T_0}^{\ddagger}$ (J mol^{-1} K^{-1})
Te Kowhai	A	20	34	-4364	34943	-185
	B	21	36	-3642	37775	-176
	C	21	35	-3914	37585	-176
Horotiu	A	17	33	-3153	22716	-214
	B	19	35	-2880	27217	-195
	C	20	37	-2754	30139	-187
Te Rapa	A	20	39	-2208	28724	-192
	B	17	32	-3396	22647	-214
	C	18	35	-2708	23808	-211

Table A.4 Temperature response parameters for SOM respiration (soil with no added glucose) derived from the macromolecular rate theory equation for three replicates of each soil type

Soil Type	Replicates	T_{inf} (°C)	T_{opt} (°C)	$\Delta C_P^{\ddagger}$ (J mol^{-1} K^{-1})	$\Delta H_{T_0}^{\ddagger}$ (J mol^{-1} K^{-1})	$\Delta S_{T_0}^{\ddagger}$ (J mol^{-1} K^{-1})
Te Kowhai	A	n/a	n/a	13	39922	-176
	B	n/a	n/a	869	32781	-201
	C	n/a	n/a	576	36591	-189
Horotiu	A	n/a	n/a	1170	1170	-199
	B	n/a	n/a	12	53290	-123
	C	n/a	n/a	-208	55127	-117
Te Rapa	A	n/a	n/a	414	36292	-184
	B	n/a	n/a	817	35608	-182
	C	n/a	n/a	933	32247	-193

Table A.5 Temperature response parameters for total soil respiration (soil with added carbon substrate) derived from the macromolecular rate theory for three replicates of each added carbon substrate

Carbon Type	Replicates	T_{inf} (°C)	T_{opt} (°C)	$\Delta C_P^{\ddagger}$ (J mol^{-1} K^{-1})	$\Delta H_{T_0}^{\ddagger}$ (J mol^{-1} K^{-1})	$\Delta S_{T_0}^{\ddagger}$ (J mol^{-1} K^{-1})
Glucose	A	22	47	-1310	26481	-200
	B	23	43	-1976	33214	-174
	C	25	47	-1743	55127	-166
Maltose	A	28	53	-1367	35946	-171
	B	32	59	-1240	39144	-157
	C	30	40	-1705	43734	-142
Glutamine	A	25	46	-1909	38005	-163
	B	24	42	-2564	41202	-152
	C	24	43	-2292	38736	-161
Dextran	A	n/a	n/a	1153	42658	-159
	B	n/a	n/a	742	53657	-123
	C	n/a	n/a	305	52454	-127
Arginine	A	33	54	-2061	57098	-105
	B	36	58	-1764	56168	-109
	C	38	63	-1513	55257	-112
Lysine	A	n/a	n/a	133	46567	-145
	B	n/a	n/a	106	48830	-138
	C	n/a	n/a	-379	49924	-134
Yeast	A	45	50	-51442	1.26E+06	4226
	B	39	42	-1.1E+05	1.84E+06	6305
	C	41	45	-50779	9.96E+05	3311

Table A.6 Temperature response parameters for carbon compound respiration (difference between total-R_S and SOM-R_S) derived from the macromolecular rate theory for three replicates of each added carbon substrate

Carbon Type	Replicates	T_{inf} (°C)	T_{opt} (°C)	$\Delta C_P^{\ddagger}$ (J mol^{-1} K^{-1})	$\Delta H_{T_0}^{\ddagger}$ (J mol^{-1} K^{-1})	$\Delta S_{T_0}^{\ddagger}$ (J mol^{-1} K^{-1})
Glucose	A	17	33	-3153	22716	-214
	B	19	35	-2880	27217	-195
	C	20	37	-2754	30139	-187
Maltose	A	22	35	-4157	40867	-158
	B	23	43	-1986	33498	-178
	C	23	40	-2836	38732	-161
Glutamine	A	19	33	-4386	31192	-187
	B	21	34	-4154	36158	-170
	C	19	31	-5440	29163	-194
Dextran	A	n/a	n/a	n/a	n/a	n/a
	B	n/a	n/a	n/a	n/a	n/a
	C	n/a	n/a	n/a	n/a	n/a
Arginine	A	24	36	-4988	53365	-122
	B	26	39	-5554	72451	-58
	C	26	40	-3842	55973	-113
Lysine	A	21	41	-2140	31392	-203
	B	18	36	-2408	24133	-230
	C	25	38	-4369	55200	-126
Yeast	A	43	47	-48836	1.07E+06	3508
	B	36	38	-1.3E+05	1.69E+06	5778
	C	33	37	-69217	8.19E+05	2701

Table A.7 Temperature response parameters for SOM respiration (soil with no added carbon substrate) derived from the macromolecular rate theory for three replicates of each added carbon substrate

Carbon Type	Replicates	T_{inf} (°C)	T_{opt} (°C)	$\Delta C_P^{\ddagger}$ (J mol^{-1} K^{-1})	$\Delta H_{T_0}^{\ddagger}$ (J mol^{-1} K^{-1})	$\Delta S_{T_0}^{\ddagger}$ (J mol^{-1} K^{-1})
Glucose	A	n/a	n/a	1170	1170	-199
	B	n/a	n/a	12	53290	-123
	C	n/a	n/a	-208	55127	-117
Maltose	A	n/a	n/a	1170	1170	-199
	B	n/a	n/a	12	53290	-123
	C	n/a	n/a	-208	55127	-117
Glutamine	D	n/a	n/a	1086	44356	-154
	E	n/a	n/a	94	47864	-143
	F	n/a	n/a	617	48308	-141
Dextran	D	n/a	n/a	1086	44356	-154
	E	n/a	n/a	94	47864	-143
	F	n/a	n/a	617	48308	-141
Arginine	G	n/a	n/a	207	57041	-114
	H	n/a	n/a	615	48326	-142
	I	n/a	n/a	631	52840	-128
Lysine	G	n/a	n/a	207	57041	-114
	H	n/a	n/a	615	48326	-142
	I	n/a	n/a	631	52840	-128
Yeast	J	n/a	n/a	-2614	189071	397
	K	n/a	n/a	21524	149336	206
	L	n/a	n/a	18429	174202	289

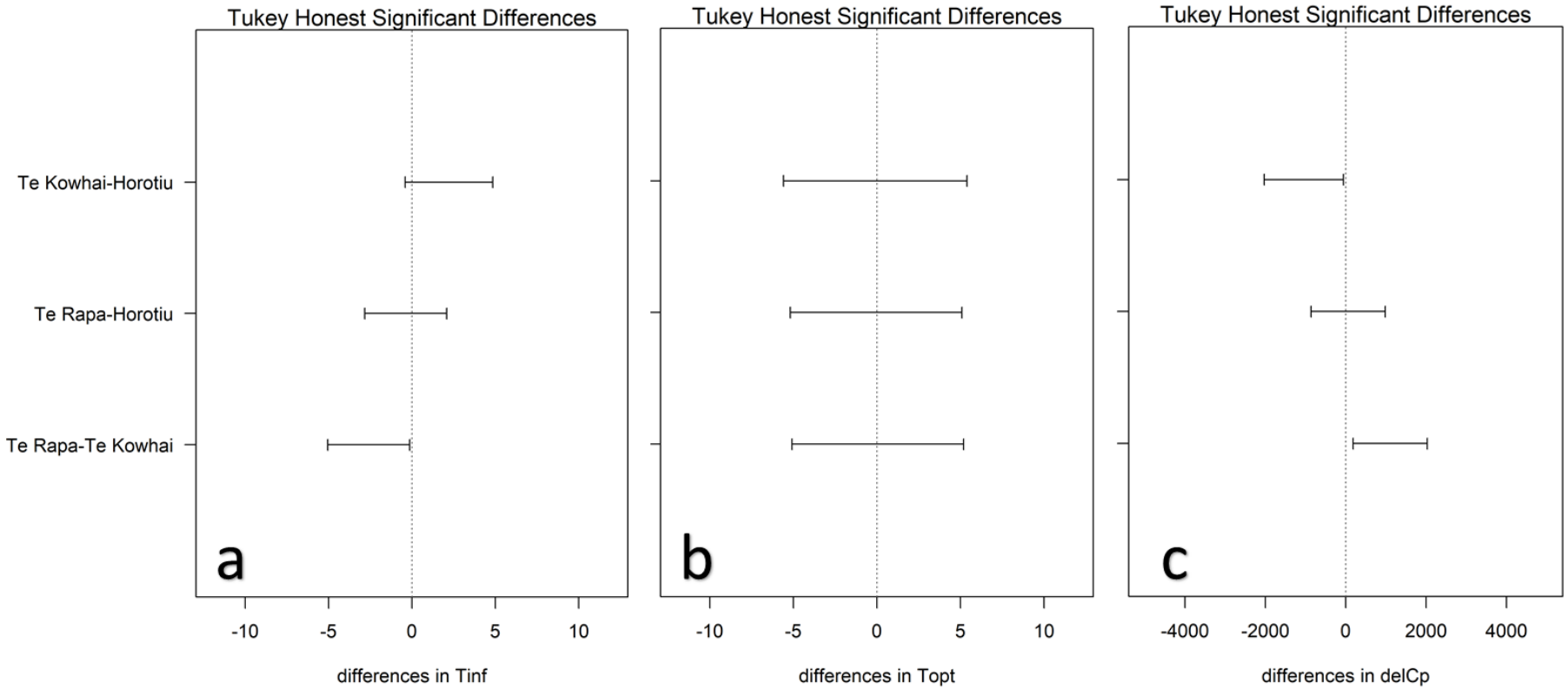

Figure A.1 Figures derived from Tukey's Honest Significant Difference test for pair-wise comparisons between the temperature response parameters of (a) temperature inflection point, (b) temperature optimum point, and (c) the change in heat capacity between the three soil types. The critical value used for alpha was 0.05

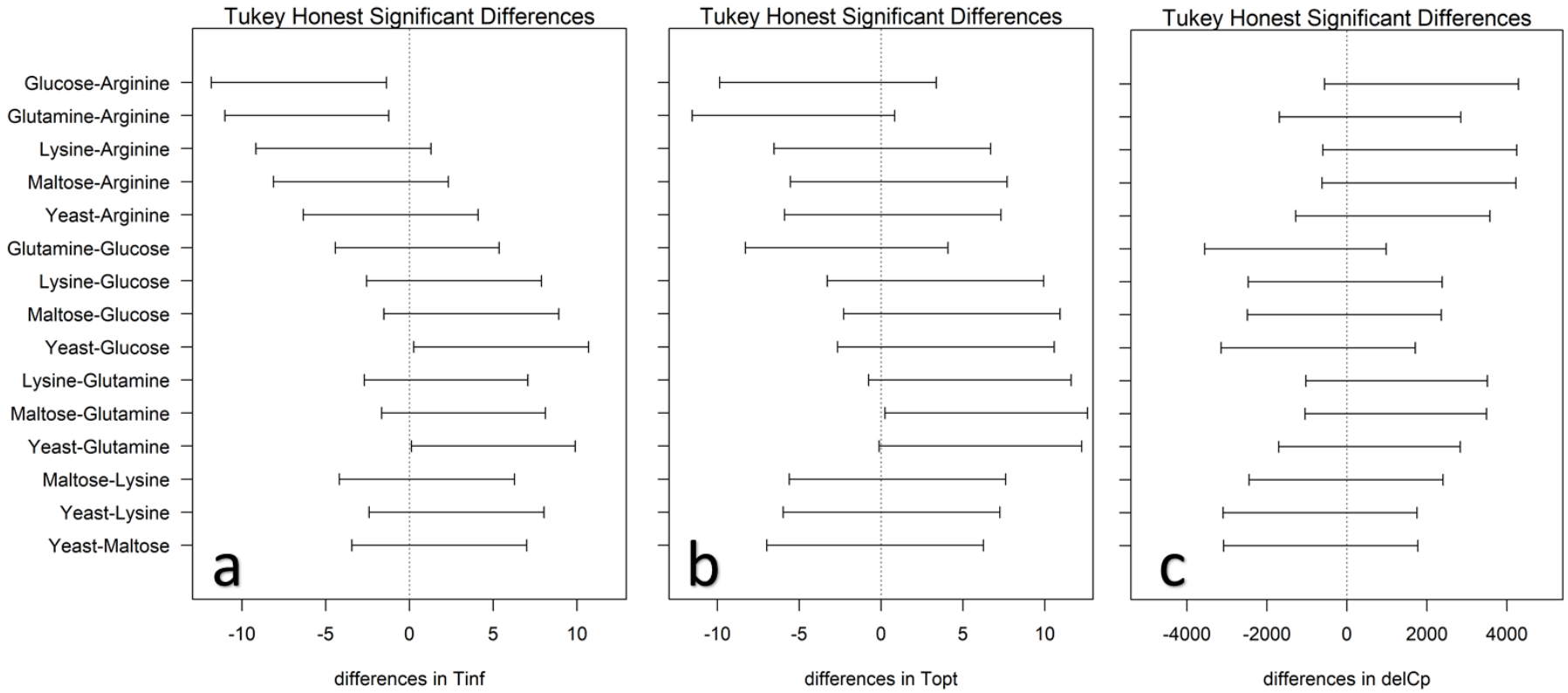

Figure A.2 Figures derived from Tukey's Honest Significant Difference test for pair-wise comparisons between the temperature response parameters of (a) temperature inflection point, (b) temperature optimum point, and (c) the change in heat capacity between all added carbon compounds. The critical value used for alpha was 0.05