TEMPERATURE SENSITIVITY OF SOIL RESPIRATION

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Abstract

Biogeochemical cycles, such as the carbon (C) cycle, are being continuously affected by anthropogenic activities such as carbon dioxide (CO_2) release from fossil fuels and the decomposition of soil organic C following some land management practices. The C cycle consists of four C pools: atmospheric CO_2, biota (mostly in vegetation), the ocean and soil organic matter (SOM), this being the largest actively cycling C pool. Respiration is the main driver of CO_2 release into the atmosphere, and is extremely sensitive to changes in moisture and temperature with small changes in these variables having a major influence on C cycling. Studies have argued in favour of both positive and negative feedbacks between CO_2 and global warming, where increasing temperatures could either increase CO_2 production, or enhance C storage. These contradicting arguments about how temperature changes will affect C exchanges, makes the understanding of how temperature change will affect temperature sensitivity of the C cycle dynamics (including respiration) critical for C modelling and budgeting.

Studies have attempted to measure temperature sensitivity of respiration when trying to understand soil response variation in temperatures. However, synthesis of current literature highlighted that laboratory methods can be problematic and introduce artefacts that obscure true temperature sensitivity. Problems highlighted included, too few temperatures treatments at which respiration was measured, use of long-term incubations that do not control microbial adaptation, lack of seasonal measurements and accounting for variability in moisture content.

A new laboratory method was developed for this thesis, which allowed for rapid determination of soil respiration rate at a wide range and number of temperatures to overcome some of the observed drawbacks frequently seen in the literature. A temperature block allowed simultaneous measurements of soil respiration rates over five hours at 44 different temperatures between ~4 and 50 °C. The objective of this thesis was to test this method on a variety of conditions (including different soil types, sampling season, range of moisture contents and pre-incubation temperatures) to understand how temperature sensitivity (T_{m_sens}) of soil respiration might change with different sample collection and processing approaches.

Seasonal measurements of respiration rates from different soil types collected from a single farm found a significant interaction for T_{m_sens} between soil type and season over the year. This interaction indicated that temperature sensitivity of soil respiration differed between soil types depending on season and thus in order to assess temperature sensitivity of soil respiration from a site, samples should be taken from all soil types at the location. T_{m_sens} was not dependent on season alone suggesting that a single sampling per year may be sufficient to estimate temperature sensitivity for a soil type, at least at a site with moderate changes in temperature during the year. Surprisingly, respiration rate response to temperature was not very sensitive to variations in soil moisture. Pre-incubated soils sampled for 10 months at different temperatures similarly resulted in no significant change in T_{m_sens} suggesting that temperature sensitivity of soil respiration can be accurately determined using soils stored at various temperatures and that microbial populations are relatively stable in response to incubation temperature.

Overall the developed method was able to rapidly assess temperature sensitivity of several soils through time under a variety of treatments and suggested overall the microbial population did not change rapidly and retained its temperature sensitivity. The success of this method allows for future testing of other hypothesis with regard to temperature sensitivity of respiration.

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Table of Contents

Abstract ..... **ii**

Acknowledgements ..... **iv**

Table of Contents ..... **vi**

List of Figures ..... **x**

List of Tables ..... **xiv**

Chapter 1. Introduction **2**

1.1 Background 2

1.2 Aims and objectives 3

1.3 Thesis Layout 4

Chapter 2. Literature Review **6**

2.1 Global carbon cycle 6

2.2 Controls of microbial respiration 8

2.2.1 Substrate availability 8

2.2.2 Moisture 9

2.2.3 Aggregation 9

2.2.4 Flooding 10

2.2.5 Drought 10

2.2.6 Freeze-thaw cycles/permafrost 10

2.3 Importance of temperature 11

2.3.1. Extrinsic effects 12

2.3.2. Intrinsic effects 13

2.4 Theory and models of temperature dependence 14

2.4.1 Arrhenius 14

2.4.2 Lloyd and Taylor 15

2.4.3 Macromolecular Rate Theory 16

2.5 Determining temperature sensitivity of soil biochemistry 18

2.5.1 Previous Approaches (theory) 18

2.5.2 Previous approaches (laboratory methods) 19

2.5.3 Number of incubation temperatures 21

2.5.4 Length of incubation time 22

2.5.5 Seasonal variation 23
2.5.6 Moisture content effects 23
2.6 Research needs 24

Chapter 3. Method Development 26
3.1 Introduction 26
3.1.1 Overview of method 26
3.2 Temperature block 27
3.2.1 Temperature linearity across the block 30
3.3 Gas sampling and analysis 31
3.3.1 Gas analysis and calculation of CO_2 concentration 31
3.3.2 Gas transport in syringes 32
3.4 Incubation of soil samples 33
3.4.1 Incubation length and sample weight 33
3.4.2 Respiration linearity 34
3.5 Summary 36

Chapter 4. Methods 38
4.1 Introduction 38
4.1.1 Site description 39
4.2 Soil types effects on temperature response 41
4.2.1 Sampling method 41
4.3 Seasonality 42
4.3.1 Methodology 43
4.4 Moisture content control on temperature response 43
4.4.1 Methodology 43
4.5 Pre-incubation 44
4.5.1 Methodology 44
4.6. Laboratory analysis 45
4.6.1 Moisture content and moisture factor 45
4.6.2 Maximum water holding capacity 46
4.6.3 Respiration measurements at different temperatures 47
4.7 Model fitting 48
4.7.1 Calculating T_{opt} and T_{m_sens} 49
4.8 Statistical analysis 49

Chapter 5. Temperature Sensitivity of Soil Respiration 50

5.1 Abstract 50
5.2 Introduction 52
5.3 Methods 55
5.3.1 Soil Type/Season. 57
5.3.2 Moisture content 57
5.3.3 Pre-incubation 57
5.3.4 Respiration rate measurement 58
5.3.5 Model fitting 59
5.3.6 Calculating T_{opt} and T_{m_sens} 60
5.3.7 Statistical analysis 60
5.4 Results 60
5.4.1 Soil type and seasonal comparison 61
5.4.2 Moisture content 62
5.4.3 Pre-incubation comparison 66
5.5 Discussion 70
5.5.1 T_{opt} and T_{m_sens} 70
5.5.2 Soil Type/Seasonal effects 70
5.5.3 Moisture content 72
5.5.4 Pre-incubation at different temperatures 73
5.6 Conclusions 74

Chapter 6. Conclusions and Future Research 76
6.1 Conclusions 76
6.2 Future work 77

References 80

Appendix A. Raw Data 92

List of Figures

Figure 2.1: Global carbon cycle. Adapted from Schlesinger and Andrews (2000). Pool values in Pg C, flux values in 10^{15} g C yr^{-1}. Values combined from Jobbagy & Jackson (2000), Schlesinger and Andrews (2000). 6

Figure 2.2: Conceptual diagram of carbon movement (solid arrows) from solid phase soil organic matter (SOM), through dissolved organic matter (DOC), simple DOC, and microbes before being respired as CO_2. Dashed arrows indicate steps that have a degree of temperature dependence and steps are divided based on whether they are intrinsic or extrinsic. 11

Figure 3.1: Temperature block with water bath on the left end and a heater on the right. (1400 mm in length) Insert (a.) shows Hungate tubes in place, stopped on the right hand side and bunged and crimped on the left. Insert (b.) shows Hungate tube with 3 g of soil. 28

Figure 3.2: Example data of soil respiration of a Horotiu silt loam collected from three separate paddocks (C21a, C35a C20) at Scott Farm. Each point represents an separate respiration rate measured at a specified temperature.. 29

Figure 3.3: Temperature of block over 18 hours, as measured by thermistors at seven slot positions across the block. Stabilisation of temperature gradient occurs within 4 hours. 29

Figure 3.4: Measured temperature at each slot with temperature block set at -5 at cold end to 70 °C at hot end. Linear fit with R^2 value of 0.999. Black squares indicate positions of thermistors subsequently used in each respiration measurement to calculate temperature gradient during routine measurements. 30

Figure 3.5: Peak height for 0.2, 0.4, 0.6, 0.8, 1.0 and 2.0 mL injections of 1% CO_2 standard in nitrogen gas. Data fit with a linear regression (excluding the 2 mL data point) as well as a polynomial regression (including the 2 mL data point). 32

Figure 3.6: Average peak heights of 1% CO_2 standard in nitrogen gas (1 mL) after left in rubber bungs for 0, 0.12, 0.5, 1 and 2.5 hour. Note: y-axis does not start at the origin (0). 33

Figure 3.7: Example data of soil respiration of a Te Kowhai silt loam collected from three separate paddocks (D2a, D15 C11) at Scott Farm. For temperatures between 0 and 32 °C, 4 g of soil was incubated for 6 h and for 32 - 50°C soil was incubated for 4 hour. Note: Break in respiration rate is indicated by arrow. 34

Figure 3.8: CO_2 concentration respired by 4 g of soil at 7 different temperatures after incubation lengths of 1, 2 and 6 hours. 35

Figure 3.9: Linear regression between two linear regression slopes (R^2:0.99) .compared to 1:1 line (Dashed). Slope through zero (y-axis) is the slope of a linear regression using only the 6 hour respiration data and zero. The actual slope (x-axis) is the slope of a linear regression using 1, 2 and 6 hour data. .. 36

Figure 4.1: Picture of Scott Farm (Outlined in white). Picture from Google Earth 23rd April 2015. Retrieved 20th December 2015. Paddocks sampled are also outlined with main soil types identified. Horotiu (H), Te Kowhai (TK), Te Rapa (TR). 40

Figure 4.2: Respiration calculated for a Horotiu soil and fitted with both MMRT (R^2= 0.99) and Arrhenius (R^2= 0.96) models. 48

Figure 5.1: Satellite view of Scott Farm (Outlined in white). Google Earth 23rd April 2015. Retrieved 20th December 2015. Replicate paddocks sampled are outlined with main soil types identified. Horotiu (H), Te Kowhai (TK), Te Rapa (TR). 56

Figure 5.2: Respiration calculated from a Horotiu soil and fitted with both MMRT (R^2= 0.99) and Arrhenius (R^2= 0.96) models. 59

Figure 5.3: Respiration rate calculated for a Horotiu soil over seven moisture contents (20, 30, 40, 50, 60, 70 and 80%). Temperature response within moisture content was fitted with MMRT and then smoothed across moisture with a loess function. 63

Figure 5.4: Respiration rate calculated for a Te Kowhai soil over seven moisture contents (20, 30, 40, 50, 60, 70 and 80%). Temperature response within

moisture content was fitted with MMRT and then smoothed across moisture with a loess function. 64

Figure 5.5: Respiration rate calculated for a Te Rapa soil over seven moisture contents (20, 30, 40, 50, 60, 70 and 80%). Temperature response within moisture content was fitted with MMRT and then smoothed across moisture with a loess function. 65

Figure 5.6: Total CO_2 produced by soil samples over. Samples incubated at either 4, 10, 20 or 35 °C for up to 10 months (T0=initial sampling, T1=2 months, T2=4 months, T3=6 months, T4=8 months and T5=10 months). 67

Figure 5.7: Average respiration rate response for the T0 sample. Four data point temperatures highlighted in black represent the data points selected for Figure 5.8. 68

Figure 5.8: Respiration rate response to pre-incubation time measured at the four incubation temperatures 69

Figure 5.9: MMRT fits for the T0 respiration rates using all 36 data points provided by the temperature block, compared to fits using only four points. 69

List of Tables

Table 2.1: Summary of previous approaches used to measure temperature sensitivity (Q10). Variable indicates what the study was observing to effect temperature sensitivity of respiration (Pre-inc= Pre-incubation effects; Var= Variation; Soil Pools= Labile and Non-labile fractions; MC= Moisture content; Fert= N and P fertilisation; C min= carbon mineralisation) Method states the method of CO2 measurement used (Cha= Chamber; IRGA= Infra-red gas analyser; Inj= injection; O-A= Open Air; GC= Gas chromatography; TGA= tunable diode laser absorption spectrometer; C-L= Closed Loop; SL= Soda and Lime method; MS= isotope mass spectrometer; NaOH= NaOH titration). No. T specifies how many incubation temperatures respiration was measured at (c. = continuous temperature change; brackets show minimum and maximum temperatures (°C)). Inc t indicates the amount of time soil was incubated at the incubation temperatures (h= hours; d= days; w= weeks; m= months). No. S is the number of times sampling occurred per year. Finally MC indicates how many moisture contents were used. 19

Table 5.1: Averaged, calculated $\Delta C_P^{\ddagger}$, T_{m_sens} and T_{opt} values for respiration rates measured for three soil types (Horotiu, Te Kowhai and Te Rapa) from three paddocks each. Rates were calculated from samples taken four times over the year to capture seasonality. 61

Table 5.2: Result of two- way ANOVA analysis of T_{m_sens} using soil type and seasonal differences. DF = degrees of freedom, SS = sum of squares, MS = Mean squares. Significant difference indicated by a P-value <0.05 (Bold text)..... 61

Table 5.3: P-values for pairwise comparison between soil types (Horotiu, Te Kowhai, and Te Rapa) within seasons (Summer, Autumn, Winter and Spring). Significant difference indicated by a P-value <0.05 (Bold text)..... 62

Table 5.4: P-values gained as a result of pairwise comparison between seasons (Summer, Autumn, Winter and Spring) within soil types (Horotiu, Te Kowhai, and Te Rapa). Significant difference indicated by a P-value <0.05. 62

Table 5.5: Averaged, calculated $\Delta C_P^{\ddagger}$, T_{m_sens} and T_{opt} values for respiration rates measured over 10 months (T0=initial sampling, T1=2 months, T2=4 months, T3=6 months, T4=8 months and T5=10 months)..... 66

Table 5.6: Result of two- way ANOVA analysis on T_{m_sens} using incubation temperature and incubation length. DF = degrees of freedom, SS = sum of squares, MS = Mean squares. Significant difference indicated by a P-value <0.05..... 67

Table A.1: Calculated temperature (T) (°C) and measured respiration rate (Rs) (µg C g^{-1} h^{-1}) for a Horotiu, Te Kowhai and Te Rapa soil collected during summer 2015. 92

Table A.2: Calculated temperature (T) (°C) and measured respiration rate (Rs) (µg C g^{-1} h^{-1}) for a Horotiu, Te Kowhai and Te Rapa soil collected during autumn 2015. 93

Table A.3: Calculated temperature (T) (°C) and measured respiration rate (Rs) (µg C g^{-1} h^{-1}) for a Horotiu, Te Kowhai and Te Rapa soil collected during winter 2015. 94

Table A.4: Calculated temperature (T) (°C) and measured respiration rate (Rs) (µg C g^{-1} h^{-1}) for a Horotiu, Te Kowhai and Te Rapa soil collected during spring 2015. 95

Table A 5: Calculated temperature (T) (°C) and measured respiration rate (Rs) (µg C g^{-1} h^{-1}) for a Horotiu soil before pre-incubation (Time Zero)..... 96

Table A.6: Calculated temperature (T) (°C) and measured respiration rate (Rs) (µg C g^{-1} h^{-1}) for a Horotiu soil after 2-months of pre-incubation at 35, 20, 10 and 4°C (2 replicates per incubation temperature) (Time One)..... 97

Table A.7: Calculated temperature (T) (°C) and measured respiration rate (Rs) (µg C g^{-1} h^{-1}) for a Horotiu soil after 4-months of pre-incubation at 35, 20, 10 and 4°C (2 replicates per incubation temperature) (Time Two). 98

Table A.8: Calculated temperature (T) (°C) and measured respiration rate (Rs) (µg C g^{-1} h^{-1}) for a Horotiu soil after 6-months of pre-incubation at 35, 20, 10 and 4°C (2 replicates per incubation temperature) (Time Three). 99

Table A.9: Calculated temperature (T) (°C) and measured respiration rate (Rs) (µg C g^{-1} h^{-1}) for a Horotiu soil after 8-months of pre-incubation at 35, 20, 10 and 4°C (2 replicates per incubation temperature) (Time Four). 100

Table A.10: Calculated temperature (T) (°C) and measured respiration rate (Rs) (µg C g^{-1} h^{-1}) for a Horotiu soil after 10-months of pre-incubation at 35, 20, 10 and 4°C (2 replicates per incubation temperature) (Time Five). 101

Table A.11: Calculated temperature (T) (°C) and measured respiration rate (Rs) (µg C g^{-1} h^{-1}) for a Horotiu soil wetted up to 7 different moisture contents (20, 03, 40, 50, 60, 70 and 80%). 102

Table A.12: Calculated temperature (T) (°C) and measured respiration rate (Rs) (µg C g^{-1} h^{-1}) for a Te Kowhai soil wetted up to 7 different moisture contents (20, 03, 40, 50, 60, 70 and 80%). 103

Table A.13: Calculated temperature (T) (°C) and measured respiration rate (Rs) (µg C g^{-1} h^{-1}) for a Te Rapa soil wetted up to 7 different moisture contents (20, 03, 40, 50, 60, 70 and 80%). 104

Chapter 1. Introduction

1.1 Background

The carbon (C) cycle is being continuously affected by anthropogenic activities such as carbon dioxide (CO_2) release from fossil fuels and the decomposition of soil organic carbon through land management practices, such as intensive cultivation and deforestation (Rousk & Bengtson, 2014). The four C pools are atmospheric CO_2, biota (mostly in vegetation), soil organic matter (SOM) and the ocean (Janzen, 2004). Respiration of previously fixed carbon by plants, fungi and microbes drive the release of C back into the atmosphere (Davidson & Janssens, 2006; Gougoulias *et al.*, 2014). An increase in atmospheric CO_2 has been suggested to cause a positive feedback loop, where increases in CO_2 concentration, increase soil respiration resulting in more CO_2 being released (Schlesinger & Andrews, 2000; Kirschbaum, 2000; Fang & Moncrieff, 2001). Other studies have instead argued for a potential negative feedback between increasing CO_2 and warming, where increasing temperatures cause increased soil C storage (following increased plant growth and detrital inputs to soil) thereby reducing atmospheric CO_2 (Conant *et al.*, 2011). Contradictory arguments and lack of definitive evidence, makes understanding the role of temperature changes on the dynamics of the C cycle a key area of interest, with a focus on SOM decomposition, frequently measured as the rate of respiration (Fang & Moncrieff, 2001).

Microorganisms are responsible for the majority of soil respiration, and there are a number of biophysical properties that control microbial function and their rate of respiration (Conant *et al.*, 2011; Davidson & Janssens, 2006). These properties include substrate availability, moisture content and temperature (Davidson & Janssens, 2006, Schlesinger, 1984; Tiemann & Billings, 2011; von Lutzow *et al.,* 2007). Additionally, effects that do not directly affect microbe function such as aggregation, flooding, drought, freeze-thaw cycles and land-use can inhibit or enhance the movement of gases (e.g. oxygen) or substrates. These processes overall can result in changes to respiration rates (Conant *et al.*, 2011; Davidson & Janssens, 2006). Soil respiration is very sensitive, in particular, to changes in temperature making the study of respiration rate response to temperature change an important area of research (Conant *et al.*, 2011; Davidson & Janssens, 2006).

Many studies have attempted to measure temperature sensitivity of respiration when trying to understand soil response to variation in temperatures. A review of literature (Section 2.5) highlighted that some laboratory methods for measuring temperature sensitivity can be problematic and introduce artefacts that obscure *in situ* responses to temperature and temperature sensitivity of respiration. Approaches for measuring the temperature dependence of soil respiration rates often include long incubation times (weeks to months) and use a limited number of incubation temperatures (often less than six). Long incubation times may allow thermal adaptation of microbial populations, or significant substrate loss, leading to results that do not represent *in situ* soil responses (Bradford, 2013; Kirschbaum, 2004). Additionally only measuring respiration rates at few temperatures allows for the fit and justification of many different predictive models leading which can lead to inaccurate extrapolation.

1.2 Aims and objectives

The overall aim of the study was to develop and test a rapid laboratory method for measuring soil respiration response at different temperatures to further the understanding of temperature sensitivity of soil respiration.

Specific objectives were:

- To develop a rapid laboratory methodology for measuring temperature sensitivity of respiration in soils collected from the field.
- To determine dependence of temperature sensitivity on soil type, season of collection, pre-incubation temperature and moisture content.

For the measurements of temperature dependence of soil respiration, samples collected from the field, the key hypotheses were:

- That temperature sensitivity of soil respiration would not be significantly different between soil types or season of collection and
- That temperature sensitivity of soil respiration would dependant on pre-incubation temperature and moisture content.

1.3 Thesis Layout

Chapter 2 reviews literature on the general controls of microbial respiration in soil and with a specific focus on importance of temperature. The review also overviews previously used methods for measuring soil respiration response to temperature changes and their strengths and weaknesses.

Chapter 3 contains the details on how the laboratory method used in this thesis was developed and describes the final methodology used in the remainder of the thesis.

Chapter 4 includes a full methodological description of the field and laboratory experiments as well as the statistical analysis used in this thesis for completeness.

Chapter 5 contains the main experimental part of this thesis as it presents the data gathered during the study and discusses the developed methodology and changes to temperature sensitivity of soil respiration under the various experimental manipulations. This chapter has been written in the form of a paper for later adaptation to submit to a peer-reviewed journal. Consequently, there is some repetition of material from the introductory, literature review and method chapters.

Chapter 6 includes the summary and conclusions for the study and recommendations for future research.

Appendix 1 includes the raw temperature and respiration data measured for all 101 samples processed during this thesis.

Chapter 2. Literature Review

2.1 Global carbon cycle

Biogeochemical cycles involve the movement and transformation of elements through the biosphere, lithosphere, hydrosphere and atmosphere of the earth (Bolin, 1981). As these cycles govern most natural processes, the modelling of these cycles and how they respond to change, is critical for the understanding of ecosystem functioning (Rousk & Bengtson, 2014). Currently, biogeochemical cycles, in particular the carbon (C) cycle, are being continuously affected by anthropogenic activities such as carbon dioxide (CO_2) release from fossil fuels and the decomposition of soil organic carbon through intensive cultivation (Rousk & Bengtson, 2014). Global concern, of climate change and soil degradation and their potential effects on the current and future environment strengthen the need for better modelling and measurement of natural biogeochemical processes that are affected by and could contribute to such change (Fang & Moncrieff, 2001). The global C cycle consists of four main pools of active C and the chemical, physical and biological processes, which drive C exchanges between them (Schlesinger, 1995) (Figure 2.1).

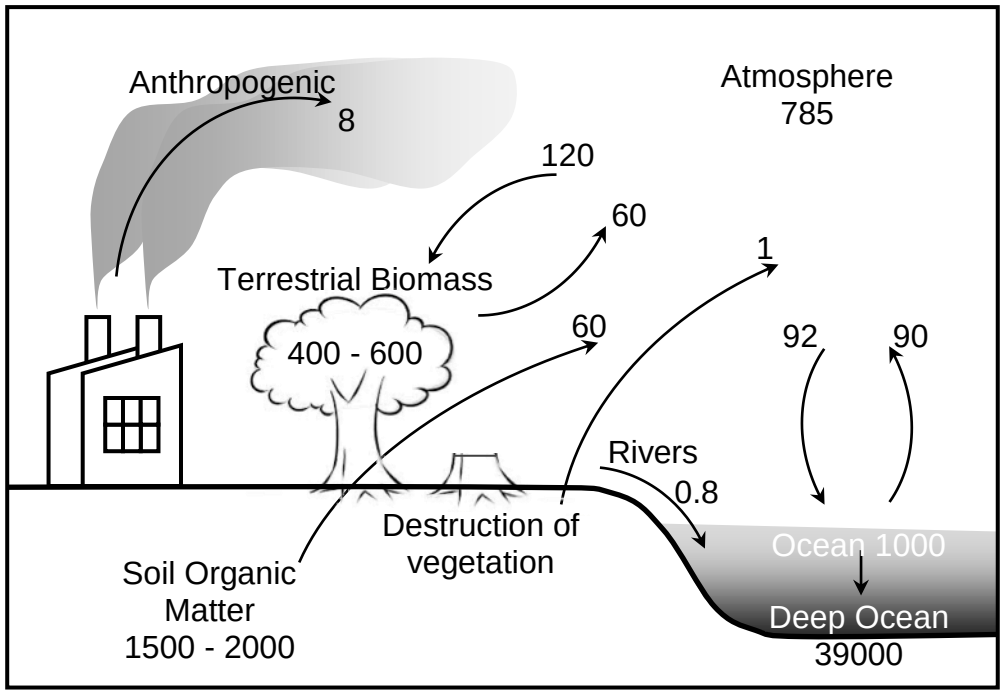

Figure 2.1: Global carbon cycle. Adapted from Schlesinger and Andrews (2000). Pool values in Pg C, flux values in 10^{15} g C yr^{-1}. Values combined from Jobbagy & Jackson (2000), Schlesinger and Andrews (2000).

The four C pools are atmospheric CO_2, biota (mostly in vegetation), soil organic matter (SOM) and the ocean (Janzen, 2004). The largest pool of C is that of the ocean with C reserves of approximately 39,000 Pg C (Schlesinger, 1995; Janzen, 2004), most of this however, is inactive sitting in deep ocean layers. Only about 1000 Pg C is potentially cycling within the upper ocean making it a relatively small active pool of C (Schlesinger, 1995; Janzen, 2004). The atmosphere contains approximately 785 Pg C as CO_2, the biota pool, although harder to measure, is comparable to this around 400 – 600 Pg C (Schlesinger, 1995; Janzen, 2004). The largest actively cycling pool is that of SOM with approximately 1500 – 2000 Pg C in multiple organic forms (Jobbagy & Jackson, 2000; Davidson & Janssens, 2006)

The storage of organic matter (OM) in soils is dominated by the balance of inputs and outputs of C. Photosynthesis by plants, including algae, provides the major pathway for the fixation of atmospheric C into organic biomass. Several microbial populations known as photo and chemoautotrophs can also fix atmospheric C into OM (Gougoulias *et al.*, 2014). Respiration of previously fixed carbon by plants, fungi and primarily microbes drive the release of C back into the atmosphere (Davidson & Janssens, 2006; Gougoulias *et al.*, 2014). Respiration is very sensitive to changes in moisture and temperature (Davidson & Janssens, 2006; Conant *et al.*, 2011) and small changes in these variables can have a major influence on how much CO_2 is released back to the atmosphere (Fang & Moncrieff, 2001). Potentially, global warming could cause an increase in the release of CO_2 from the soil resulting in positive feedback on the atmospheric CO_2 and global change (Kirschbaum, 2000). As total international emissions of CO_2 from soil respiration, estimated at 7.5 Gt C yr^{-1} (Schlesinger & Andrews, 2000), are already close to that of the highest contributor of CO_2 emissions, burning fossil fuels which produce 9.7 Gt C yr^{-1} (Quéré *et al.*, 2014), any increase in soil respiration could cause significant increases to total atmospheric CO_2 and intensify and positive feedback occurring (Schlesinger & Andrews, 2000; Kirschbaum, 2000; Fang & Moncrieff, 2001). Other studies have instead argued for a potential negative feedback between CO_2 and warming, where increasing temperatures cause increased soil C storage (through increased plant growth, and detrital inputs) thereby reducing atmospheric CO_2 (Conant *et al.*, 2011). Conflicting arguments, and lack of definitive evidence makes understanding the role of temperature changes on the dynamics of the C cycle a key area of interest, focusing

on decomposition, frequently measured as the rate of respiration (Fang & Moncrieff, 2001).

In this literature review, the controls of microbial respiration in soil are examined (Section 2.2). The importance of temperature is presented last and in most detail (Section 2.3), as this controlling factor is the focus of this thesis.

2.2 Controls of microbial respiration

Soil respiration rate is affected by a range of biophysical properties that impact microbial function. These influences include substrate availability, moisture content and temperature (Davidson & Janssens, 2006, Schlesinger, 1984; Tiemann & Billings, 2011; von Lutzow *et al.,* 2007). Additionally indirect effects such as aggregation, flooding, drought, freeze-thaw cycles and land-use can also influence the rate of soil respiration (Conant *et al*., 2011; Davidson & Janssens, 2006; Oades, 1988). These factors do not directly affect microbe function but can inhibit or improve movement of gases (e.g. oxygen) or substrates resulting in changes to respiration rates. Temperature can also have indirect effects as changes in temperature can limit gas and substrate movement.

2.2.1 Substrate availability

Soil organic carbon (SOC) is not always readily available for decomposition. Different fractions have varying residence times and cycling rates that can be used to divide carbon their conceptual pools, labile, stabilized and passive (von Lutzow *et al.,* 2007). The labile pool is readily active with a turnover-time of less than 10 years. It includes microbes and particulate organic carbon, typically within the top 1 m of soil (Schlesinger, 1984; von Lutzow *et al.,* 2007). The stabilised pool contains physically protected and complexed C that can have a decadal turnover rate, whilst the passive pool (including carbonates) has an expected turnover rate of millennia (Schlesinger, 1984).

The labile fraction contains C readily available for decomposition, whilst in the stabilised and passive pools, C is typically bound or protected in some manner. Soil with a high proportion of labile fraction will produce CO_2 readily, those with low proportions of labile C will have less CO_2 production. These pools, however, are

closely connected and changes to land-use, management, moisture content and temperature can drive carbon exchange between these pools increasing or decreasing decomposition rates (Davidson & Janssens, 2006; Schlesinger, 1984).

2.2.2 Moisture

Soil moisture controls microbial respiration in three major ways, (i) by directly affecting microbial physiology (growth, enzyme production etc.), (ii) by affecting plant activity that contributes C to soil through litter and exudation, and (iii) by increasing substrate availability by providing water films for C diffusion (Manzoni *et al.*, 2012). These effects can often be confounded by one another in field experiments, but overall, all mechanisms can have a pronounced effect on overall soil respiration (Tiemann & Billings, 2011). Microbes tend to have optimum water content at which they function at maximum efficiency (Davidson *et al.*, 2000; Sierra *et al.*, 2015) when moisture drops below this optimum, physiological stress begins to occur. Initially microbes reduce activity to maintain survival, in these situations carbon use efficiency has been found to increase, leading to less CO_2 production and more C used for biomass production or maintenance (Manzoni *et al.*, 2012) Eventually, less adaptive microorganisms begin to die, leading to overall reduction in decomposition (Tiemann & Billings, 2011). Many studies have shown that in a dry soil, an increase in precipitation rate or water availability can directly increase soil microorganism activity leading to increased decomposition and respiration (Davidson *et al.*, 2000; Liu *et al.*, 2009). Additionally, plant growth can also be stimulated resulting in an increase of available carbon substrate, again promoting microbial activity (Liu *et al.*, 2009), however, when soil moisture exceeds the optimum, soil can become water saturated displacing air (oxygen in particular). The removal of this oxygen creates an anoxic environment in which only anaerobic decomposition can occur. Anaerobic decomposition tends to be slower than aerobic decomposition, decreasing OM turnover (Davidson *et al.*, 2000; Maltby & Immirzi, 1993). Oxygen diffusion is much slower through water than through air also limiting oxygen supply to microbes.

2.2.3 Aggregation

Aggregation involves the binding of soil particles physically and chemically to one another creating aggregates of varying sizes. Aggregation occurs in all soils to some

degree, the degree of which is dependent on various factors such as soil type, climate conditions, land-use and land-use management (Davidson & Janssens, 2006; Oades, 1988). Aggregation can reduce respiration by protecting OM within aggregates from microbial consumption. This protection occurs by two means, physically and chemically (Davidson & Janssens, 2006; Oades, 1988). Physical OM protection occurs when OM is trapped inside aggregates during formation. Due to the isolation from air and water outside the aggregate, enzymes cannot diffuse to the trapped C and microorganisms cannot access this OM through small space. This isolation can restrict respiration in highly aggregated soils (Davidson & Janssens, 2006; Oades, 1988). Chemical protection occurs when OM is bonded though electrostatic or covalent bonds to mineral surfaces. Once adsorbed the OM can no longer be decomposed due to the large amount of energy that would be required to break the chemical bonds (Oades, 1988).

2.2.4 Flooding

When flooded, soil pore spaces are filled with water, displacing air filled space. This increase in water within pore spaces increases the distance of diffusion of oxygen and enzymes, slowing down their movements between active micro sites an microbes reducing the decomposition rate (Davidson & Janssens, 2006) (Section, 2.2.2)

2.2.5 Drought

During drought conditions, a reduction of water within soil pore space is observed (McHale *et al.*, 2005, Wang *et al.*, 2014). This reduction of pore water thins out the layer of water surrounding soil particles. A thinner layer of water is believed to reduce the movement of SOM throughout soil micro sites, reducing the availability of consumable C and thus respiration rates (McHale *et al.*, 2005, Wang *et al.*, 2014). Additionally, for some soil types, drought conditions can result in the deposition of hydrophobic molecules over OM further restricting diffusion of C by excluding water (McHale *et al.*, 2005). Long-term drought eventually may lead to reductions in C inputs due to restricted plant-growth as well as direct moisture and temperature stresses on microbial respiration (Wang *et al.*, 2014).

2.2.6 Freeze-thaw cycles/permafrost

In addition to slowing microbial metabolic rates due to low temperatures, freeze-thaw cycles and permafrost can also restrict microbial access to SOM (Davidson &

Janssens, 2006). When frozen, SOM is protected from microbial consumption due to a lack of liquid water for diffusion of C and microbial enzymes decreasing respiration (Miken *et al.*, 2002). Subsequently, during thaw cycles formation of liquid water, increasing diffusion releasing an abundance of C is available for microbial consumption. A positive feedback loop has also been suggested as a consequence of warming areas subjected to permafrost or freeze-thaw cycles (Davidson & Janssens, 2006). When global or local temperatures increase, there can be a reduction in permafrost depth and extent, and a shortening of the frozen stage of freeze-thaw cycles can occur. The reduction in ice exposes SOM, normally frozen, to decomposition and increasing soil respiration as a result (Davidson & Janssens, 2006).

2.3 Importance of temperature

All chemical reactions are temperature dependant. As temperature increases molecular kinetic energy increases leading to more collisions occurring, at higher energies, effectively increasing rate of reaction (Brown *et al.*, 2010). Biochemical processes in soil, including respiration, tend to be strongly temperature dependant in two ways (Figure 2.2). Firstly, through indirect (extrinsic) effects on the solubility and diffusion of substrate, and secondly, through the direct (intrinsic) effect on microbial metabolism, driven by enzyme kinetics (Conant *et al.*, 2011).

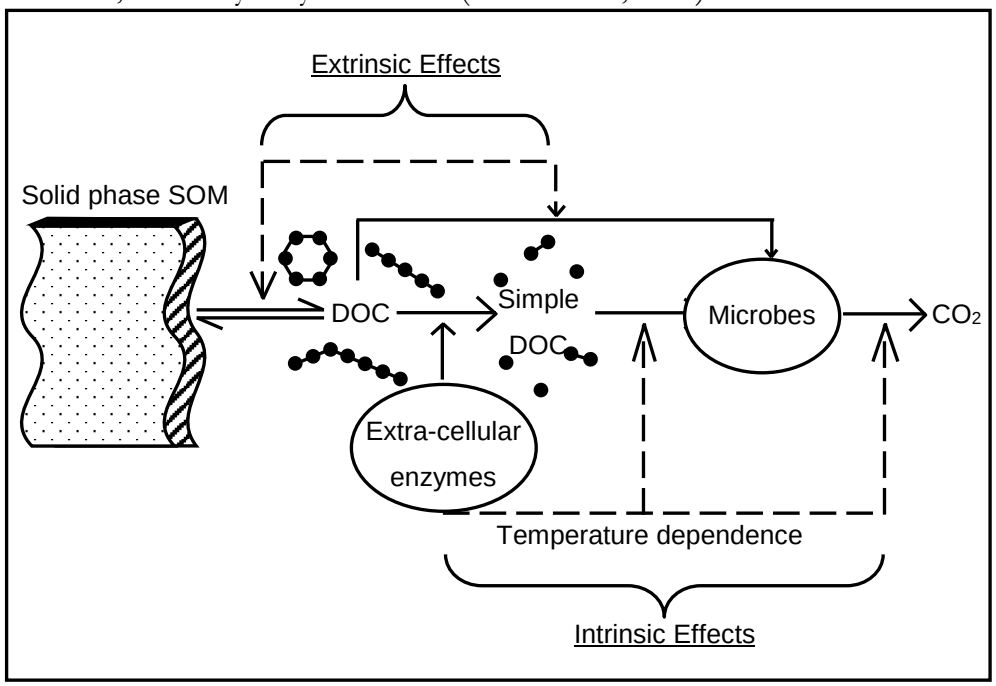

Figure 2.2: Conceptual diagram of carbon movement (solid arrows) from solid phase soil organic matter (SOM), through dissolved organic matter (DOC), simple DOC, and microbes before being respired as CO_2. Dashed arrows indicate steps that have a degree of temperature dependence and steps are divided based on whether they are intrinsic or extrinsic.

2.3.1. Extrinsic effects

Extrinsic effects tend to vary spatially depending on soil type, climate and multiple other, possibly confounded, variables (Conant *et al.*, 2011; Davidson & Janssens, 2006). The main extrinsic effect that is temperature dependant is the solubility of soil constituents (Dalias *et al.*, 2001; Conant *et al.*, 2011).

Changes in temperature can change the ability of substrate to mineralize or bind to clay and SOM depending on the affinity and quality of the substrate (Dalias *et al.*, 2001; Conant *et al.*, 2011). High-affinity substrate has stronger chemical interactions between mineral and OM and tends to form stronger, harder to break bonds. Soils with high-affinity substrate could also be considered soil with lower-quality substrates as stronger bonds require high energy to break making this substrate less available to microbes. Low-affinity soil has weaker chemical interactions between minerals and OM requiring less reaction energy to separate SOM.

For a high-affinity substrate, the rules of equilibrium thermodynamics are used to describe how substrate availability reacts to temperature changes (Conant *et al.*, 2011). Le Chatelier's principle states, "When a system at equilibrium is subjected to change in concentration, temperature, volume, or pressure, then the system readjusts itself to counteract the effect of the applied change and a new equilibrium is established."(Brown *et al.*, 2010). This means as temperature increases the equilibrium reaction for substrate sorption will favour the state that minimises the creation of heat, which in the case of high-affinity substrates is endothermic desorption (rather than exothermic adsorption) releasing SOM for consumption (Conant *et al.*, 2011).

In contrast to high-affinity substrate, some low-affinity substrate reactions can involve endothermic adsorption. When temperature increases, the opposite effect occurs and the binding of low-affinity substrate is promoted, reducing its availability for consumption (Conant *et al.*, 2011). In addition to this, as desorption occurs for high-affinity substrate, binding sites are exposed increasing the amount of low-affinity adsorption that can occur, further removing consumable low-affinity substrate.

Overall the change in substrate availability as temperature increases affects the respiration rate by changing the overall quality of substrate available for microbial consumption (Conant *et al.*, 2011). As low-quality (high-affinity) substrates are more complex they require more energy to consume. When more, low-quality substrate becomes available compared to high-quality (due to temperature change), microbes are forced to use more energy to consume substrate and thus produce more CO_2 in response (Conant *et al.*, 2011).

2.3.2. Intrinsic effects

The primary drivers of SOM decomposition respiration are microbes. This strongly suggests the effects that temperature has on microbial function will mostly dictate any temperature related changes observed in CO_2 fluxes (Li *et al.*, 2014). In spite of this somewhat simple argument, in truth, understanding microbial controls over CO_2 emissions is challenging. There are often contradictory responses found in experimental studies combined with a lack of knowledge about the soil organisms themselves; their spatial diversity, community compositions and seemingly varying responses to climate change (Zhang *et al.*, 2013). Further complications arise from interactions between multiple drivers (warming, moisture, OM changes etc.) that can confound outcomes making it hard to relate results to a single variable change (Balser *et al.*, 2010). By better understanding microbial decomposition and responses to varying drivers, there is potential to further the understanding of CO_2 responses to climate change (Zhang *et al.*, 2013). Of particular interest is the direct effect temperature has on the microbial degradation of SOM and CO_2 production (Fang & Moncrieff, 2001).

Microbial respiration is the most common variable measured to quantify decomposition of OM in soil. However, in reality the relationship between decomposition rates and factors such as temperature is driven by a series of microbial and enzyme-mediated processes (Steinweg *et al.*, 2013). In order to access organic C within the soil, the C must first be broken down into simple molecules to facilitate the uptake of C and ultimately the respiration of CO_2. To accomplish this, microbes produce extracellular enzymes that catalyse chemical reactions to breakdown large structures of soil organic carbon (SOC) into dissolved organic carbon (DOC) (Li *et al.*, 2014). These enzyme-mediated reactions are typically

sensitive to temperature changes, such that when other variables are non-limiting, an increase in temperature accelerates the rate of reaction (Bradford, 2013; Schipper *et al*, 2014). Bengtson and Bengtsson (2007) suggested that the rates of the production of these enzymes and of DOC are the rate-limiting step in the decomposition process. This leads to the hypothesis that improved understanding of the temperature dependence of enzyme-mediated reactions will assist in accurately modelling respiration responses (Steinweg *et al.*, 2013). The temperature sensitivity of SOM and its decomposition is still highly debated (Steinweg *et al.*, 2013; Bradford, 2013). A variety of methods and models have been used in an attempt to predict and measure temperature related changes, from which contradictory results are often derived (Conant *et al.*, 2011). This makes the development and testing of reliable new models essential in continuing the examination of temperature sensitivity and its future implications.

2.4 Theory and models of temperature dependence

There are many models of temperature dependence (Davidson & Janssens, 2006; Del Grosso *et al.*, 2005) including conceptual, theoretical and empirical models.

The models outlined here are the Arrhenius equation, the empirical adjustment of the Arrhenius equation by Lloyd and Taylor (Lloyd & Taylor, 1994) and Macromolecular Rate Theory (MMRT) (Arcus *et al.*, In press; Hobbs *et al.*, 2013; Schipper *et al.*, 2014).

2.4.1 Arrhenius

The Arrhenius model was developed in 1889 to describe chemical reaction. The equation is based on the activation energy (E_A) of reactions, which is essentially an energy peak or transition state that reactants must achieve in order to create its products (Transition State Theory) (Hobbs *et al.*, 2013; Schipper *et al.*, 2014; Sierra, 2012). Transformations typically include bond interactions, which can be of either high or low energies. The Arrhenius function aims to model reaction rates with respect to temperature to determine how fast these transformations of differing energy will occur in relation to temperature (Equation 2.1) (Schipper *et al.*, 2014). It is generally accepted that chemical reaction rates in decomposition largely follow the Arrhenius equation (Sierra, 2012).

$$k = Ae^{-E_A/RT} \quad \text{(Eq. 2.1)}$$

Where A is the pre-exponential factor, E_A is the activation energy of the reaction, *R* is the universal gas constant (8.314 J mol^{-1} K^{-1}) and *T* is absolute temperature (K) (Sierra, 2012).

By definition, the Arrhenius equation asserts that low-quality substrates have high activation energies to degrade and thus the rate of reaction is slow whilst high-quality substrates have low activation energies and is thus faster to react (Sierra, 2012). In this case, it could be said that activation energy is used as a measure of substrate quality as activation energy determines the rate of reaction (Sierra, 2012). Even though it is the most commonly used equation in soil respiration studies, Lloyd and Taylor (1994) found that after studying the residuals of an Arrhenius relationship fitted to respiration vs. temperature data from six published papers, the Arrhenius equation underestimated rates at low temperatures and overestimated rates at high temperatures. This led to their development of a similar, more flexible equation aimed to reduce the problems of the Arrhenius function when modelling biochemical reactions.

2.4.2 Lloyd and Taylor

Respiration involves many changing populations of different organisms, all of which potentially have different complex reactions and different temperature sensitivities (Lloyd & Taylor, 1994). Lloyd and Taylor (1994) showed that the activation energy (E_A) for these systems was not constant, decreasing with increasing temperature potentially further than expected due to enzyme deactivation at high temperatures. They did not have theoretical explanation for this decrease in activation energy. The Lloyd and Taylor model uses a semi-empirical equation developed by Kavanau (1951) to include a declining E_A in the Arrhenius model. Lloyd and Taylor (1994) rearranged this equation to a standard temperature of 10 °C (Equation 2.2). By making the equation relevant to a standard temperature the activation energy (E_0) and temperature (T_0) are forced to be constant, but E_0 is no longer representative of theoretical E_A (Lloyd & Taylor, 1994).

$$R = R_{10}e^{E_o\left(\frac{1}{283.15-T_0}-\frac{1}{T-T_0}\right)} = R_{10}e^{308.56\left(\frac{1}{56.02}-\frac{1}{T-227.13}\right)} \quad \text{(Eq. 2.2)}$$

Where with E_0 = 308.56 K and T_0 =227.13 K.

This equation gave a random set of residuals when fitted to soil respiration data measured at different temperatures in the studies they utilised. Consequently, this model was an improvement over the Arrhenius equation across a wide range of temperatures while still maintaining an exponential like form generally observed in respiration data (Lloyd & Taylor, 1994). The Lloyd and Taylor equation has had widespread acceptance as a useful equation for use in modelling (2527 citations on Google Scholar retrieved March 2016). However the Lloyd and Taylor equation remains an empirical model adjusting E_A as temperature changes. While this equation is useful and well used, it does not give a theoretical explanation for temperature dependence of respiration.

Neither the Lloyd and Taylor and the Arrhenius equations account for a decline in enzyme or microbial activity in higher temperature due to an increase in respiration costs (requires too much energy to complete complex reactions) and/or heat stress (enzyme denaturation) (Manzoni *et al.*, 2012). This indicates that respiration, in reality, does not follow the traditional exponential shape of the Arrhenius or, even with modifications, the Lloyd and Taylor model, but reaches a temperature optimum (T_{opt}) where respiration is maximal (Schipper *et al.*, 2014). Above this temperature, a decline in rate of activity is observed that has commonly been attributed to enzyme denaturation at high temperatures (Schipper *et al.*, 2014). However, enzyme denaturation most often only occurs at temperatures greater than the observed T_{opt} and so denaturation is an incomplete explanation for decreases in respiration at higher temperatures. (Hobbs *et al*, 2013).

2.4.3 Macromolecular Rate Theory

Macromolecular rate theory (MMRT) was developed to account for the temperature optimum of enzyme activity, unlike simple exponential models (Sierra, 2012). MMRT is a theoretical extension of the Arrhenius equation accounting for thermodynamic properties of biological macromolecules (i.e. enzymes) and includes a temperature dependence of activation energy, derived from a large, negative change in heat

capacity (C_P) of the enzyme during catalysis (Arcus *et al.*, In press; Hobbs *et al.*, 2013). This equation results direct prediction of a T_{opt} for enzymatic processes without needing to include enzyme denaturation (Schipper *et al.*, 2014). The theory was derived from the Arrhenius equation, expanded using the Transition State Theory where the E_A in the equation is substituted with the difference in Gibbs free energy between the ground state and transition state ($\Delta G^{\ddagger}$). The pre-exponential constant (A) is further divided into Planck's constant (*h*), Boltzmann's constant (k_B) and κ which represents the mechanical effects of transition-state crossing (assumed as 1). The heat capacity difference ($\Delta C_P^{\ddagger}$) between the two states determines the temperature dependences of $\Delta G^{\ddagger}$ and thus the reaction rate (Hobbs *et al.*, 2013; Schipper *et al.*, 2014) (Equation 3.3).

$$ln(k) = ln\left(\frac{k_B T}{h}\right) - \frac{\Delta H_{T_0}^{\ddagger} + \Delta C_P^{\ddagger}(T - T_0)}{RT} + \frac{\Delta S_{T_0}^{\ddagger} + \Delta C_P^{\ddagger}(lnT - lnT_0)}{R} \qquad \text{(Eq. 2.3)}$$

Where T_0 is a reference temperature, and $\Delta H_{T_0}^{\ddagger}$ and $\Delta S_{T_0}^{\ddagger}$ are the difference in enthalpy and entropy between the ground state and the transition state, respectively, at T_0 (Arcus *et al.*, In press; Hobbs *et al.*, 2013; Schipper *et al.*, 2014). $\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$ are both temperature dependent and consequently, the relationship between rate and temperature is negatively curved in an *ln* (*k*) versus T plot. This curvature occurs when values of $\Delta C_P^{\ddagger}$ are large and negative and results in prediction of T_{opt} (Hobbs *et al.*, 2013).

It has been proposed that accounting for heat capacity (C_P) is critical for accurately predicting soil respiration response to temperature as it does not simply add flexibility to the model by adding extra variables, but physically describes biological processes within enzyme reactions (Schipper *et al.*, 2014). Changes in heat capacity are a measure of the change in flexibility of the enzyme during catalysis (Hobbs *et al.*, 2013). For a folded protein, the number of vibration and rotational modes within the protein accounts for the majority of a molecules heat capacity and thus an enzyme with more modes tend to have greater heat capacity (Hobbs *et al.*, 2013). As enzymes tightly bind the substrate, the enzymes is stabilised until further reactions occur (Hobbs *et al.*, 2013; Schipper *et al.*, 2014). The enzyme-substrate complex has fewer numbers of structural arrangements (conformation states) as some modes are locked

in place reducing the heat capacity of the transition state relative to the unbound enzyme ($\Delta C_P^{\ddagger}$) (Hobbs *et al.*, 2013; Schipper *et al.*, 2014).

If this change in heat capacity was not accounted for (i.e. $\Delta C_P^{\ddagger} = 0$), the reaction rate would increase exponentially, as predicted by the Arrhenius functions (the MMRT equation collapses to Arrhenius equation). Schipper *et al.* (2014) tested the MMRT model, against literature data of a variety of soil processes measure a different temperature to determine whether the model produced accurate fits for enzyme-meditated processes such as respiration, methane production and nitrification. They concluded that the model produced better fits for data, particularly at low temperatures. Whether this new model has broader applicability to soil processes and scales reasonably to ecosystems needs further testing.

2.5 Determining temperature sensitivity of soil biochemistry

There is considerable interest in determining how the rates of biological processes will increase as temperature increases, which is referred to as temperature sensitivity.

2.5.1 Previous Approaches (theory)

The temperature sensitivity of many biological processes in soil is most commonly described using the Q_{10} model and very commonly used to describe changes in respiration with increasing temperature (Equation 2.4) (Hyvönen *et al.*, 2005). Q_{10} is the ratio of the rate observed at one temperature to the rate observed at a temperature 10 °C lower (Tjoelker *et al.*, 2001).

$$Q_{10} = \frac{k_{T+10}}{k_T} \qquad \text{(Eq. 2.4)}$$

Where k is the decomposition rate (in the case of this thesis respiration rate = Rs) for a given temperature.

Most Q_{10} values are calculated from the results of different models fitted to respiration data, such as the Arrhenius and Lloyd Taylor models, which aim to model temperature responses (Fang & Moncrieff, 2001). The Q_{10} model is therefore used in

conjunction with these to provide a measure of sensitivity, which can be compared to other relative sensitivities in the literature (Sierra, 2012).

2.5.2 Previous approaches (laboratory methods)

Many studies attempt to measure temperature sensitivity of respiration when investigating the soil response to daily, seasonal or even interannual variation in temperatures (Davidson *et al.*, 2000). When measuring temperature sensitivity, some laboratory method can be problematic and introduce artefacts that obscure true temperature sensitivity. Common problems include; a lack of temperatures that respiration is measured at, use of long term incubations, a lack of seasonal measurements and including variability in moisture content. There are very many laboratory studies of respiration response to temperature and other variables. A few of these studies are summarised in Table 2.1. While not an exhaustive list of studies, this collection is useful to explore a number of shortcomings observed in literature.

Table 2.1: Summary of previous approaches used to measure temperature sensitivity (Q10). Variable indicates what the study was observing to effect temperature sensitivity of respiration (Pre-inc= Pre-incubation effects; Var= Variation; Soil Pools= Labile and Non-labile fractions; MC= Moisture content; Fert= N and P fertilisation; C min= carbon mineralisation) Method states the method of CO2 measurement used (Cha= Chamber; IRGA= Infra-red gas analyser; Inj= injection; O-A= Open Air; GC= Gas chromatography; TGA= tunable diode laser absorption spectrometer; C-L= Closed Loop; SL= Soda and Lime method; MS= isotope mass spectrometer; NaOH= NaOH titration). No. T specifies how many incubation temperatures respiration was measured at (c. = continuous temperature change; brackets show minimum and maximum temperatures (°C)). Inc t indicates the amount of time soil was incubated at the incubation temperatures (h= hours; d= days; w= weeks; m= months). No. S is the number of times sampling occurred per year. Finally MC indicates how many moisture contents were used.

Reference	Variable	Method	No. T	Inc t	No. S	MC
Andrews *et al.*, 2000	Pre-inc	Cha IRGA	3 (4-40)	48 d	2	1
Arevalo *et al.*, 2012	Land use	IRGA inj	3 (7-21)	370 d	1	1
Barrett *et al.*, 2006	Pre-inc	IRGA inj	3 (5-20)	120 d	1	2
Bekku *et al.*, 2003	Soil type	O-A IRGA	6 (5-40)	12-18 h	1	1
Bekku *et al.*, 2003	Soil type	O-A IRGA	6 (2-28)	12-18 h	1	1
Bekku *et al.*, 2003	Soil type	O-A IRGA	6 (2-20)	12-18 h	1	1
Bradford *et al.*, 2010	Pre-inc	IRGA inj	3 (10-30)	77 d	1	1

Table 2.1 continued

Reference	Variable	Method	No. T	Inc t	No. S	MC
Chen *et al*., 2010	Spatial var	GC	15 (10-30)	24 h	1	1
Conant *et al*., 2008a	Pre-inc	IRGA inj	2 (25-35)	336 d	1	1
Conant *et al*., 2008b	Pre-inc	IRGA inj	1 (4-4)	450 d	1	1
Conant *et al*., 2008b	Pre-inc	IRGA inj	1 (15-15)	450 d	1	1
Conant *et al*., 2008b	Pre-inc	IRGA inj	1 (25-25)	450 d	1	1
Conen *et al*., 2006	Substrate type	GC	4 (5-35)	≤ 6 d	1	1
Conen *et al*., 2008	Substrate type	GC	4 (5-35)	3-105 h	1	1
Cross and Grace, 2010	Soil pools	TGA	5 (5-30)	8 w	2	1
Cusack *et al*., 2010	N addition	GC	2 (21-31)	245 d	1	1
Elberling & Brandt, 2003	Ecosystem	GC	5 (-10-20)	6-8h	1	1
Elberling *et al*., 2006	Landscape elements	GC	24 (-1-12)	13 d	1	1
Fang & Moncrieff, 2001	Pre-inc/MC	Cha IRGA	10 (10-40)	120 h	1	3
Fang *et al*., 2005	Soil pools	Cha IRGA	10 (4-44)	2 h	1	1
Fierer *et al*., 2003	Soil pools Fert Addition	C-L IRGA	6 (10-35)	30 h	3	4
Fierer *et al*., 2006	Spatial var	C-L IRGA	5 (10-30)	1-24 h	7-20	1
Fissore *et al*., 2009	Ecosystem	GC	2(10-30)	525 d	1	1
Gershenson *et al*., 2009	Substrate type	SL	4 (0-30)	1 h	1	1
Gillabel *et al*., 2010	Soil pools	MS	2 (25-35)	176 d	1	1
Haddix *et al*., 2011	Soil type	IRGA	3 (15-35)	588 d	1	1
Hamdi *et al*., 2011	Pre-inc	NaOH	4 (20-50)	28 d	1	1
Hartley & Ineson, 2008	Substrate type	O-A IRGA	3 (10-20)	124 d	1	1
Hartley *et al*., 2008	Pre-inc	Cha IRGA	2 (2-10)	200 d	1	1
Holland *et al*., 2000	Ecosystem	GC	4 (15-55)	24 h	1	1
Koch *et al*., 2007	Pre-inc	NaOH	7 (0-30)	25 d	3	1
Leifeld and Fuhrer, 2005	Substrate type	Chamber	4 (5-35)	159 d	1	1
Liu *et al*., 2006	Soil pools	SL	2 (10-30)	5 w	1	2

Table 2.1 continued

Reference	Variable	Method	No. T	Inc t	No. S	MC
Mikan *et al*., 2002	Pre-inc	C-L IRGA	(-10-14)	28 d	1	1
Miller *et al*., 2007	C min	IRGA inj	3 (-2-5)	4 m	1	2
Neff & Hooper, 2002	Substrate	IRGA inj	2 (10-30)	365 d	1	1
Phillips *et al*., 2012	Soil Horizon	GC	5 (-15-5)	< 28 d	1	1
Reichstein *et al*., 2000	C min	C-L IRGA	3 (5-25)	104 d	1	1
Reichstein *et al*., 2005	Substrate type MC/ Pre-inc	Chamber	c. (7-23)	90 d	1	2
Song *et al*., 2010	C min	NaOH	3 (5-20)	28 d	1	1
Vanhala *et al*., 2007	Soil pools	MS	5 (10-33)	24 h	1	1
Wang *et al*., 2010	Soil pools	NaOH	4 (5-20)	40 d	1	4
Wickland & Neff, 2008	Drainage type	IRGA inj	2 (10-20)	57 d	1	5
Xu *et al*., 2012	Pre-inc	NaOH	6 (5-35)	170 d	1	1
Zhang *et al*., 2007	Soil pools	NaOH	2 (20-25)	114 d	1	1
Zhu & Cheng, 2011	Land use	NaOH	2 (20-25)	122 d	1	1

2.5.3 Number of incubation temperatures

To determine soil respiration changes with increasing temperature, soil incubations need to be made at a range of temperatures. This is frequently limited by the availability of incubators that can be set at specified temperatures. As observed in Table 2.1 most studies of respiration response to temperature measurements use under 10 temperatures, five being the most common number, leading to a rather limited small number of points in a data set. A lack of data points allows for the fit and justification of many predictive models with little ability to discriminate between them (e.g. Arrhenius, Lloyd and Taylor and MMRT). Some papers have attempted to measure respiration response to temperature in systems with controlled increases in temperature changes, such as Bekku *et al*., (2003) who used a water bath to increase a soil sample temperature over several hours. However, the method used still only resulted in six respiration measurements during the temperature change due to the need to stabilise temperature and then make measurements. To accurately test and compare different models a larger number of incubation temperatures are needed before sensible conclusions can be drawn about temperature response and sensitivity.

2.5.4 Length of incubation time

To determine soil respiration rates at different temperatures, soil needs to be incubated at each temperature for a specified time interval to allow for measurement of CO_2 production. As observed in Table 2.1, many studies tend to have soils incubating for a long period of time before and between CO_2 samplings (Inc t). In some cases, this incubation time is needed to observe changes in respiration rate and temperature sensitivity in the long-term (Conant *et al.*, 2008) but a large number studies tend to measure collected soil respiration over extended periods and use values to infer temperature sensitivity of *in situ* CO_2 production (Cusack *et al.*, 2010; Fierer *et al.*, 2006). This inference may not be justifiable as often, with long incubation periods, a decline in respiration rate response to temperature that can occur after some time (Bradford 2013; Conant *et al.*, 2008). This reduction has been attributed to thermal adaptation of microbial populations to higher temperatures over time, and thus respiration responses measured after incubation no longer reflect the microbial population at the time of sampling Bradford (2013). Thermal adaptation, as described by Bradford (2013) is a term, which includes direct (rather than indirect) organism responses to temperature over multiple timescales, which may appear as a physiological change. Within in the literature the term "thermal acclimation" is often used interchangeably with "thermal adaptation". Both terms typically are used to explain the decrease in respiration rate responsiveness to temperature increase that can occur after some time (Bradford, 2013, Kirschbaum, 2004).

In addition to thermal adaptation, another explanation commonly proposed to explain a reduction in respiration rate response to increasing temperature is greater the consumption of carbon resources at higher temperatures during long-term incubations (Kirschbaum, 2004). After litter enters a system, with time CO_2 production will increase as the biological system responses and consumes the available substrate. However, if the input rate remains constant, but the respiration increases in response to temperature, the increased respiration rate can only be sustained for a limited time before available substrate is exhausted. This decrease in available substrates results in a decline in respiration rate even at higher temperatures (Kirschbaum, 2004). This explanation can account for the observed reduction in respiration rate response to increasing temperature without adaptation.

There is wide debate (Bradford *et al.*, 2008; Conant *et al.*, 2008b; Kirschbaum, 2004) about whether thermal adaptation occurs during long incubation studies or if changes in respiration rate response are attributed only to changes in the consumption of SOC. Modelling studies such as Kirschbaum (2004) and Knorr *et al.*, (2005) found that thermal adaptation was not needed to explain changes in soil respiration rate due to temperature and response could be explained by substrate depletion. Other studies such as Bradford *et al.*, (2008) and Hartley *et al.,* (2009) observed some thermal adaptation. Overall, while there is uncertainty about the responsible mechanisms, there is a temperature effect on respiration during long-term incubations, which can confound our understanding of temperature sensitivity of *in situ* respiration.

2.5.5 Seasonal variation

While often studies of temperature sensitivity focus on the mean annual temperature of sampling sites there has been little study on how temperature sensitivity might vary seasonality. To observe seasonal effects in soil respiration rates at different temperatures samples need to be taken at multiple times during the year to allow for any *in situ* seasonal changes in microbial population or changes in enzyme production by microbes to be examined (Bradford, 2013; Davidson *et al.*, 2000). As observed in Table 2.1 temperature sensitivity studies tend to look at variables such as land use and pre-incubation and are only sampled on one occasion throughout the year. Although temperature based seasonal effects on soil respiration can be mimicked in the laboratory by changing incubation temperature over time, *in situ* changes caused by temperature change cannot and do not account for changes in other seasonally dependent variables such as changing carbon inputs from plant growth. A lack of seasonal sampling ignores potential seasonal effects on temperature sensitivity thus it is unclear whether temperature sensitivity varies throughout time as microbial populations adapt to seasonal changes.

2.5.6 Moisture content effects

Moisture content is typically a highly studied factor in respiration response to temperature (Sierra *et al.*, 2015, Davidson *et al.*, 2000). It is generally considered that microbes tend to have optimum water content at which they function at maximum

efficiency although this optimum moisture content has not been well described (Sierra *et al.*, 2015, Davidson *et al.*, 2000). As observed in Table 2.1 the generalization of an optimum moisture content likely leads to studies using a small range of a lack moisture contents in respiration studies. Indeed, most studies use one moisture content, often field capacity (about 50 to 60%), ignoring potentially important interactions between moisture content and temperature change that occur *in situ*.

2.6 Research needs

Enhancing the understanding of C cycle dynamics in response to climate change are driving the need for better predictions of changing C stocks through more accurate methodology and modelling of C exchanges in response to temperature, including soil respiration.

To address research gaps identified in section 2.5, a method of measuring soil respiration rate is required which includes short incubation times to reduce any potentially negative effects of thermal adaptation, and has multiple incubation temperatures to allow for better fitting and justification of predictive models for more accurate carbon budgeting purposes. Additionally new methodology needs to be used applied various treatments including spatial variation, moisture content, pre-incubation temperature and seasonal variation to ensure the method is robust and can measure temperature sensitivity.

Chapter 3. Method Development

3.1 Introduction

A central component of this thesis was to be able to determine temperature sensitivity of soil respiration (Section 2.5). Due to potential problems with current measurement techniques (Section 2.5.2) an improved method was required to accurately measure temperature sensitivity. The main objective of the work reported in this chapter was to develop a quick and reliable method for measuring soil respiration at a wide range of temperatures. This chapter describes the development of the general method and how limitations were overcome to refine general laboratory procedures including:

- The use of the temperature block, stability and temperature linearity (Section 3.2)
- Gas sampling methodology (Section 3.3)
- Soil sample size and incubation timing (Section 3.4)
- Respiration linearity with time (Section 3.5)
- Summary (Section 3.6)

The method developed here was used in the next component of the thesis that examined specific questions related to temperature sensitivity of soil respiration.

3.1.1 Overview of method

An overview of the final method is given here. Please see sections 3.2, 3.3, 3.4 and 3.5 for a description of how the final design was decided.

The temperature block for incubating soil (Section 3.2) was left on overnight to stabilise the temperature gradient. Thermistors spaced evenly across the block measured and recorded seven temperatures along the block to allow calculation of the temperature at each location. With the antifreeze coolant set at -5 °C and the heater at 55 °C the temperature gradient across the block stabilised at approximately 2 – 50 °C (Section 3.2.1)

Soil (3 ± 0.5 g, 2 mm sieved) was added to 120 Hungate tubes. Tubes were evenly distributed along the gradient (40 tubes maximum per row). Once distributed, rubber stoppers were inserted into tops of tubes to prevent gas loss, and the time when inserting all rubber stoppers was recorded. Once stopped, the tubes were also sealed using aluminium crimps as an extra precaution against gas loss. The block was then closed with a clear Perspex lid to help maintain a stable internal temperature.

After five hours of incubation (from the first rubber stopper placement), the Perspex lid was lifted and, whilst in the block, the crimps were removed and 1 mL of gas removed via an insulin syringe and needle from each tube, in the same order the tubes were sealed. These syringes have the needle welded onto the syringe barrel minimising the opportunity for gas loss. The time taken to sample the gas from all tubes was also recorded.

After sampling needles were inserted into large rubber bungs for transportation to the laboratory for CO_2 analysis using an infra-red gas analyser (IRGA) within one hour. A standard curve was produced for the IRGA using 1% CO_2 standard (Section 3.3.1), before and after the soil gas samples were injected.

3.2 Temperature block

One of the limitations of previous approaches for measuring the response of respiration rate to increasing temperature is that frequently respiration is only measured at a limited number of temperatures (e.g. 5, 15, 25, 35 °C). Limited temperature points mean that a wide variety of models (Section 2.4) can be fitted without being able to distinguish between them. Often the number of temperatures that can be tested is limited by availability of incubators that can be set to specific temperatures. To increase the number of data points and consistent model fits, a temperature gradient was set up across a large aluminium block to give a large range of incubation temperatures.

This temperature block was custom built originally for determining temperature response of microorganisms in pure culture and consisted of an aluminium block (1400 x 130 x 190 mm), which is heated using a heater at one end, and cooled with anti-freeze cycled through a water bath at the other end (Figure 3.1). There are three

rows of 44 slots (20 mm) drilled into the block at 10 mm intervals giving a total of 132 slots. A clear Perspex lid sits over the block to help maintain an even temperature gradient whilst incubations are occurring (Figure 3.1). The sides of the block were insulated using polystyrene.

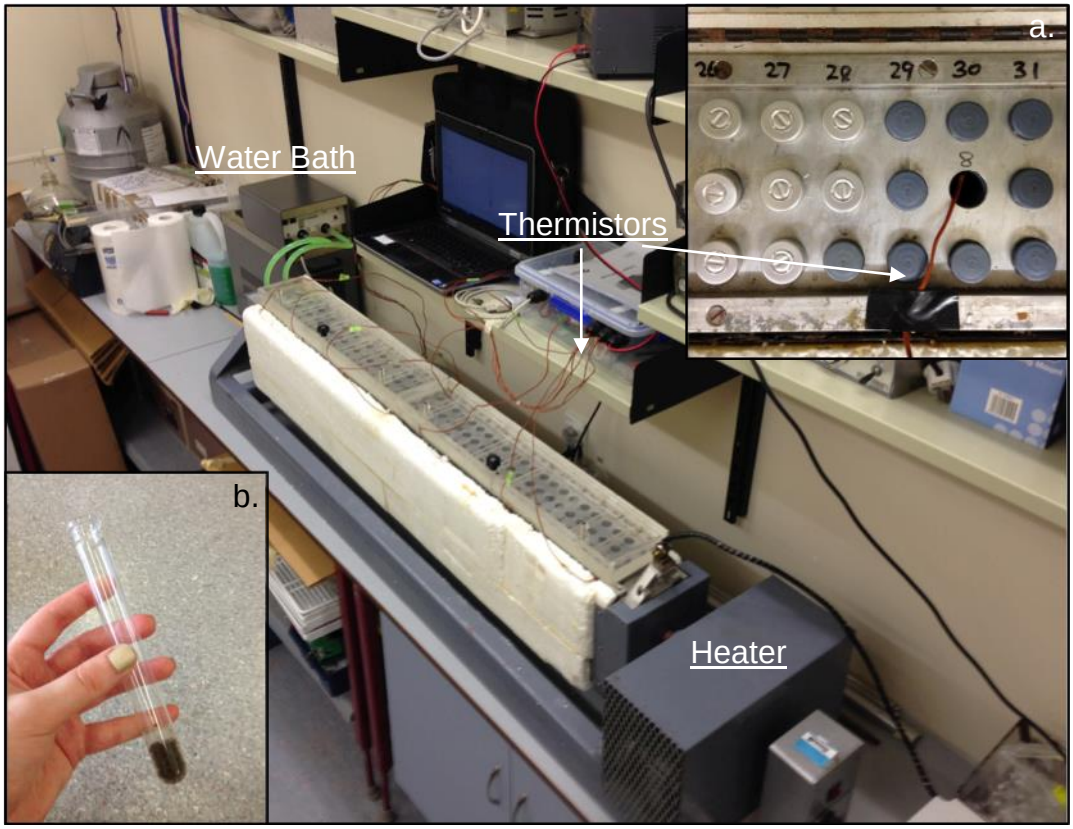

Figure 3.1: Temperature block with water bath on the left end and a heater on the right. (1400 mm in length) Insert (a.) shows Hungate tubes in place, stopped on the right hand side and bunged and crimped on the left. Insert (b.) shows Hungate tube with 3 g of soil.

Small soil samples, contained within 10 mm diameter glass Hungate tubes were inserted into each slot for incubation (Figure 3.1). Seven thermistors measure temperature consistently during incubation period. (Figure 3.3) provides an example display of the temperature measured at these seven points along the block during the course of an incubation.

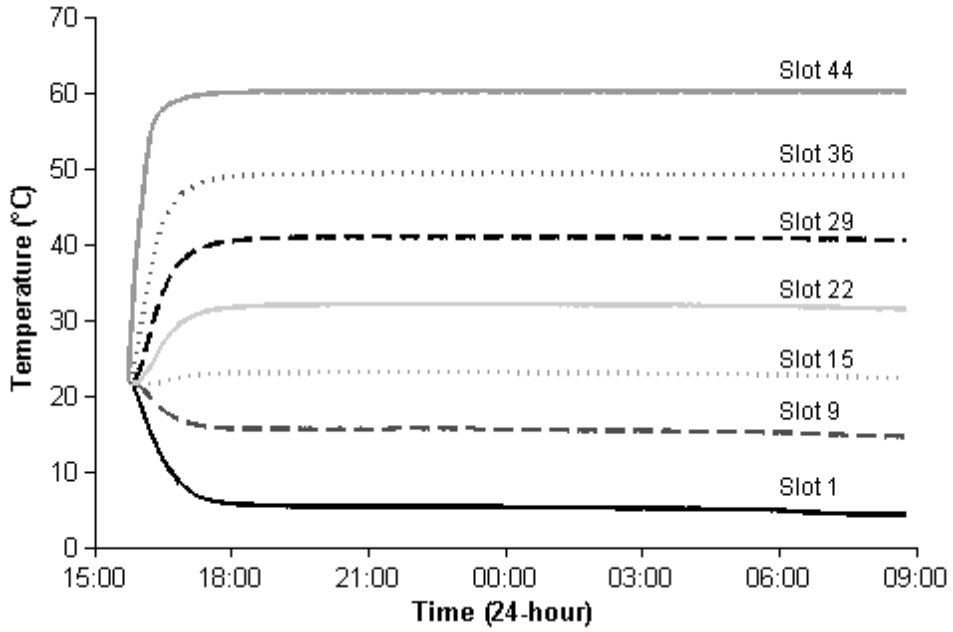

Figure 3.3: Temperature of block over 18 hours, as measured by thermistors at seven slot positions across the block. Stabilisation of temperature gradient occurs within 4 hours.

After overnight equilibration, temperature stability was high (Figure 3.3). The use of this temperature block allowed for measurements of soil respiration at 40 different temperatures (Figure 3.2) during a 5 hour incubation with accumulated CO_2 measured using an IRGA (Section 3.3.1).

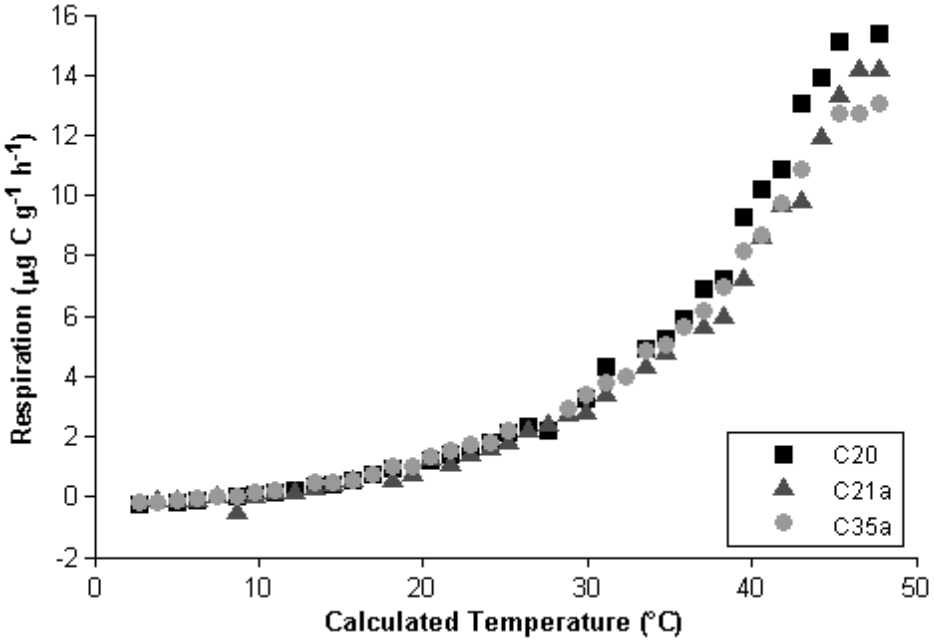

Figure 3.2: Example data of soil respiration of a Horotiu silt loam collected from three separate paddocks (C21a, C35a C20) at Scott Farm. Each point represents an separate respiration rate measured at a specified temperature.

3.2.1 Temperature linearity across the block

3.2.1.1 Temperature gradient

While there was good stability of temperature at seven locations along the length of the block during each incubation, temperature at each slot needed to also be accurately calculated for each experimental run. In a preliminary experiment, to ensure the temperature gradient was even across the block, thermistors were placed in each of 44 slots after the temperature gradient had been stabilised, and the temperature at each position measured. The result was a linear gradient from one end of the block to the other (Figure 3.4).

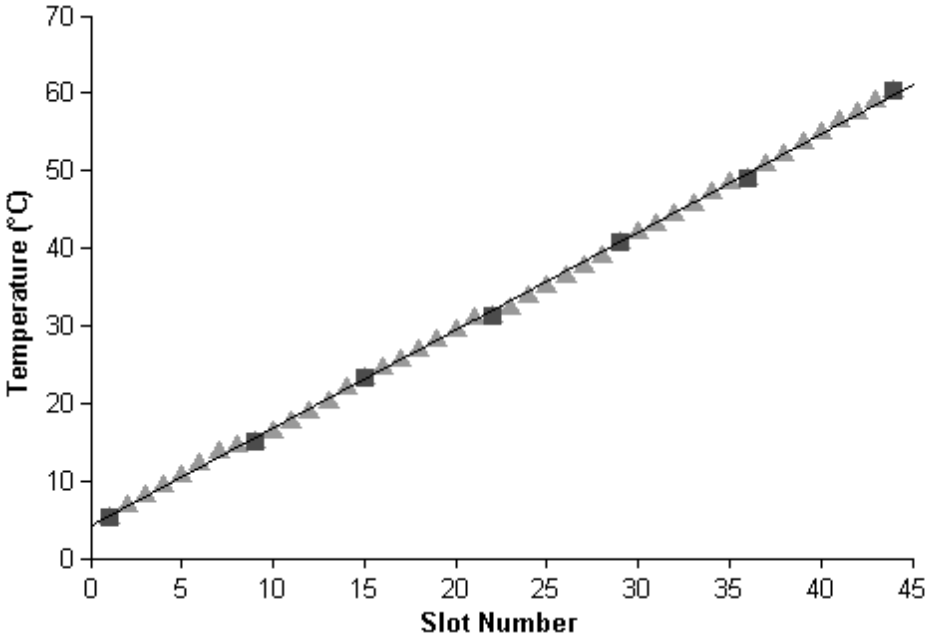

Figure 3.4: Measured temperature at each slot with temperature block set at -5 at cold end to 70 °C at hot end. Linear fit with R^2 value of 0.999. Black squares indicate positions of thermistors subsequently used in each respiration measurement to calculate temperature gradient during routine measurements.

This stable, linear gradient means the temperature at each slot (and therefore each soil sample) can easily be calculated from only a few temperature measurements along the length of the block. For all subsequent experiments, the block had seven thermistors evenly placed along the block, measuring temperature every minute throughout the incubation period (Figure 3.4). Linear regression of temperature and slot number was then used to calculate the temperature of each slot.

3.2.1.2 Temperature consistency through incubation time

A small upwards drift in temperature of about 1 °C was observed over the incubation period (5 hours). This drift occurred across the block and was dependant on changes in room temperature which increased from morning to afternoon.

For routine measurements the temperature for each slot was averaged over the total incubation period and the change was small (1 °C) compared to the temperature range (~2 – 55 °C) on the block. Consequently, this small change in temperature was not considered problematic. It was not possible to maintain the room temperature more precisely, however, in experiments with a smaller temperature range, the external temperature change may have a greater effect on resulting respiration rate and experiments would benefit from a greater regulation of room temperature.

3.3 Gas sampling and analysis

Respiration analysis required the measurement of CO_2 concentration within a withdrawn sample. The simplest way to quantify CO_2 concentration in a small, contained sample is through removing a specified amount of headspace gas, using a needle and syringe, and subsequent analysis using an infra-red gas analyser (IRGA). Gas samples were collected in 44 individual ultra-fine insulin syringes (Becton, Dickinson and Company. These syringes have the advantage of a very fine needle that is directly welded onto the barrel eliminating gas loss at the needle/barrel junction.

3.3.1 Gas analysis and calculation of CO_2 concentration

Collected CO_2 was measured using a LI-6262 CO_2/H_2O analyser (LI-COR, Lincoln, Nebraska) coupled to a chart recorder. A standard curve was created before each set of sample analysis by injecting varying volumes of a 1% CO_2 standard in nitrogen gas (Beta-standard 1.00 ± 0.4%, ISO Guide 34 Certificate). Standard curves were constructed from peak heights of triplicate injections of 1 mL, 0.8 mL, 0.6 mL, 0.4 mL and 0.2 mL were injected before and after injections of experimental samples. When CO_2 concentrations in injected samples exceeded the highest standard (1 mL of 1% CO_2), a 2 mL standard was also included.

Typically a linear regression is used for standard curve fitting. For standard injections less than 1 mL a linear fit appeared adequate, however with the inclusion of a 2 mL injection linear injection, a linear regression was no longer reasonable (Figure 3.5).

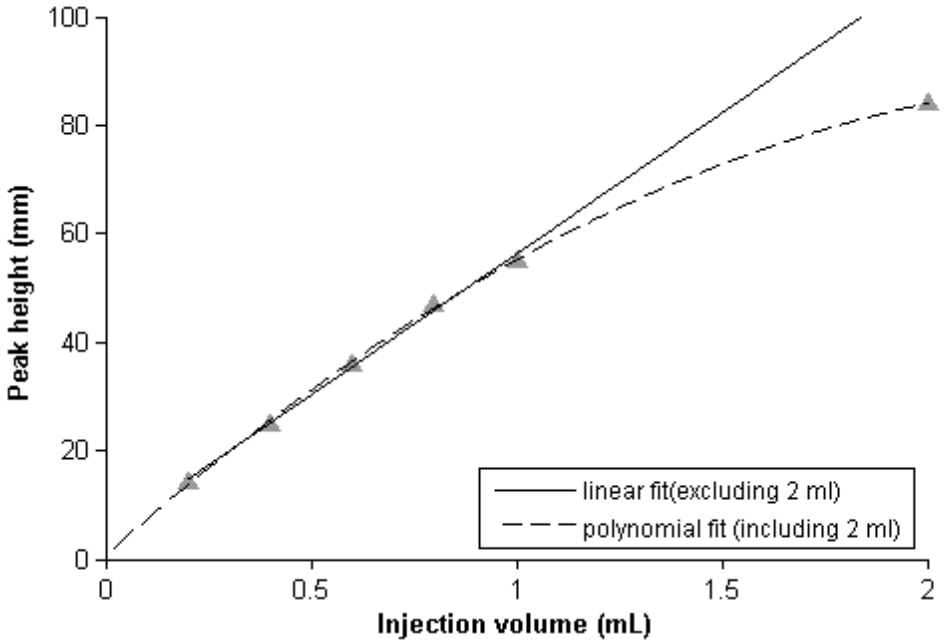

Figure 3.5: Peak height for 0.2, 0.4, 0.6, 0.8, 1.0 and 2.0 mL injections of 1% CO_2 standard in nitrogen gas. Data fit with a linear regression (excluding the 2 mL data point) as well as a polynomial regression (including the 2 mL data point).

After this analysis, gas samples that would exceed the upper standard (1 mL of 1% CO_2) were reduced to 0.5 mL injections to bring the CO_2 concentrations within the linear range of the standard curve.

3.3.2 Gas transport in syringes

For this thesis, the two key experimental instruments (the temperature block and the IRGA) were housed in separate laboratories about a 5-minute walk apart. As the gas was removed using a syringe after a specific incubation time, all samples were taken at one time and transported to the IRGA for analysis. Syringe needles were inserted into rubber bungs immediately after sampling. To determine whether there was loss of gas during transport, the syringe and needles while inserted into rubber bungs were tested for potential leakage. Triplicate samples (1 mL) of 1% CO_2 standard were taken using syringes and placed into rubber bungs. These standards were subsequently analysed by IRGA after 0, 0.12, 0.5, 1 and 2 hour (Figure 3.6).

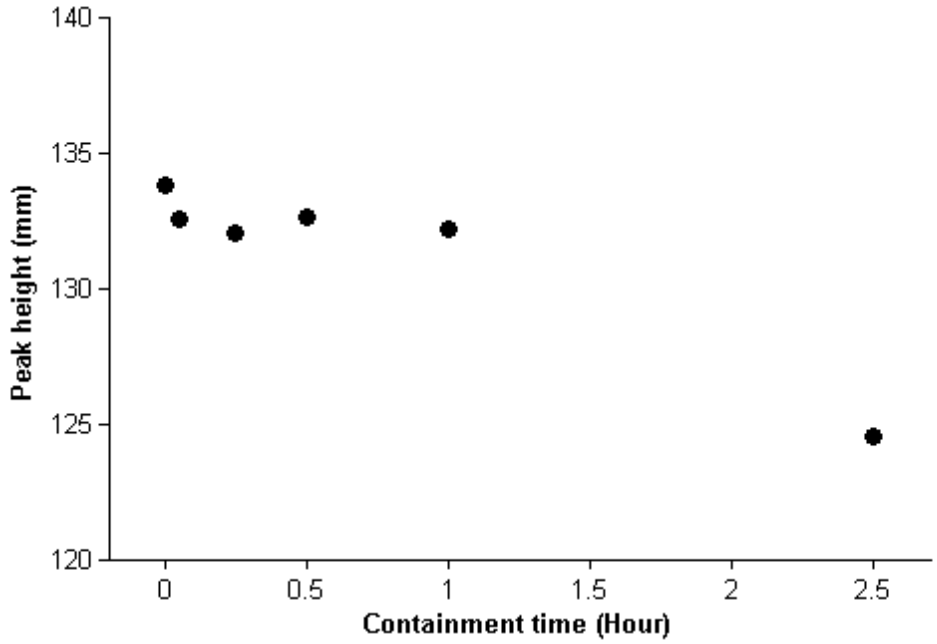

Figure 3.6: Average peak heights of 1% CO_2 standard in nitrogen gas (1 mL) after left in rubber bungs for 0, 0.12, 0.5, 1 and 2.5 hour. Note: y-axis does not start at the origin (0).

The results from this experiment showed that the insulin syringes with welded needled have minimal to no gas leakage within 1 hour. There was a 5% reduction in peak height between 1 and 2.5 hour. Throughout this thesis, the time between sampling and analysis was less than 1 hour.

3.4 Incubation of soil samples

3.4.1 Incubation length and sample weight

To produce measurable CO_2 concentrations across the range of incubation temperatures, adequate soil sample sizes and incubation periods are required. The soil weight was initially set at 4 g with an incubation time of 6 hours. This approach produced a detectable respiration rate at low temperatures (<10 °C); however, at higher temperatures, too much CO_2 production resulted in peak heights above that in the standard curve.

To ensure accurate measurement of CO_2 production at lower temperatures and avoid excessive CO_2 concentration at higher temperatures, a split in soil weight and incubation length between two halves of the block was tested. For the split-block

approach, Hungate tubes at the low temperature end of the block had 4 g of soil added and incubated for 6 hours. For the high temperature end, 2 g of soil was incubated for 4 hours. The results from this split-block method resulted useful data within each section of the block but examination of the graphs indicated that the production rates for each end of the block did not align (Figure 3.7).

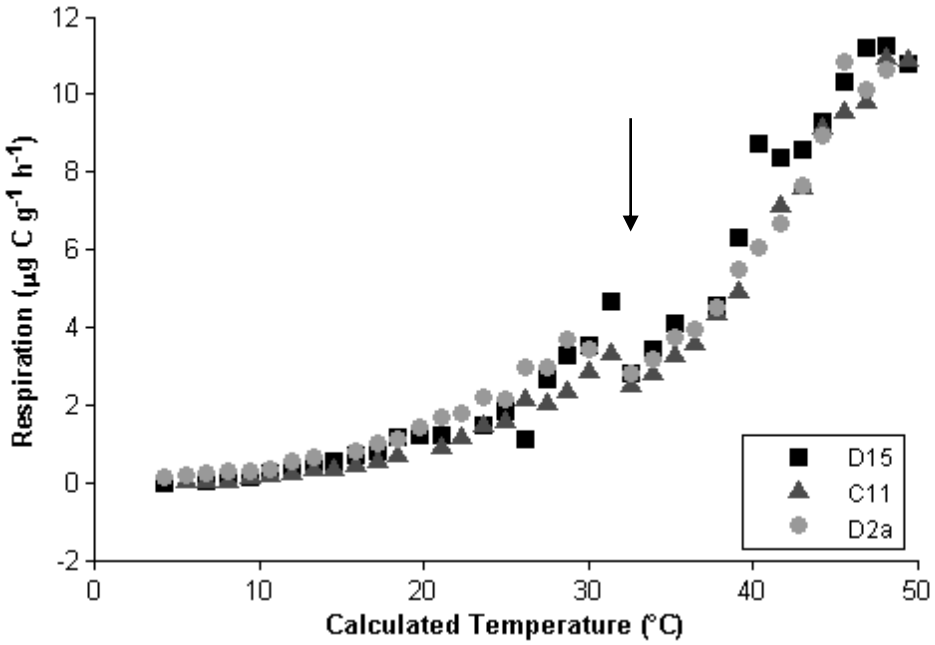

Figure 3.7: Example data of soil respiration of a Te Kowhai silt loam collected from three separate paddocks (D2a, D15 C11) at Scott Farm. For temperatures between 0 and 32 °C, 4 g of soil was incubated for 6 h and for 32 - 50°C soil was incubated for 4 hour. Note: Break in respiration rate is indicated by arrow.

A final approach was to use 3 g of soil with a 5 hour incubation time. This final method resulted in adequate CO_2 concentrations at both ends of the temperature block. Nonetheless, there remained concern that for the very cold temperatures (<10 °C) insufficient CO_2 was produced to be measured accurately for full inspection of temperature sensitivity at these low temperatures. While this method was used for the remainder of this thesis, caution is applied to interpretation of low temperature data.

3.4.2 Respiration linearity

This part of method development was concluded by Dr Tanya O'Neill as this thesis was being started. The description of results is presented here for completeness.

Respiration rates were calculated as the amount of CO_2 produced per g of soil divided by increment of time. However, it was uncertain about whether there was linear production of CO_2 during the incubation. If respiration increased exponentially this would indicate microbial growth rather than *in situ* response. To test for respiration linearity, 4 g of Horotiu Silt Loam was added to Hungate tubes with the block set between -5 and 30 °C. Additional air (3 mL) was added to each sealed tube. Gas samples (1 mL) were taken after 1, 2 and 6 hours and analysed using the IRGA. Respiration rate was calculated following section 4.6.3. Data suggested a rapid rise in CO_2 concentration during the 1st hour with subsequently a slower respiration rate (Figure 3.8).

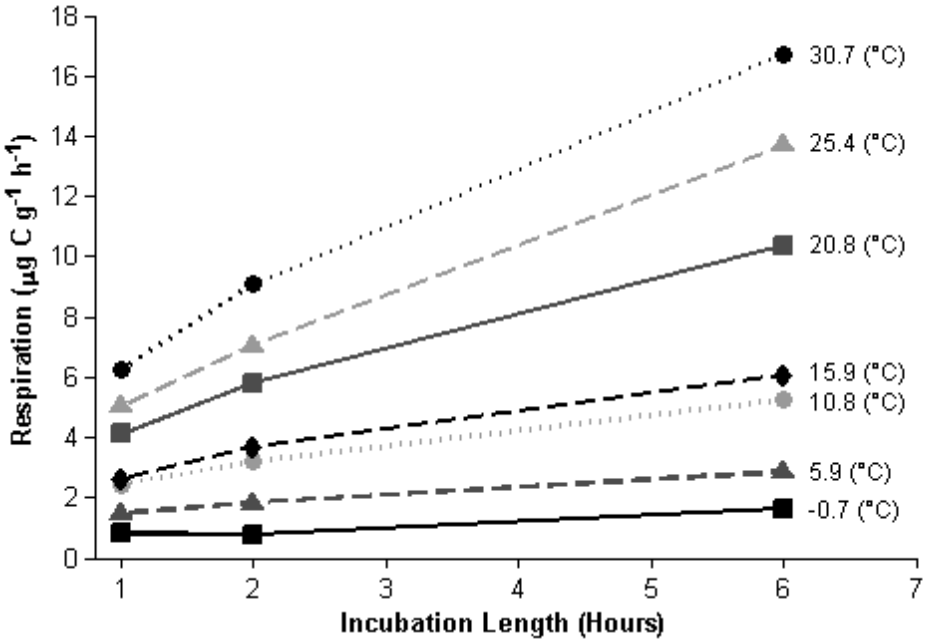

Figure 3.8: CO_2 concentration respired by 4 g of soil at 7 different temperatures after incubation lengths of 1, 2 and 6 hours.

When calculating a respiration rate based on one measurement point at the end of incubation, a linear respiration rate throughout the incubation period is assumed (although checking this concept is frequently absent in reported literature). A comparison of calculated rates between 1 and 6 hours, and 0 and 6 hours demonstrated that sampling only once (at 6 hours) overestimates CO_2 respiration rates (Figure 3.9). It is not known what might contribute to this initial rapid increase in headspace CO_2 may be due to a change in equilibrium between dissolve CO_2 and headspace CO_2.

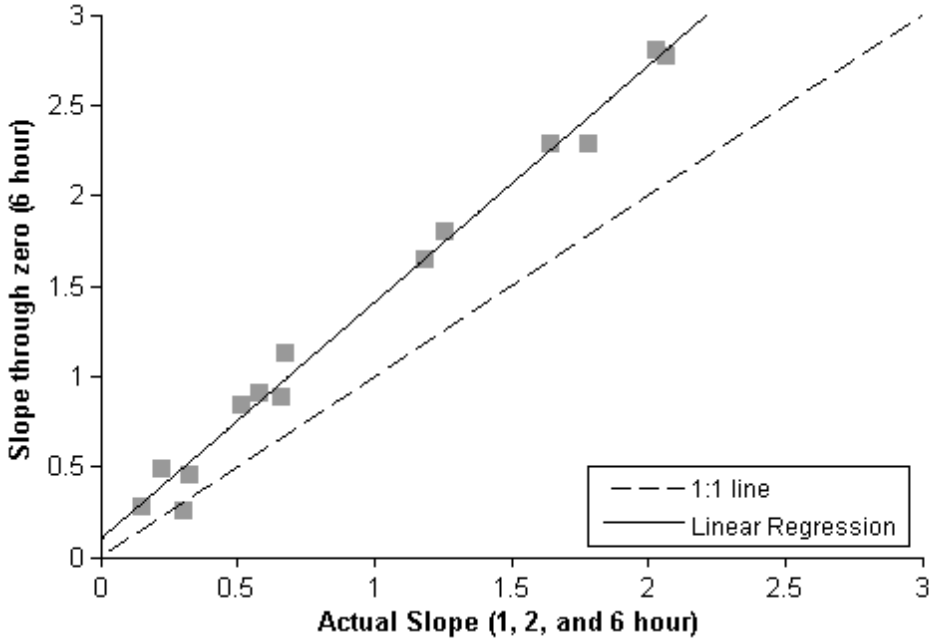

Figure 3.9: Linear regression between two linear regression slopes (R^2:0.99) .compared to 1:1 line (Dashed). Slope through zero (y-axis) is the slope of a linear regression using only the 6 hour respiration data and zero. The actual slope (x-axis) is the slope of a linear regression using 1, 2 and 6 hour data.

For this thesis, a one-point measurement after 5 hours was used and represents a relative respiration rate rather than absolute respiration rate but these are highly correlated. This decision reflects a compromise to allow measurement of more soil samples. However, this experiment does highlight the potential need for multiple gas samplings, over an incubation period, in order to observe actual respiration rates.

3.5 Summary

During the development of this method, key findings were:

- Block temperature was linear along the block and remains stable (within 1 °C) throughout incubation periods.
- Gas can be held in needle and syringes for up to an hour, allowing for transportation of samples.
- The best methodology for determining respiration for a temperature range of ~2 to 55 °C was use of 5 g of soil incubated for 5 hours.
- Respiration rate was not linear with time but estimates of respiration using an average 6 hour incubation was strongly correlated to linear production

between 1 and 6 hours. For pragmatic reasons a one-time point sampling was selected.

- CO_2 standard curves can be non- linear above 1 mL of 1% CO_2 standard and so smaller volumes of samples with high concentration were injected to stay within the linear region of standard curve.

These points were taken into consideration when finalising the method used for the remainder of this thesis (See section: 3.1.1).

Chapter 4. Methods

4.1 Introduction

The focus of this thesis was to create an accurate and easy approach for measuring respiration rates of soil collected from the field at a range of temperatures. As covered in Chapter 3, a method was created that measured temperature response of a single soil sample addressing a number of important laboratory procedures. To test the limitations of this method for routine measurement of soil respiration in samples collected from the field, it was necessary to determine whether factors associated with field sampling or soil preparation also altered the temperature dependency of respiration.

In this chapter several key factors that influence sample collection and preparation were examined and the main objectives were:

- to determine whether different soil types collected from a single farm had different respiration response to temperature after collection. If temperature sensitivity varied between different soils collected from a small area (e.g. a farm) this would imply that assessment of temperature sensitivity required multiple soil sampling (Section 4.2).
- to determine whether seasonal change in temperature prior to collection would alter the respiration sensitivity to temperature. If temperature sensitivity depended on season this implies multiple sampling events per year was necessary to characterise each site (Section 4.3).
- determine respiration rate response to temperature change over varying soil moisture contents and identify optimum water content for measuring temperature sensitivity. If temperature sensitivity varied with differing soil moisture contents this would imply the adjustment of samples moisture content was required for optimum response (Section 4.4).
- observe whether pre-incubation of soil at different temperatures has an effect on subsequent respiration rate response to temperature change. If temperature sensitivity depended on pre-incubation temperature this implies samples required the same incubation conditions, or preferably were analysed immediately after collection (Section 4.5).

This chapter gives a full description of the methods used. These will also be summarised in Chapter 5 but in a format more suited for journal publication. There will therefore be some repetition between Chapters 3, 4 and 5.

4.1.1 Site description

Scott Farm (Vaile Rd, Newstead, Hamilton, New Zealand.) is a research dairy farm with variable stocking rate, run by DairyNZ (Figure 4.1). This location was selected due to its close proximity to the University for quick and easy sampling of a wide range of soils under the same climate. Annual rainfall at the site was 1031.2 mm and average annual temperature was 14.4 °C.

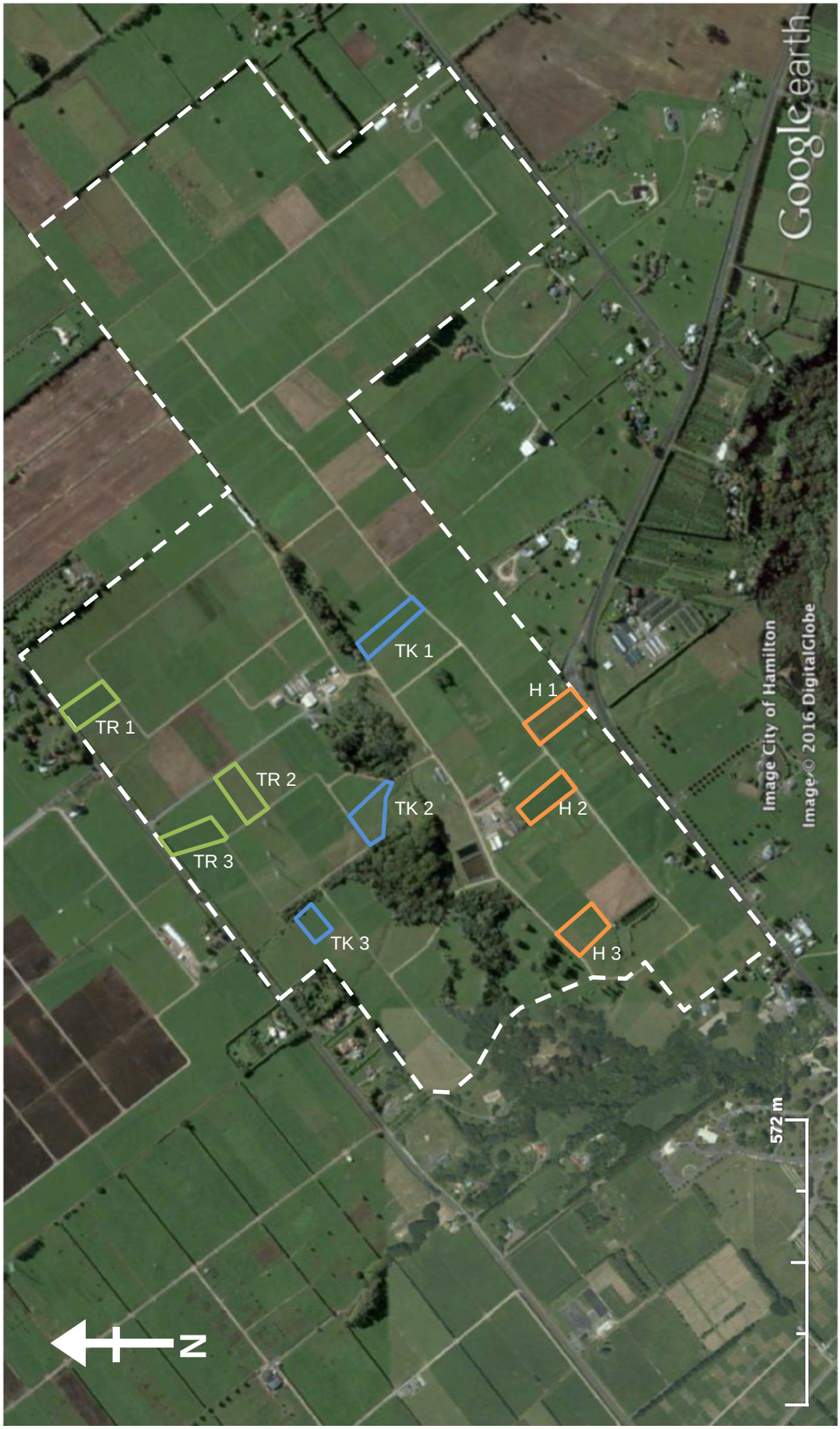

Figure 4.1: Picture of Scott Farm (Outlined in white). Picture from Google Earth 23rd April 2015. Retrieved 20th December 2015. Paddocks sampled are also outlined with main soil types identified. Horotiu (H), Te Kowhai (TK), Te Rapa (TR).

4.2 Soil types effects on temperature response

The main objective for this section was to determine whether different soil types had different respiration response to temperature after collection from a single farm.

Primary soil types found at Scott Farm include Horotiu, Te Kowhai, Bruntwood, Te Rapa, and Matangi soils. For this experiment, Horotiu, Te Kowhai and Te Rapa were chosen for sampling due to the difference in their types.

Horotiu silt loam is classified as a Typic Orthic Allophanic Soil and is described as well-drained with high P retention in the top soil. The parent material is derived from alluvium with tephric profile material. The topsoil has a loamy silt texture, with the subsoil having very fine to coarse loamy sand and loamy silt textures. The most significant feature of the Horotiu soil is its high allophanic content. Allophane increases phosphorus (P) retention, which limits its availability for plant growth (Singleton, 1991).

Te Kowhai silt loam is classified as a Typic Orthic Gley Soil and is described as poorly drained with medium P retention in the top soil. The parent material is derived from alluvium with tephric profile material and has a loamy silt texture with fine to medium sized gravel also present. The main difference between Te Kowhai and Horotiu soils is lower P retention and a slower drainage regime with a high water table. A high water table can restrict root growth and limit decomposition due to anoxic conditions (Singleton, 1991).

Te Rapa humic silt loam is classified as a Peaty Orthic Gley Soil and is described as poorly drained with low P retention in the top soil. The parent material is derived from alluvium with tephric profile material. The major feature of this soil is the presence of peat material and also moderately acidic topsoil (Singleton, 1991).

4.2.1 Sampling method

Initially three separate paddocks were chosen for each soil type to allow for farm scale variance between soil types (Figure 4.1). Paddocks were chosen in consultation with DairyNZ staff to ensure no other trials were occurring on the paddocks and

that they were part of the regular farm rotation. A total of 500 g was collected from each paddock (0-75 mm) using a bucket sampler and placed in a plastic bag, resulting in nine soil samples (three soils by three paddocks). Bucket sampler cores were taken throughout each paddock by taking samples every 10 to 20 steps, avoiding sampling on or next to cowpats. Around 50 to 70 cores were taken per paddock.

Once sampled, the soils were sieved through 2 mm mesh and a sub-sample taken from each bag for moisture content analysis. This analysis was completed using the method outlined in Section 4.6.1.

After moisture content analysis, water was added to adjust the content to 60% maximum water holding capacity (MWHC), which was determined using soil sub-samples according to the method outlined in Section 4.6.2.

After moisture adjustment the bags were plugged with cotton wool to allow for gas exchange and left at room temperature for two days before measurement of respiration rate at a range of temperatures (Section 4.6.3). In other studies, a pre-incubation of one week is used to allow any initial increase of respiration after re-wetting of soil samples to stabilise before analysis occurs. However, as the objective was to minimise adaptation of microbial population subsequent to sampling, a two-day pre-incubation was used.

After measurement of respiration rate data was fitted with MMRT and T_{opt} and T_{m_sens} calculated as described in section 4.7 and statistical analysis was completed (Section 4.8)

4.3 Seasonality

The main objective for this section was to determine whether seasonal change in temperature prior to collection would alter the respiration sensitivity to temperature

Scott Farm experiences a wide range of temperatures during the year. In summer maximum air temperatures range between 21 °C and 26 °C with winters being cooler and maximums ranging between 10 °C and 14 °C.

4.3.1 Methodology

Soil samples from Horotiu, Te Kowhai and Te Rapa soils were collected seasonally (Beginning of March, June, September and December) using sampling protocols described in section 4.2

Once sampled, the soils were sieved through 2mm mesh and a sub-sample taken from each bag for moisture content analysis. This analysis was completed using the method outlined in Section 4.6.1.

After moisture content analysis, water was added to adjust the content to 60% MWHC, which was determined using soil sub-samples according to the method outlined in Section 4.6.2. After moisture adjustment the bags were plugged with cotton wool allowing for gas exchange and left at room temperature for two days before measurement of respiration rate at a range of temperatures (Section 4.6.3)

After measurement of respiration rate data was fitted with MMRT and T_{opt} and T_{m_sens} calculated as described in section 4.7 and statistical analysis was completed (Section 4.8)

4.4 Moisture content control on temperature response

The objective for this section was to determine respiration rate response to temperature change over a wide range of soil moisture contents to identify optimum water content for measuring temperature sensitivity.

4.4.1 Methodology

One paddock for each soil type (Horotiu, Te Kowhai and Te Rapa) was chosen in consultation with DairyNZ to ensure no other trials were occurring on the paddocks and that they were part of the regular farm rotation. A total of 4 kg was collected from each paddock using a 75 mm by 20 mm bucket sampler and placed in a plastic bag, resulting in three large soil samples. Bucket sampler cores were taken randomly across the paddock, taking samples every 10 to 20 steps avoiding sampling on or next to cowpats. Approximately 400 to 500 cores were taken per paddock per soil type.

Once sampled, the soils were 2 mm sieved, homogenised and separated into 8 bags per soil type (500 g each) resulting in 24 samples for the three soils. Two sub-samples were taken from each bag for moisture content analysis. This analysis was completed using the method outlined in section 4.6.1.

After moisture content analysis, varying amounts of water was added to seven samples of each soil to adjust the moisture contents to 20, 30, 40, 50, 60, 70 and 80% MWHC which was determined using soil sub-samples according to the method outlined in Section 4.6.2.

After moisture adjustment the bag were sealed with cotton wool providing an outlet for gas exchange and left at room temperature for one week before measurement of respiration rate as described in section 4.6.3 Lastly respiration rate data was fitted with MMRT and T_{opt} and T_{m_sens} calculated as described in section 4.7 and statistical analysis was completed (Section 4.8)

4.5 Pre-incubation

The main objective for this section was to observe whether time of pre-incubation of soil at different temperatures has an effect on subsequent respiration rate response to temperature change.

4.5.1 Methodology

The Horotiu Silt Loam was the selected soil type for this experiment as it is well drained and easy to sieve and handle in large quantities. A paddock was chosen in consultation with Dairy NZ, which was under normal farm management and could have substantial amounts of soil removed. From this paddock, 50 kg of soil was obtained from digging 20 blocks (20 x 20 x 10 cm) of soil. The holes were back filled with other Horotiu topsoil, provided by Scott Farm.

Once sampled, the soil was sieved using a 2 mm mesh and a placed into two large containers for mixing. Once homogenised, the soil was separated into 84 samples of 500 g. Six subsamples were taken for moisture content analysis. This analysis was completed using the method outlined in Section 4.6.1.

After moisture content analysis, water was added to adjust the content to 60% MWHC, which was determined using soil sub-samples according to the method outlined in Section 4.6.2. The bags were then reweighed and the weight noted for future moisture adjustment.

After moisture adjustment, the bags were plugged with cotton wool allowing for gas exchange. Four samples were analysed for respiration rate, as described in section 4.6.3 after a two days of pre-incubation (Time Zero). After analysis, the remaining 80 bags were distributed for incubation at four temperatures (4, 10, 20 and 35 °C).

To maintain 60% MWHC throughout the incubation period, every two weeks the samples were weighed and water added to reach the initial recorded weight.

Every two months, two bags of soil from each temperature were randomly chosen to for measurement of respiration rate response to temperature. First two subsamples were taken from each bag for moisture content analysis (Section 4.6.1). If needed, the moisture content was adjusted to 60% MWHC and left at their individual temperatures for 2 days before analysis of respiration rate (Section 4.6.3).

After measurement of respiration rate data was fitted with MMRT and T_{opt} and T_{m_sens} calculated as described in section 4.5 and statistical analysis was completed (Section 4.6)

4.6. Laboratory analysis

4.6.1 Moisture content and moisture factor

Soil moisture is an important controller of a range of microbial processes in soil. Therefore, accurate monitoring and control of moisture content during these experiments was necessary.

To adjust moisture content for soil samples, a moisture factor can be used based on a ratio between oven dry soil mass and wet soil mass as described by Blackmore *et al.* (1987). Approximately 3 g of sample soil was added to a pre-weighed aluminium tin and weighed. The soil and tin were then placed in an oven at 105 °C for 24 to 48

hours for drying and cooled in a desiccator and re-weighed until constant weight was reached. Moisture factor (MF) was determined using equation 4.1.

$$MF = \frac{(M_w - M_t)}{(M_{od} - M_t)} \quad \text{(Eq. 4.1)}$$

Where M_W is the mass of the wet soil and aluminium tin (g), M_t was the mass of the aluminium tin (g), and M_{OD} was the mass of the oven-dried soil and aluminium tin (g).

Moisture content (MC) was determined using equation 4.2.

$$MC = (1 - MF) \times 100 \quad \text{(Eqn. 4.2)}$$

4.6.2 Maximum water holding capacity

The maximum amount of water soil can hold against drainage is the MWHC. Soil properties such as particle size and organic matter content can influence MWHC can vary between soil types and thus different soils can retain different amounts of water. This means soils may, at the same MC, have different amounts of available water and thus when wetting a soil, different amounts of water need to be added to achieve similar water availabilities. For this thesis due to differences in soil type, in order to maintain equal water availability in soils 60% MWHC was used instead of 60% MC for re-wetting soils.

The method of Harding and Ross (1964) was used to determine water holding capacity. Three glass funnels, with 200 mm of 10 mm plastic tubing attached to the ends, were suspended on a retort stand. The tubing was bent over itself by 20 mm and crimped with an adjustable crimp to prevent water flow. Inside each funnel, a small amount of glass wool (approximately a 30 mm circle) was laid as a filter to prevent soil from passing through. On top of the wool, soil was placed in the funnel and lightly compressed until a 10 mm rim was left between the soil surface and the funnel edge. Once soil was settled, water was gently sprayed on the surface until water was seen to pool. Spraying then ceased to allow water to soak into the soil. This sequence was repeated until the soil was saturated and water had filled the

tubing below. Afterwards, water was added to the funnel until there was 5 to 10 mm of water overlying the soil surface. The funnels were then covered and left for 24 hours to ensure the soil was fully saturated. After 24 hours the tubing was uncrimped and water allowed to flow freely into a glass beaker. The soil was then left for another 24 hours to drain. Once any free water had drained and none could be seen pooling on the soil surfaces, the moisture factor and content of the soils was determined using methods described in section 4.6.1. This moisture content is considered the MWHC of the soil. With the moisture factor of the 100% MWHC soil known, the moisture factor at 60% was calculated for moisture adjustments as made in sections 4.2, 4.3, 4.4 and 4.5.

4.6.3 Respiration measurements at different temperatures

For more detailed description of this process see section 3.1.1.

The temperature block for incubating soil (Section 3.2) was left on overnight to stabilise the temperature gradient at approximately 2 – 50 °C.

Soil (3 ± 0.5 g, 2 mm sieved) was weighed into 120 Hungate tubes. Tubes were evenly distributed along the gradient. Once distributed, rubber stoppers were inserted and sealed using aluminium caps. The block was then closed with a clear Perspex lid.

After five hours of incubation (from the first rubber stopper placement), the Perspex lid was lifted and, whilst in the block, the crimps were removed and 1 mL of gas removed via an insulin syringe and needle from each tube. The time taken to sample the gas was also recorded.

After sampling, needles were inserted into large rubber bungs for transportation to the laboratory for CO_2 analysis using an infra-red gas analyser (IRGA) within one hour. A standard curve was produced for the IRGA using 1% CO_2 standard (Section 3.3.1), before and after the soil gas samples were injected. Respiration rate (Rs) is calculated using equation 4.3.

$$Rs = \left[\left(\left(\frac{H_s/V_i}{H_{st}/V_i}\right) - \left(\frac{H_b/V_i}{H_{st}/V_i}\right)\right) \times S \times V \times 10^3\right] \div (ODW \times t)) \quad \text{(Eq. 4.3)}$$

Where Rs is respiration rate in μL CO_2 g $soil^{-1}$ h^{-1}. H_s is the peak height of the sample (mm). V_i is the injection volume (mL). H_{st} is the peak height of the standard (1% CO_2) (mm). H_b is the peak height of the blank. S is the CO_2 concentration in the standard (1% = 0.01 mL CO_2 / mL gas). V is the headspace volume in the Hungate tube. ODW is the oven dry weight of the soil (g) and t is the incubation time.

4.7 Model fitting

Initially data was fitted using both MMRT (Equation 2.3) and Arrhenius (Equation 2.1) models (Figure 4.2) using MATLAB R2012a.

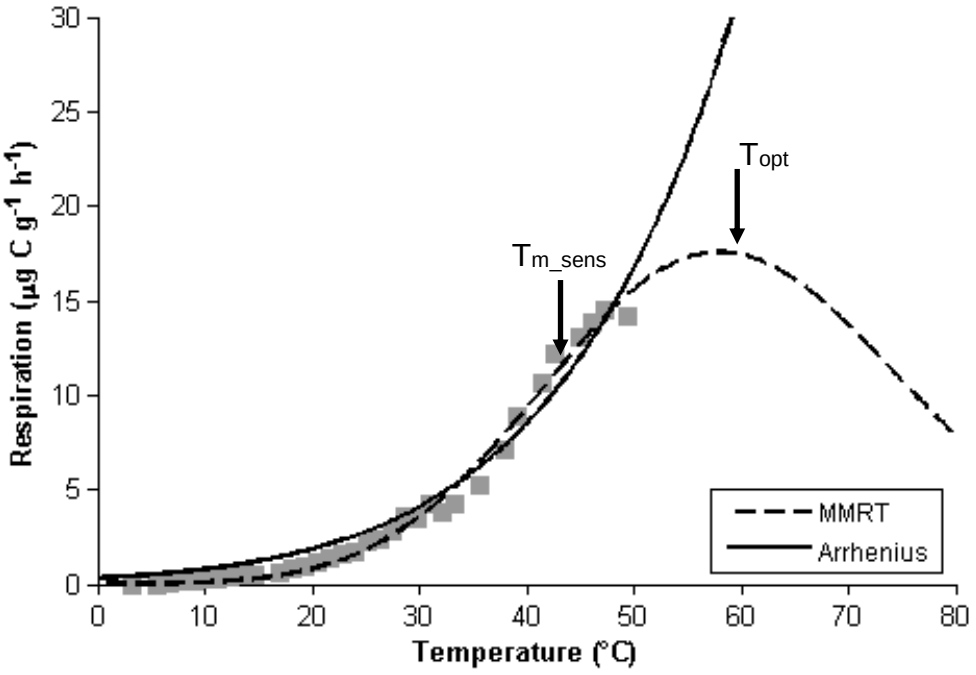

Figure 4.2: Respiration calculated for a Horotiu soil and fitted with both MMRT (R^2= 0.99) and Arrhenius (R^2= 0.96) models.

Both equations gave reasonable fits but MMRT was chosen for all subsequent fits because of its greater theoretical power.

The data also fit using the natural log (*ln*) version of MMRT to reduce the high leverage of greater respiration at higher temperatures.

4.7.1 Calculating T_{opt} and T_{m_sens}

Temperature optimum (T_{opt}) and temperature of maximum sensitivity (T_{m_sens}) were used to compare soil respiration rates between experimental data. When fitted with MMRT, values are given for coefficients $\Delta C_P^{\ddagger}$, $\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$. These can then be used to calculate T_{opt} and T_{m_sens} (Hobbs *et al.*, 2013) (Eq. 4.6 & 4.7).

$$T_{opt} = \frac{\left(\Delta H^{\ddagger} - \left(\Delta C_P^{\ddagger} \times 280\right)\right)}{\left(\left(-\Delta C_P^{\ddagger}\right) - 8.314\right)} \quad \text{(Eq. 4.6)}$$

$$T_{m\_sens} = \frac{T_{opt}}{1 + \left(2.883 / \left(\sqrt{-\Delta C_P^{\ddagger}}\right)\right)} \quad \text{(Eq. 4.7)}$$

4.8 Statistical analysis

Statistical differences between the T_{m_sens} of all soil types, seasons, soil type in individual seasons, season in individual soils incubation temperatures, and incubation sampling times were analysed using two-way analysis of variance (ANOVA). Where main effects were significant post hoc comparisons of treatments were made.

Assumptions of normality and equal variances were checked using standard residual plots.

Chapter 5. Temperature Sensitivity of Soil Respiration

5.1 Abstract

Soil respiration is extremely sensitive to changes in moisture and temperature and small changes in these variables can have a major influence carbon (C) cycling. However, there are conflicting arguments about how temperature changes will ultimately affect C exchanges, makes understanding the role of temperature changes on the dynamics of the C cycle crucial for future C modelling and budgeting.

A new laboratory method was developed for this thesis to allow rapid determination of soil respiration rate at a wide range and number of temperatures. A temperature block allowed simultaneous measurements of soil respiration rates within five hours at 44 different temperatures between ~4 and 50 °C. The main objective was to test this method on a range of conditions (including different soil types, sampling season, range of MCs and pre-incubation temperatures) to understand how temperature sensitivity of soil respiration might change with different sample collection and processing approaches.

Respiration rate data was analysed using the newly developed macromolecular rate theory (MMRT), which allows direct calculation of the temperature at which respiration has maximum temperature sensitivity (T_{m_sens}).

Seasonal measurements of respiration rates from different soil types collected from a single farm found a significant interaction for T_{m_sens} between soil type and season over the year indicating that temperature sensitivity of soil respiration differed between soil types depending on season. Therefore, to assess temperature sensitivity of soil respiration from a site, samples should be taken from all soil types at the location. T_{m_sens} was not dependant on season suggesting that a single sampling per year may be sufficient to estimate temperature sensitivity for a soil type. Surprisingly, respiration rate response to temperature was not very sensitive to quite large changes in soil moisture. Pre-incubated soils sampled for 10 months at different temperatures

also resulted in no significant change in T_{m_sens} suggesting that temperature sensitivity of soil respiration can be accurately determined using soils stored at various temperatures and that microbial populations are relatively stable in response to incubation temperature.

5.2 Introduction

The largest active pool in the carbon (C) cycle is the turnover of soil organic carbon (SOC), with microbial decomposition of SOC accounting for half of terrestrial carbon dioxide (CO_2) emissions (Davidson & Janssens, 2006; Jobbagy & Jackson, 2000). Decomposition of SOC is considered extremely sensitive to changes in moisture and temperature (Conant *et al.*, 2011; Davidson & Janssens, 2006) so that small changes in these variables, particularly temperature, can have a major influence on how much CO_2 is released to the atmosphere (Conant *et al.*, 2011; Davidson & Janssens, 2006; Fang & Moncrieff, 2001).

Potentially, global warming could cause an increase in the release of CO_2 from the soil by increasing microbial respiration resulting in positive feedback on the atmospheric CO_2 concentrations and global change (Kirschbaum, 2000). Other studies have instead argued for a potential negative feedback between CO_2 and warming, where increasing temperatures cause increased soil C storage (through increased plant growth and detrital inputs to soil) thereby reducing atmospheric CO_2 (Conant *et al.*, 2011). Contrary arguments and lack of definitive evidence makes understanding the role of temperature changes on the dynamics of the C cycle a key area of interest, focusing on decomposition as frequently measured as the rate of respiration (Fang & Moncrieff, 2001). Additionally, temperature changes affect microbes both directly, though physiological changes (i.e. the responsiveness of enzymes kinetics to temperature), and indirectly through altering other important variables including aggregation, flooding, drought, and freeze-thaw cycles. The ability to predict how climate change will affect the C balance of soil depends on the understanding of the temperature dependence of respiration.

There are many models of temperature dependence (Davidson & Janssens, 2006) including conceptual, theoretical and empirical models. The models used in this thesis are the Arrhenius equation, the empirical adjustment of the Arrhenius equation by Lloyd and Taylor (Lloyd & Taylor, 1994) and the Macromolecular Rate Theory (MMRT) (Schipper *et al.*, 2014).

The Arrhenius model was developed in 1889 to describe chemical reaction. The equation is based on the activation energy (E_A) of reactions, which is essentially an energy peak that reactants must overcome to create its products (Transition State Theory) (Hobbs *et al.*, 2013; Schipper *et al.*, 2014; Sierra, 2012). The Arrhenius function aims to model reaction rates with respect to temperature to determine how fast these transformations of differing energy will occur in relation to temperature (Equation 5.1)

$$k = Ae^{-E_A/RT} \quad \text{(Eq. 5.1)}$$

Where A is the pre-exponential factor, E_A is the activation energy of the reaction, *R* is the universal gas constant (8.314 J mol^{-1} K^{-1}) and T is absolute temperature (K) (Sierra, 2012).

The Lloyd and Taylor model uses a semi-empirical equation developed by Kavanau (1951) to include a declining E_A in the Arrhenius model. Lloyd and Taylor (1994) rearranged this equation to a standard temperature of 10 °C (Equation 5.2). By making the equation relevant to a standard temperature the activation energy (E_0) and temperature (T_0) are forced to be constant, but E_0 is no longer representative of theoretical E_A (Lloyd & Taylor, 1994).

$$R = R_{10}e^{E_o\left(\frac{1}{283.15-T_0}-\frac{1}{T-T_0}\right)} = R_{10}e^{308.56\left(\frac{1}{56.02}-\frac{1}{T-227.13}\right)} \quad \text{(Eq. 5.2)}$$

Where with E_0= 308.56 K and T_0=227.13 K.

This equation has generally resulted in improved fit between soil respiration data and temperature.

Macromolecular rate theory (MMRT) was developed to account for the temperature optimum of enzyme activity, unlike other simple exponential models. MMRT is a theoretical extension of the Arrhenius equation accounting for thermodynamic properties of biological macromolecules (i.e. enzymes) and includes a temperature dependence of activation energy, derived from a large, negative change in heat

capacity (C_P) of the enzyme during catalysis (Equation 5.3) (Arcus *et al.*, In press; Hobbs *et al.*, 2013).

$$ln(k) = ln\left(\frac{k_B T}{h}\right) - \frac{\Delta H^{\ddagger}_{T_0} + \Delta C^{\ddagger}_P (T - T_0)}{RT} + \frac{\Delta S^{\ddagger}_{T_0} + \Delta C^{\ddagger}_P (lnT - lnT_0)}{R} \qquad \text{(Eq. 5.3)}$$

Where T_0 is a reference temperature, $\Delta C^{\ddagger}_P$ is change in heat capacity during the reaction and $\Delta H^{\ddagger}_{T_0}$ and $\Delta S^{\ddagger}_{T_0}$ are the difference in enthalpy and entropy between the ground state and the transition state, respectively, at T_0 (Hobbs *et al.*, 2013; Schipper *et al.*, 2014).

Frequently, approaches for measuring the temperature dependence of soil respiration rates include long incubation times (weeks to months) and use a limited number of incubation temperatures (often less than six). Both of these choices limit the ability to adequately describe how respiration responses to increasing temperature. Long incubation times may allow thermal adaptation of microbial populations, or significant substrate loss, leading to results that do not represent *in situ* soil responses (Bradford, 2013; Kirschbaum, 2004). Measuring respiration rates at few temperatures allows for the fit and justification of many different predictive models (e.g., those above), which can lead to inaccuracies when used for extrapolation.

A new method was developed here that allows for rapid determination of soil respiration rate incubated under a large number of temperatures across a wide range (Section 3.2). An aluminium block (1400 x 130 x 190 mm) with 44 sample slots is cooled at one end and heated at the other to provide a stable temperature gradient from 0 – 55 °C. This stable gradient allows many samples of the same soil to be incubated at ~1.5 °C increments concurrently. Soil respiration is measured after a 5 hour incubation period to minimise the possibility of any potential thermal adaptation.

Preliminary studies (Section 3) demonstrated that a rapid assessment of soil respiration across a range of temperatures could be made; however, there are a number of unknowns with respect to sample collection and handling.

The primary objective here was to use the developed method to determine whether temperature sensitivity of soil respiration changed under different sample collection and processing approaches. Respiration measurements using the temperature block on soils collected from three soil types from a single farm, through different seasons. A single soil was also pre-incubated at multiple temperatures and subsamples periodically tested for temperature response for up to 8 months. Finally, the moisture content (MC) of one soil was adjusted to determine an array of soil respiration responses to temperature at different MCs. Macromolecular Rate Theory (MMRT) was used to fit data to calculate the temperature optimum (T_{opt}) and max temperature sensitivity (T_{m_sens}) of soil respiration for these factors.

The hypotheses were:

1. Soil type would not affect the respiration rate response to temperature change when soil samples were collected from a single site due to the close proximity to one another and being under the same climatic conditions,
2. Sampling season would not affect respiration rate response to temperature change as Hamilton's yearly seasonal change in temperature is small and mild,
3. MC would affect respiration rate response to temperature change with decline at high and low moisture contents, and finally
4. Pre-incubation temperature and time in pre-incubation will alter respiration rate response to temperature change.

5.3 Methods

Soil samples were collected from Scott Farm (Vaile Rd, Newstead, Hamilton, New Zealand.) which is a research dairy farm run by DairyNZ with a number of different soil types represented in close proximity to one another (Figure 5.1). Soils from this site were used in a series of experiments to test for differences in temperature responses between soils, sampling times, pre-incubation temperatures and MCs.

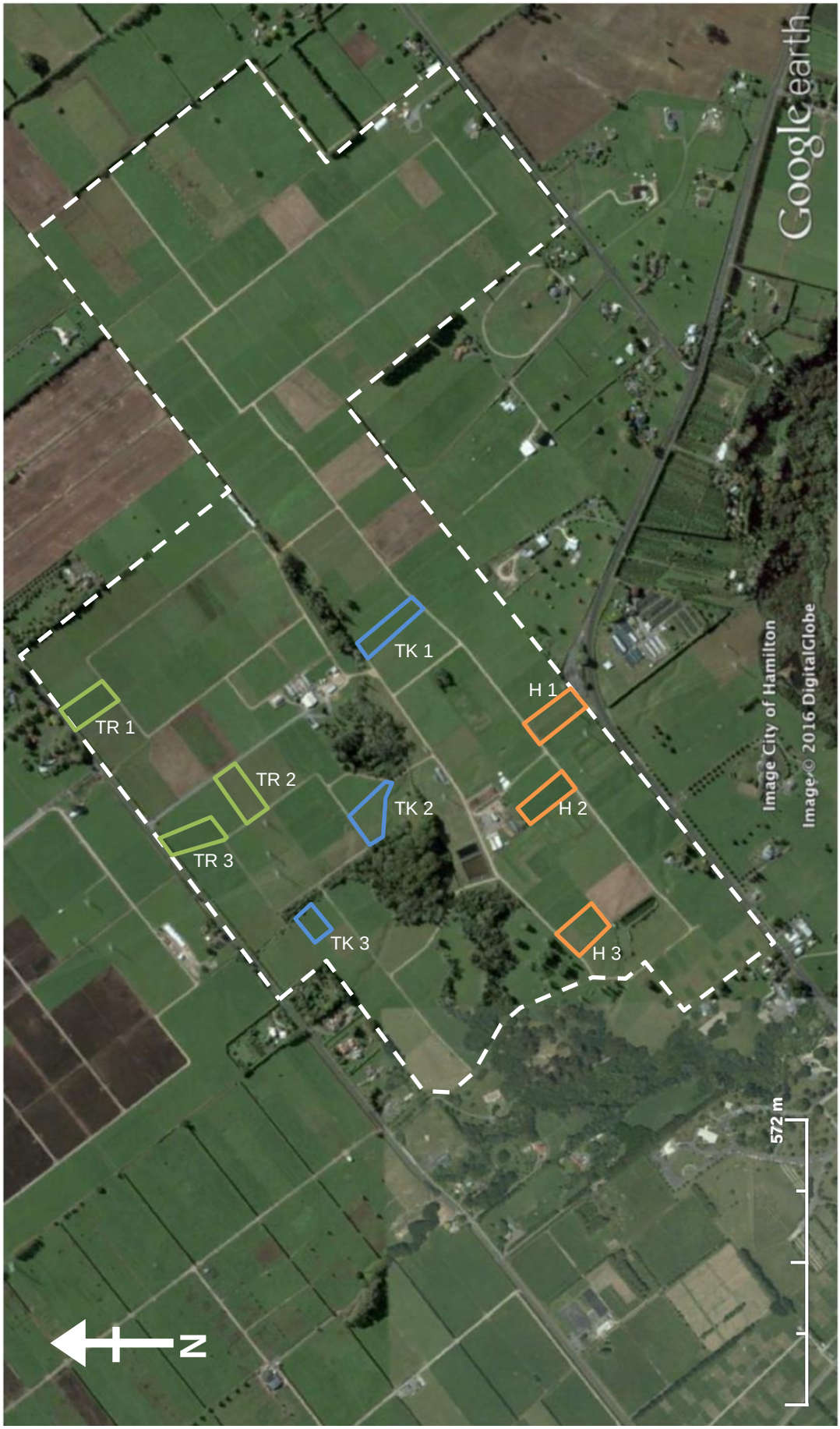

Figure 5.1: Satellite view of Scott Farm (Outlined in white). Google Earth 23rd April 2015. Retrieved 20th December 2015. Replicate paddocks sampled are outlined with main soil types identified. Horotiu (H), Te Kowhai (TK), Te Rapa (TR).

5.3.1 Soil Type/Season.

To determine whether different soils from the same locality had similar temperature responses, Horotiu, Te Kowhai and Te Rapa soils were chosen for sampling due to the difference in their mineralogy, organic matter (OM) contents and drainage.

Horotiu is classified as a well-drained allophanic soil, Te Kowhai's are poorly drained and ash soils, whilst Te Rapa soils are poorly drained organic soils (Singleton, 1991). Three separate paddocks were chosen for each soil type to include within farm variation of soil types. A total of 500 g of soil was collected from each paddock using a bucket sampler (75 mm deep, 20 mm diameter), samples were sieved (2 mm mesh) and then moisture adjusted to 60% maximum water holding capacity (MWHC) (Section 3.6.2). After adjustment soil was placed in a plastic bag that was plugged with cotton wool to allow gas exchange and left at room temperature (about 20 °C) for two days before analysis of respiration rate. This collection was repeated four times throughout the year to observe any seasonal effects (March, June, September December). In total, these seasonal measurements were made on three soils (three replicates each time) for four seasons to give a total of 36 temperature response curves.

5.3.2 Moisture content

To determine whether changes in MC altered temperature response soil samples were collected from one paddock for each soil type (Horotiu, Te Kowhai and Te Rapa), Soil (~4 kg) was collected from each paddock using a bucket sampler (75 mm deep, 20 mm diameter) before being sieved (2 mm mesh). After sieving the samples were homogenised and separated into eight, 500 g bags per soil type. Seven of the eight bags were then moisture adjusted to 20, 30, 40, 50, 60, 70 and 80% MWHC. After adjustment, the bag were plugged with cotton wool to allow air exchange and left at room temperature for one week before analysis of respiration rate as described in section 4.6.3. In total, measurements were made at the seven MCs for three soils giving a total of 21 temperature response curves.

5.3.3 Pre-incubation

To determine whether pre-incubation temperature altered subsequent temperature response a single large sample of Horotiu soil (~50 kg) was collected by removing 16

blocks of soil (20 x 20 x 10 cm) from one paddock. Soil was sieved (2 mm mesh), homogenised then separated into 84 samples (500 g). Soil in the bags were then moisture adjusted to 60% MWHC. After adjustment, the bags were plugged with cotton wool to allow for gas exchange and four of these samples were analysed for respiration rate after a two day waiting period (Time Zero). The remaining 80 bags were equally divided between four storage temperatures, 4, 10, 20 and 35 °C (20 bags per temperature). To maintain 60% MWHC throughout the incubation period, every two weeks the samples were weighed and water added to reach the initial recorded weight. Every two months for 10 months, two bags of soil from each temperature were randomly chosen for respiration rate measurements. If needed, the MC was adjusted to 60% MWHC and left at their respective temperatures for two days before analysis of respiration rate. In total, measurements made on the four pre-incubation temperatures (two replications) over 10 months, including the initial four measurements at time zero, gave a total of 44 temperature response curves.

5.3.4 Respiration rate measurement

The temperature block for incubating soil (Section 3.2) was left on overnight to stabilise a temperature gradient from about 2 – 50 °C. Thermistors spaced evenly across the block measured and recorded seven temperatures along the block to allow calculation of the temperature at each location (the temperature gradient was strongly linear with distance and stable – Section 3.4.2).

Soil (3 g ± 0.5 g, 2 mm sieved) was weighed into 120 Hungate tubes. Tubes were evenly distributed along the gradient and rubber stoppers inserted to prevent gas loss and then sealed using aluminium crimps. The block was then closed with a clear Perspex lid to help maintain a stable internal temperature.

After 5 hours of incubation the lid was lifted and, whilst in the block, the crimps were removed and 1 mL of gas removed via an insulin syringe and needle from each tube. Sampling of all 140 Hungate tubes was completed with 30 min, which was accounted for when calculating respiration rate. After sampling needles were inserted into large rubber bungs for transportation to the laboratory for CO_2 analysis using an infrared gas analyser (IRGA) within one hour. A standard curve was produced for

the IRGA using 1% CO_2 standard (Section3.3), before and after the soil gas samples were injected. Respiration rate (Rs) is calculated using equation 5.4

$$Rs = \left[\left(\left(\frac{H_s/V_i}{H_{st}/V_i}\right) - \left(\frac{H_b/V_i}{H_{st}/V_i}\right)\right) \times S \times V \times 10^3\right] \div (ODW \times t) \quad \text{(Eq. 5.4)}$$

Where Rs is respiration rate in μL CO_2 g $soil^{-1}$ h^{-1}. H_s is the peak height of the sample (mm). V_i is the injection volume (mL). H_{st} is the peak height of the standard (1% CO_2) (mm). H_b is the peak height of the blank. S is the CO_2 concentration in the standard (1% = 0.01 mL CO_2 / mL gas). V is the headspace volume in the Hungate tube. ODW is the oven dry weight of the soil (g) and t is the incubation time.

5.3.5 Model fitting

Initially data was fitted using both MMRT and Arrhenius models in MATLAB R2012a. Both equations gave reasonable fits but MMRT was selected for all subsequent fits because of its improved theoretical power and ability to capture the low temperature response and temperature optimum rather continuous increase (Figure 5.2).

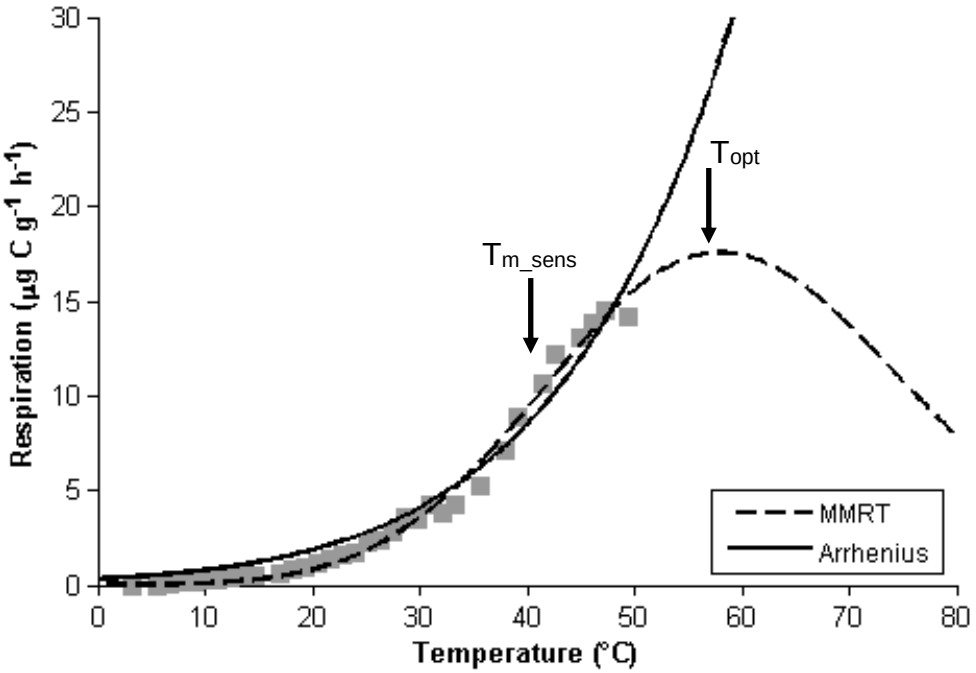

Figure 5.2: Respiration calculated from a Horotiu soil and fitted with both MMRT (R^2= 0.99) and Arrhenius (R^2= 0.96) models.

The data also fit using the natural log (*ln*) version of MMRT to reduce the high overall leverage of greater respiration at higher temperatures.

5.3.6 Calculating T_{opt} and T_{m_sens}

Temperature optimum (T_{opt}) and temperature at which respiration has maximal sensitivity (T_{m_sens}) were calculated to compare soil respiration rates between experimental data. When fitted with MMRT, values are derived for coefficients $\Delta C_P^{\ddagger}$, $\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$. These can then be used to calculate T_{opt} and T_{m_sens} (Hobbs *et al.*, 2013) (Eq. 5.5 & 5.6).

$$T_{opt} = \frac{\left(\Delta H^{\ddagger} - \left(\Delta C_P^{\ddagger} \times 280\right)\right)}{\left(\left(-\Delta C_P^{\ddagger}\right) - 8.314\right)} \quad \text{(Eq. 5.5)}$$

$$T_{m\_sens} = \frac{T_{opt}}{1 + \left(2.883 / \left(\sqrt{-\Delta C_P^{\ddagger}}\right)\right)} \quad \text{(Eq. 5.6)}$$

5.3.7 Statistical analysis

Statistical differences between the T_{m_sens} of all soil types, seasons, soil type in individual seasons, season in individual soils incubation temperatures, and incubation sampling times were analysed using two-way analysis of variance (ANOVA). Where main effects were significant post hoc comparisons of treatments were made.

Assumptions of normality and equal variances were checked using standard residual plots.

5.4 Results

Overall, 84 sets of measurements of temperature sensitivity of individual soils were made. Calculated respiration rates for each are given in Appendix 1. Each set of respiration rates for a soil were fitted individually and results for $\Delta C_P^{\ddagger}$, T_{m_sens} and T_{opt} averaged for comparisons.

5.4.1 Soil type and seasonal comparison

Temperature responses for three soil types (Horotiu, Te Kowhai and Te Rapa) were measured for respiration response for four seasons during the year and T_{m_sens} and T_{opt} calculated (Table 5.1). T_{m_sens} ranged from 37 to 50 °C whilst T_{opt} ranged from 55 to 71 °C over the course of the year.

Table 5.1: Averaged, calculated **$\Delta C_p^{\ddagger}$**, T_{m_sens} and T_{opt} values for respiration rates measured for three soil types (Horotiu, Te Kowhai and Te Rapa) from three paddocks each. Rates were calculated from samples taken four times over the year to capture seasonality.

Season	Soil Type	$\Delta C_p^{\ddagger}$ (J mol^{-1} °C^{-1})	T_{m_sens} (°C)	T_{opt} (°C)
Summer	Horotiu	-2678	37	55
	Te Kowhai	-2270	43	62
	Te Rapa	-2330	39	58
Autumn	Horotiu	-2260	45	64
	Te Kowhai	-2409	44	63
	Te Rapa	-2154	48	68
Winter	Horotiu	-2325	46	65
	Te Kowhai	-2726	42	60
	Te Rapa	-2114	50	71
Spring	Horotiu	-2351	47	66
	Te Kowhai	-2136	45	65
	Te Rapa	-2320	47	66

ANOVA analysis of T_{m_sens} resulted in a statistically significant interaction between soil and season (Table 5.2). This indicated T_{m_sens} was dependant on which soil was sampled at a particular season

Table 5.2: Result of two- way ANOVA analysis of T_{m_sens} using soil type and seasonal differences. DF = degrees of freedom, SS = sum of squares, MS = Mean squares. Significant difference indicated by a P-value <0.05 (Bold text).

Source of Variation	DF	SS	MS	F ratio	P value
Soil	2	226.424	113.212	20.894	**<0.001**
Season	3	30.485	10.162	1.875	0.165
Soil x Season	6	150.541	25.09	4.631	**0.004**
Residual	21	113.784	5.418		
Total	32	512.073	16.002		

As a significant interaction was found, pairwise comparison of T_{m_sens} within soil and season was conducted (Table 5.3 and Table 5.4). The main reason for the significant interaction appeared to have occurred during the first sampling in summer as

pairwise comparisons identified (Table 5.3). There was also a significant difference in T_{m_sens} between Horotiu and Te Kowhai soils in autumn and winter and between Te Rapa and Horotiu soils in winter.

Table 5.3: P-values for pairwise comparison between soil types (Horotiu, Te Kowhai, and Te Rapa) within seasons (Summer, Autumn, Winter and Spring). Significant difference indicated by a P-value <0.05 (Bold text).

	Summer	Autumn	Winter	Spring
Te Rapa vs. Te Kowhai	**0.007**	0.555	0.418	0.156
Te Rapa vs. Horotiu	**<0.001**	0.053	**0.012**	0.379
Horotiu vs. Te Kowhai	**0.006**	**0.037**	**0.003**	0.415

When pairwise comparisons were made of seasonal differences for soil types, there were only two significant differences observed between spring and summer, and spring and winter for the Horotiu soil (Table 5.4).

Table 5.4: P-values gained as a result of pairwise comparison between seasons (Summer, Autumn, Winter and Spring) within soil types (Horotiu, Te Kowhai, and Te Rapa). Significant difference indicated by a P-value <0.05.

	Horotiu	Te Kowhai	Te Rapa
Spring vs. Summer	**0.005**	0.433	0.388
Winter vs. Summer	0.403	0.485	0.109
Autumn vs. Summer	0.054	0.217	0.484
Spring vs. Autumn	0.402	0.087	0.764
Spring vs. Winter	**0.028**	0.220	0.629
Winter vs. Autumn	0.222	0.603	0.580

5.4.2 Moisture content

Respiration was measured at seven MCs for three soils (Horotiu, Te Kowhai and Te Rapa) and fitted with MMRT. Results were combined in a three dimensional (3D) web to visualise the effects of temperature change on respiration at different MCs (Figure 5.3, Figure 5.4 & Figure 5.5). First, MMRT was fitted to the temperature response for each moisture content and then a loess function used to interpolate between fitted curves at each moisture content.

In contrast to expectations, there was no obvious decrease in respiration at high and low MCs for the Te Rapa and Horotiu soils (Figure 5.3 and Figure 5.5). Respiration rates measured in the Te Kowhai soils did decrease more at high and low MCs (Figure 5.4).

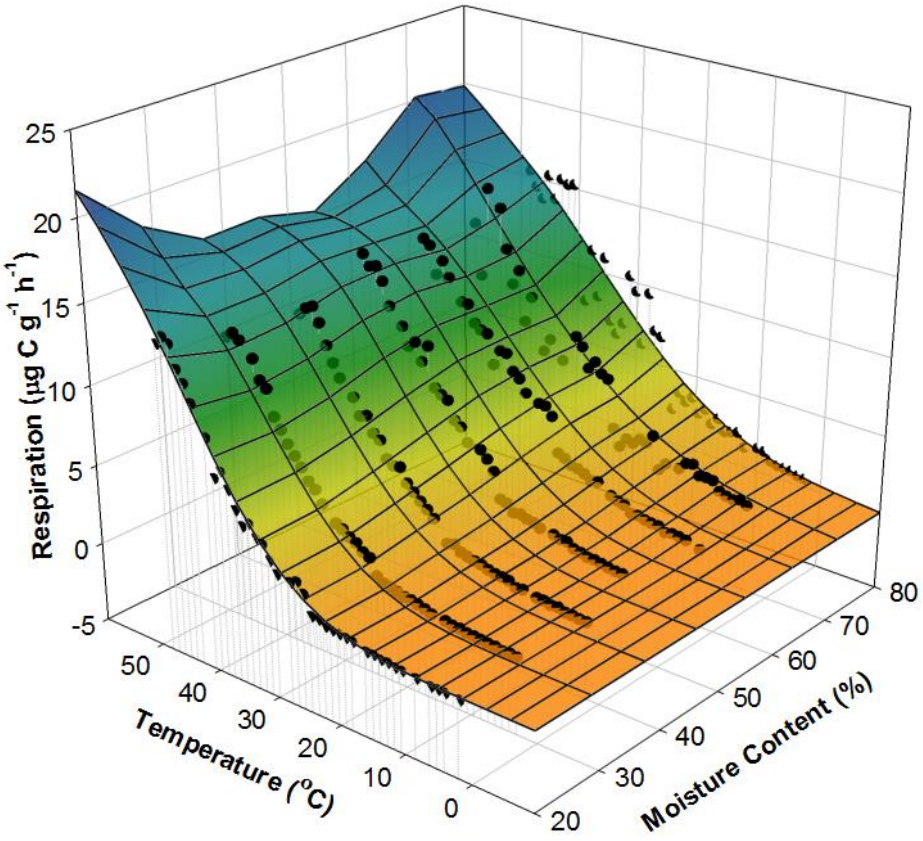

Figure 5.3: Respiration rate calculated for a Horotiu soil over seven moisture contents (20, 30, 40, 50, 60, 70 and 80%). Temperature response within moisture content was fitted with MMRT and then smoothed across moisture with a loess function.

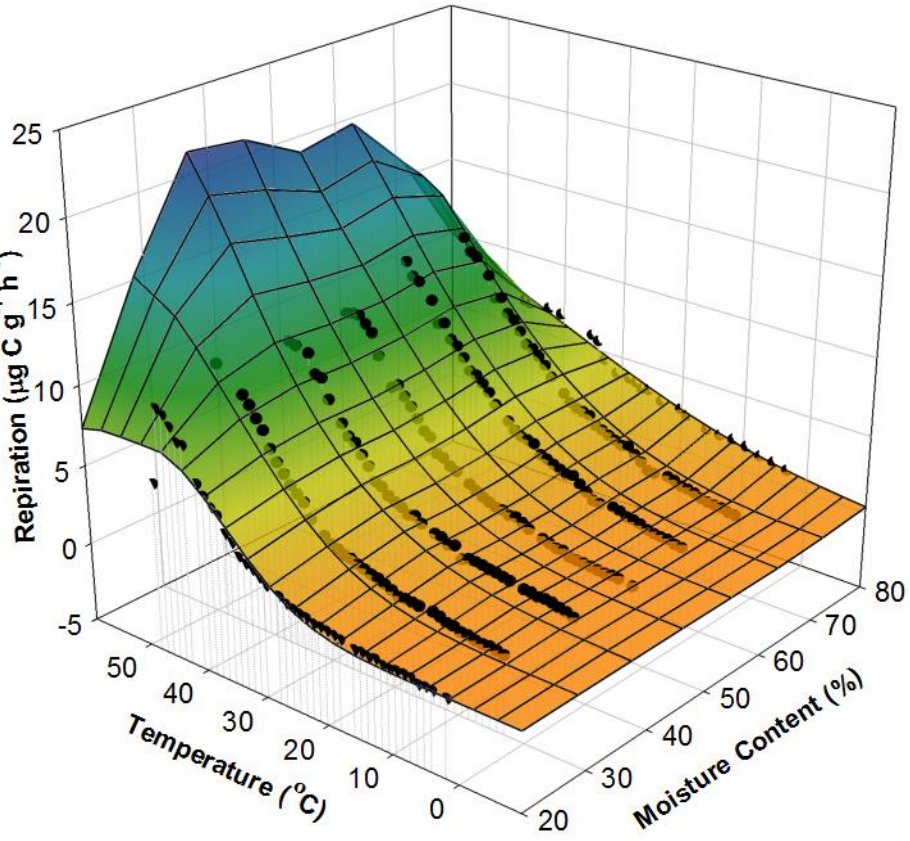

Figure 5.4: Respiration rate calculated for a Te Kowhai soil over seven moisture contents (20, 30, 40, 50, 60, 70 and 80%). Temperature response within moisture content was fitted with MMRT and then smoothed across moisture with a loess function.

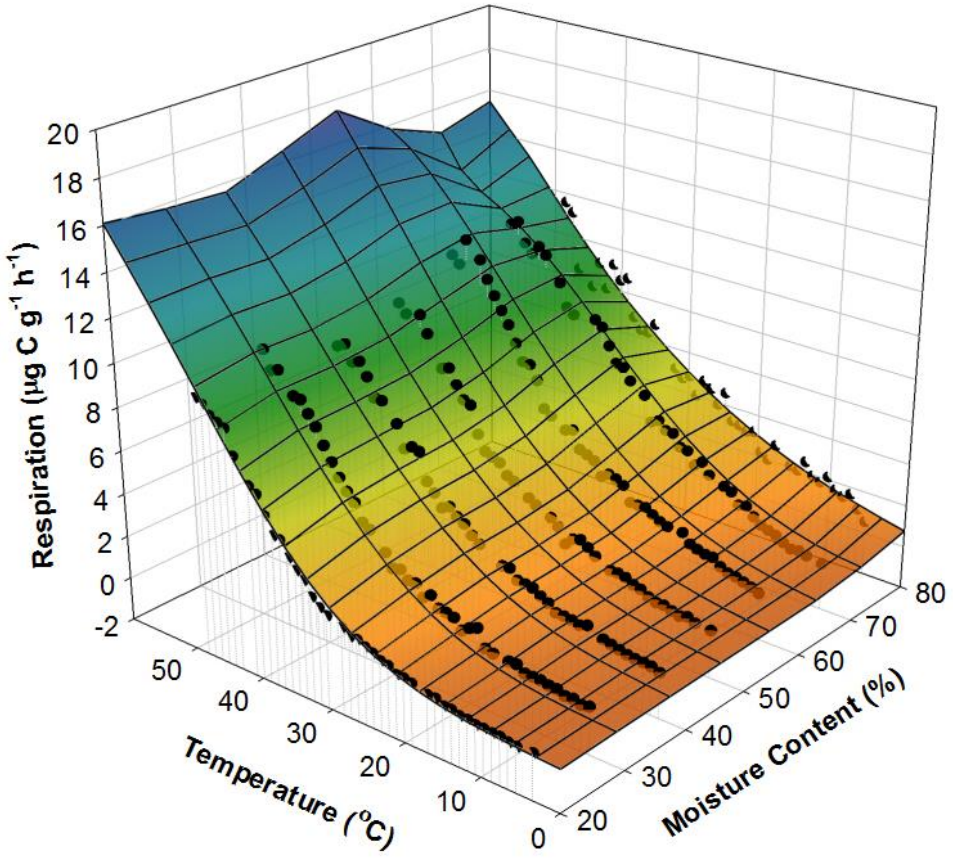

Figure 5.5: Respiration rate calculated for a Te Rapa soil over seven moisture contents (20, 30, 40, 50, 60, 70 and 80%). Temperature response within moisture content was fitted with MMRT and then smoothed across moisture with a loess function.

5.4.3 Pre-incubation comparison

Samples were pre-incubated at four different temperatures (4, 10, 20 and 35 °C) and respiration rate in response to temperature was measured every 2 months for a 10 month period. T_{m_sens} ranged from 36 to 48 °C whilst T_{opt} ranged from 54 to 69 °C over the course of the year (Table 5.5).

Table 5.5: Averaged, calculated $\Delta C\ddagger$, T_{m_sens} and T_{opt} values for respiration rates measured over 10 months (T0=initial sampling, T1=2 months, T2=4 months, T3=6 months, T4=8 months and T5=10 months).

Incubation length	Incubation temperature (°C)	$\Delta C\ddagger$ (J mol^{-1} °C^{-1})	T_{m_sens} (°C)	T_{opt} (°C)
T0	Room (~20)	-2212	44	64
T1	35	-2145	37	57
	20	-1365	44	69
	10	-2130	38	58
	4	-1092	47	75
T2	35	-2640	42	60
	20	-2200	40	60
	10	-2551	36	54
	4	-1812	42	64
T3	35	-1375	47	72
	20	-2135	48	68
	10	-2975	39	55
	4	-2469	41	59
T4	35	-2010	43	63
	20	-2124	46	66
	10	-2938	39	56
	4	-2966	39	55
T5	35	-2640	41	59
	20	-2152	44	64
	10	-2583	41	59
	4	-2756	42	59

Total CO_2 respired, with the block set at ~4 – 50 °C, over the 5 hour measurement period decreased over the 10 month sampling period. The decline was most prominent in samples stored at 35 °C (Figure 5.6).

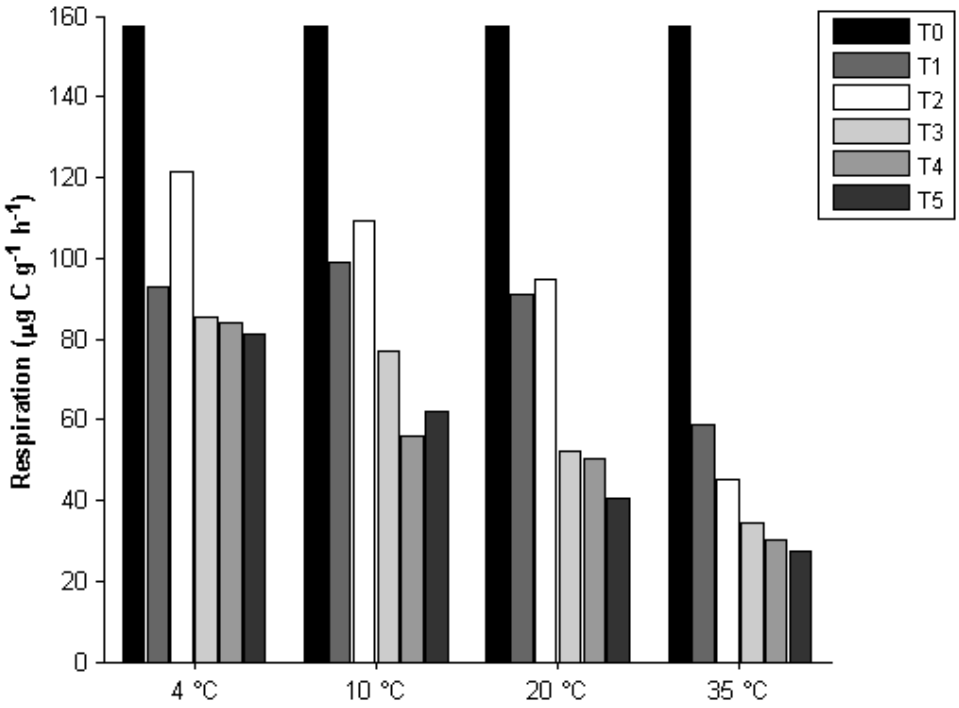

Figure 5.6: Total CO_2 produced by soil samples over. Samples incubated at either 4, 10, 20 or 35 °C for up to 10 months (T0=initial sampling, T1=2 months, T2=4 months, T3=6 months, T4=8 months and T5=10 months).

There was no significant effect of incubation temperature or time of pre-incubation or their interaction when analysed by ANOVA (Table 5.6).

Table 5.6: Result of two- way ANOVA analysis on T_{m_sens} using incubation temperature and incubation length. DF = degrees of freedom, SS = sum of squares, MS = Mean squares. Significant difference indicated by a P-value <0.05

Source of Variation	DF	SS	MS	F	P
Inc temperature	3	15.25	5.083	0.172	0.914
Inc length	6	91.978	15.33	0.517	0.788
Temperature x Length	18	706.434	39.246	1.324	0.27
Residual	20	592.656	29.633		
Total	47	1404.394	29.881		

Many previous studies have measured respiration at a few incubation temperatures. To compare results from these previous experiments with data collected here, the respiration measurement from the tube in the block closest to the long-term incubation temperature was selected to represent a one-off measurement of respiration at specified temperatures. For example, the respiration rate measured on the block at 35 °C at each sampling times (T0 to T5) was designated as the

respiration for a long-term incubation at 35 °C. This one of measurement would represent a 5 hour accumulation of CO_2 respired from a soil sample, stored at 35 °C and sampled at that one temperature, not over a range like the temperature block. The process would of result in four measurements of respiration (4, 10, 20 and 35 °C) (Figure 5.7) after each incubation time (2, 4, 6, and 10 months) (Figure 5.8). This analysis demonstrates the commonly observed decline in soil respiration with time at all temperatures.

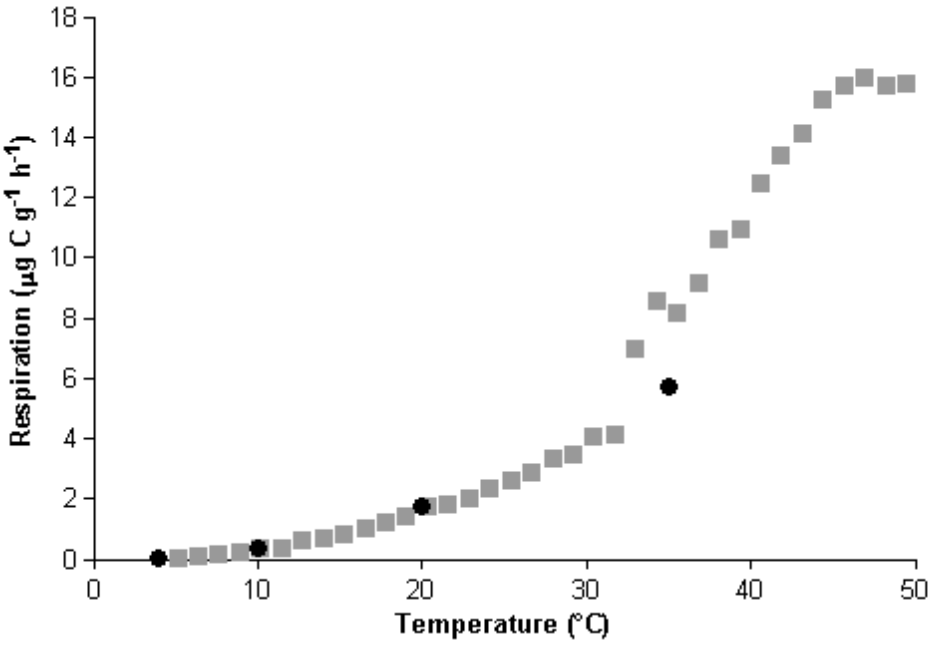

Figure 5.7: Average respiration rate response for the T0 sample. Four data point temperatures highlighted in black represent the data points selected for Figure 5.8.

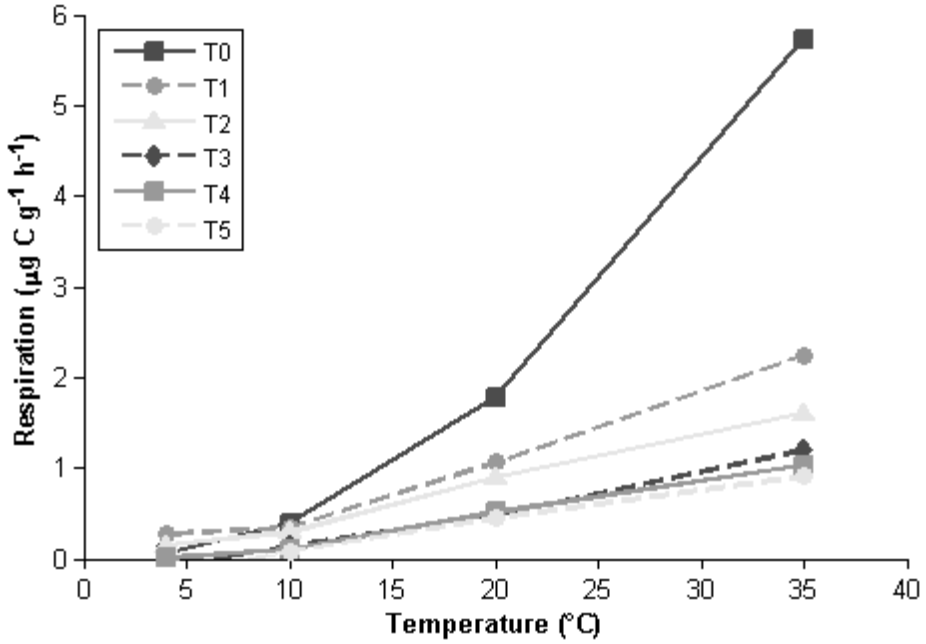

Figure 5.8: Respiration rate response to pre-incubation time measured at the four incubation temperatures.

T0 data was fit with MMRT for both the full data set provided by the block, and the separate four points to demonstrate the benefits of more data points (Figure 5.9).

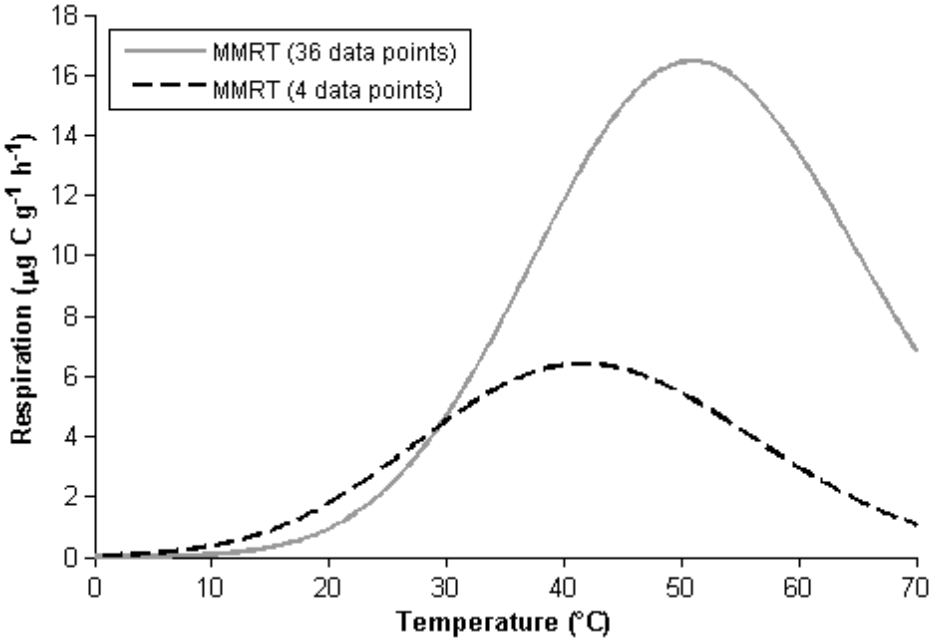

Figure 5.9: MMRT fits for the T0 respiration rates using all 36 data points provided by the temperature block, compared to fits using only four points.

5.5 Discussion

5.5.1 T_{opt} and T_{m_sens}

Values of T_{opt} were generally greater than 70 °C with T_{m_sens} around 40 to 50 °C and higher than expected. It was thought that due to average annual temperatures at the field site of 14.4 °C, soil organisms would have optimal at a temperature of around 30 °C, as is frequently observed for enzymes (Hobbs *et al.*, 2013). These higher values may have been due to multiple factors such as substrate availability, dominant microorganisms and how much root matter remained in the soil.

The development and testing of this method was designed to study temperature response sensitivity more efficiently and in greater detail than previous methods. Calculating T_{m_sens} allows for discussion of when a change in temperature will affect the soil microbial ecosystem the most. This is important for modelling changes in respiration with increases in daily to annual temperature increases as predicted due to global climate change. Additionally, T_{m_sens} is independent of the magnitude of soil respiration. T_{m_sens} is basically the change in slope of the respiration rate response to temperature and not the magnitude of the whole curve, if the magnitude of respiration decreases (due to less substrate availability), T_{m_sens} can remain the same, as observed in this study. For example, essentially the same T_{m_sens} was calculated for soils that had been pre-incubated for up to 10 months despite large declines in magnitude of respiration.

5.5.2 Soil Type/Seasonal effects

Soil types differ in a number of key factors that could potentially affect the response of soil respiration including litter input, drainage regime, temperature, and parent material. Topsoil, where majority of respiration occurs, is also most strongly affected by climatic variables, land-use and litter (Davidson *et al.*, 2000). Seasonal changes of temperature sensitivity have been attributed to changes in labile soil C fractions (Davidson *et al.*, 2000; Kirschbaum, 2013). As temperature increases, decomposition is accelerated leading to a reduction in labile C and subsequently a reduction in respiration rate (Kirschbaum, 2013). This reduction occurs more rapidly at higher temperatures and can then limit respiration in the longer-term incubations and confound assessments of temperature sensitivity. The method used here reduces this

loss of labile C and allows assessment of temperature sensitivity more closely aligned to *in situ* conditions.

In general, T_{m_sens} at Scott Farm was similar across all soil types and seasons (ranging by about 10 °C). There was a significant interaction for T_{m_sens} between soil type and season during the year (Table 5.2). This difference was most apparent when comparing the soils during the first sampling in summer. This difference was somewhat unexpected as farmers manage their soils to even out production across different soil types. Fertiliser is added to counteract P limitations, lime for acidity and irrigation for water limitations to minimise environmental differences between soils within a farm. Farm systems also typically have the same pasture type in each paddock so experience no litter differences within the system. Due to the close proximity of the soil types and their similar management regime it was expected there would be no differences found between soil types. It is important to note that these summer measurements and estimates of T_{m_sens} were the first measurements made and it is likely the subtle improvements in methodology occurred subsequently, which would have improved estimates of T_{m_sens}.

Few studies have focused on soil type and seasonal differences in temperature sensitivity of soil respiration. Most studies looking at spatial variation of temperature sensitivity were either large-scale modelling or field studies where factors such as temperature, MC and litter input control apparent temperature sensitivity of soil respiration (Mielnick & Dugas, 2000; Zheng *et al*, 2009). One laboratory study by Drake *et al*, (2013), found that in soils under three different forests types (affecting litter type and pH), soils under Ash assemblages showed marginally stronger temperature sensitivity to Oak and Hemlock. This study; however, still focused on differing litter addition rather than specifically testing for differences between soil types. Thurgood, *et al.*, (2014), found that a Ferrosol (well drained iron-oxide rich soils) had a higher temperature sensitivity values than Chromosols (poorly-drained soils). A field based study by Lohila *et al.*, (2003) looked at effects of crop and soil type on soil respiration. They found that differences in soil respiration between soil types, depended on differences in soil C contents, in particular peat soils had two to three times higher respiration than mineral based soils however, they did not look for temperature sensitivity. Additionally, studies generally did not include multiple

seasonal sampling to observe changes through time or interactions between soils and seasons.

In addition to differences in summer, T_{m_sens} in the Horotiu soil was also significantly different from T_{m_sens} in the Te Kowhai and Te Rapa soils in autumn and winter, and the T_{m_sens} in the Horotiu soil was significantly different to the T_{m_sens} of the Te Rapa soil in winter. As sieving and moisture adjustment reduces differenced in MC and aggregate protection, these differences in T_{m_sens} may have been due to changes in litter inputs, OM or microbial population changes throughout the year. Overall, a significant difference between soil types within season suggests that in order to assess temperature sensitivity of soil respiration accurately from a small area, samples of different soils must be taken.

When seasons were compared pairwise within soil type, a significant difference was observed between spring and winter, and spring and summer for the Horotiu soil only. As temperature and MC are the major factors seasonality effects, it might be expected that T_{m_sens} would responds to changes in these variables. A general lack of differences indicated there was no seasonal effect on temperature sensitivity of soil respiration so to assess temperature sensitivity of soil respiration it may not be necessary to have multiple samplings through the year. However, seasonal temperature variation at Scott Farm is not large and this hypothesis needs testing at a wide range of other sites particularly with larger temperature variations.

5.5.3 Moisture content

Moisture content is a well-studied controlling factor of respiration response in conjunction with temperature (Davidson *et al.*, 2000; Sierra *et al.*, 2015). It generally accepted that microbes tend to have optimum water content at which they function at maximum efficiency with lower rates when soils are very wet or very dry (Davidson *et al.*, 2000; Sierra *et al.*, 2015). Respiration rate responses to changing MC differed between soils and were generally flat for Horotiu and Te Rapa and more curved for Te Kowhai (Figure 5.3, Figure 5.4, Figure 5.5). For the Horotiu soil (Figure 5.3), when fitted with MMRT, there was some visible curvature in response to moisture for the 40, 50 and 60% MC, whilst the Te Kowhai (Figure 5.4) showed lower rates for the 20 and 80% MC. The Te Rapa (Figure 5.5) soils showed no visible

curvature in response to moisture at all. It may be that even higher or lower MCs in soils are required before declines in respiration occur. This lack of shape indicates respiration in different soils may not always have a defined optimum MC in contrast to what is generally accepted (Sierra *et al.*, 2015) but rather varies for soil type. If correct, to assess temperature sensitivity of soil respiration accurately, multiple soil MCs must be tested for some soil types to determine optimum MC for each sample. These measurements have rarely been conducted but represent an important area for further research to inform modelling approaches.

5.5.4 Pre-incubation at different temperatures

Measurement of temperature sensitivity for soils pre-incubated at different temperature prior to analysis showed no significant effects for T_{m_sens} for either pre-incubation temperature and length, or their interactions (Table 5.6). A reduction in absolute respiration rate with time occurred, particularly in the 35 °C incubation (Figure 5.6). This decline in absolute respiration rate is common in incubation studies due to consumption of available substrate that limits decomposition and respiration rate (Fang *et al.*, 2005; Hassan *et al.*, 2015; Winkler *et al.*, 1996). Changes to temperature sensitivity due to pre-incubation temperature, however, are still widely debated (Bradford *et al.*, 2008; Fang *et al.*, 2005; Kirschbaum, 2004; Winkler *et al.*, 1996). No significant change to temperature sensitivity during the 10 months suggests microbial communities are very robust to changes in temperature and do not change easily. This robustness is consistent with Fang *et al.* (2005) whom, over a 108 day incubation, found insignificant differences in temperature sensitivity of decomposition between more labile (respired early) and less labile (respired later) substrate.

On the other hand, many studies have observed changes to temperature sensitivity of decomposition/respiration during incubations (Giardina & Ryan, 2000; Liski *et al.*, 1999; Luo *et al.*, 2001; Melillo *et al.*, 2002) who found changes to temperature sensitivity with time that was not explained by loss of substrate. Overall, a lack of change in T_{m_sens} indicated that pre-incubation temperature and length does not have a large effect on temperature sensitivity so that when assessing temperature sensitivity of soil respiration, pre-incubation temperature may not be critically

important. However this may also deserve further investigation to determine how widely true this might be.

5.6 Conclusions

In general, T_{m_sens} did not vary as much as hypothesised being generally similar between soils and seasons and varying little when measured after changes in MC, time of incubation or temperature of pre-incubation. Overall, this suggests the microbial community is mainly a function of longer-term climate at the site of collection.

However, some difference in temperature sensitivity of soil respiration was worth noting. Seasonal measurements of different soil types at Scott Farm found a significant interaction in T_{m_sens} between soil type and season over the year (Table 5.2). When soil types were compared pairwise within seasons, a significant difference was found between all three soils at the summer sampling. This could have been due to these being some of the first measurements made with subsequent refining increasing the accuracy of measurements. In addition to summer, some differences were observed in pairwise comparisons of soil type (between Te Kowhai and Horotiu soils in autumn and winter, and between Horotiu and Te Rapa soil in winter). These significant differences indicate that potentially the temperature sensitivity of soil respiration could differ between soil types and to assess temperature sensitivity of soil respiration accurately, within a small farm, samples should be taken from all soil types at the location. However, these differences did not seemingly respond in a uniform way across seasons and some further work may be necessary to fully clarify our observations and conclusions.

In terms of seasonality, when pairwise comparisons within soil types, only identified two significant differences (between spring and winter, and spring and summer for the Horotiu soil). This general lack of difference indicated there was no obvious seasonal effect on temperature sensitivity of soil respiration and to assess temperature sensitivity of soil respiration accurately multiple samplings over the year may not be required.

Changes in respiration with changing MC showed a dependence on soil type producing different 3D surfaces of respiration rate in response to temperature and MC. For Horotiu and Te Rapa soils, respiration responses did not appear to decline as much as might have been expected at higher and lower MC. In contrast, Te Kowhai soils had lowered respiration response at wetter and drier MCs.

Pre-incubation of soil samples for 10 months showed no dependence of T_{m_sens} with either pre-incubation temperature and/or length (Table 5.6). This lack of difference indicated that temperature sensitivity of soil respiration could be accurately determined using soils stored at various temperatures and only slowly changes if at all with time.

On the whole, measuring temperature sensitivity, including T_{m_sens}, was generally robust to differences in soil, time of the year sampling took place and pre-incubation treatment. However, subtle differences may still need to be accounted for that requires ongoing investigation.

Chapter 6. Conclusions and Future Research

6.1 Conclusions

A method was developed to study temperature sensitivity that was more efficient than other methods allowing respiration rates to be measured from a small soil sample at 40 different temperatures. This large number of data points allowed for fitting of a new theoretical model, MMRT, which estimates temperature at which sensitivity in maximal.

This method was tested on soil samples collected from the field to determine factors that might alter temperature sensitivity and T_{m_sens}. For the seasonal/soil type experiment, a significant interaction in T_{m_sens} between soil type and season was observed for some seasons. When separated out into pairwise comparisons, several significant differences were found between soil types within seasons, whilst only two significant differences were observed between seasons within soil types. The number of significant differences between soil types indicated that temperature sensitivity of soil respiration does differ somewhat between soil types and order to assess temperature sensitivity of soil respiration accurately, within a small farm, samples should probably be taken from a range of soils. Conversely, the lack of significant differences between seasons indicated there is no obvious seasonal effect on temperature sensitivity of soil respiration and only one sampling over the year is required.

Analysis of respiration response to changes in moisture content proved inconclusive with each soil type producing very different 3D surfaces of respiration rate, response to increasing temperature and MC. This result differs from many other studies reported in the literature such as Sierra *et al.* (2015) who predicted an optimum MC at which respiration rate occurs.

Finally the respiration response to temperature change measurement using pre-incubated samples over 10 months found no significant interaction in T_{m_sens} between

pre-incubation temperature and length which is consistent with Fang *et al.*, (2005). The lack of difference indicated that temperature sensitivity of soil respiration can be accurately determined using soils stored at various temperatures and lengths. Furthermore this result and previous findings in this thesis suggests the microbial community is relatively robust with respect to temperature sensitivity.

6.2 Future work

This thesis was primarily focused on method development and so main suggestions are on improving the method. There were slight problems with modelling fitting in a few situations, which could require a thorough investigation into how fitting can be improved if similar situations occur. Additionally some more work on respiration rate linearity over time (Section 3.4.2) would be useful, as multiple samplings over time could be incorporated into the methodology to increase the accuracy of respiration rate measurements.

Additionally, some repetition of experiments conducted here could be useful. This thesis found that pre-incubation temperature did not appear to be critically important as T_{m_sens} did not change between soils pre-incubated at different temperatures. However, this unexpected result deserves further investigation to determine how widely true this lack of response to pre-incubation temperature might be, particularly for a range of soils collected from different climatic regions.

Temperature sensitivity of respiration at differing MCs was found vary between soil types. These measurements have rarely been conducted and, again investigation of a wider range of soils represents an important area for further research to inform modelling approaches.

Other future work could revolve around the practical use of this method for analysis of temperature sensitivity in other soil situations. A New Zealand wide set of observations of temperature sensitivity would potentially give greater insight into differences observed between soil types. Seasonal samplings in areas with greater climatic variability similarly could reveal seasonal effects not seen in this study. Examination of temperature sensitivity in irrigated compared with non-irrigated farm paddocks would give insight into how farming practises might affect soil respiration,

in particular with respect to difference in moisture content. This examination could be expanded into investigating responses from multiple land-uses such as horticulture or sheep/cattle farming, rather than just dairy farming.

Overall, if effective, this method could be used for almost any situation and could replace current methods of temperature sensitivity analysis.

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Appendix A. Raw Data

Table A.1: Calculated temperature (T) (°C) and measured respiration rate (Rs) (μg C g^{-1} h^{-1}) for a Horotiu, Te Kowhai and Te Rapa soil collected during summer 2015.

		Horotiu						**Te Kowhai**						**Te Rapa**					
C20		C21a		C35		C11		C2a		D15		A10		D5		D7	
T	Rs	T	Rs	T	Rs	T	Rs	T	Rs	T	Rs	T	Rs	T	Rs	T	Rs
5.39	0.08	5.39		5.39	0.21	4.20	-0.03	4.20		4.20	0.14	4.71	0.31	4.71		4.71	0.12
6.65		6.65	0.04	6.65	0.34	5.49		5.49	0.01	5.49	0.18	5.99		5.99	-0.02	5.99	0.19
7.91	0.16	7.91	0.17	7.91	0.30	6.79	0.05	6.79	0.05	6.79	0.22	7.27		7.27	0.05	7.27	0.26
9.17	0.25	9.17	0.16	9.17	0.44	8.08	0.14	8.08	0.05	8.08	0.31	8.54	0.50	8.54	0.19	8.54	0.33
10.42	0.38	10.42	0.25	10.42	0.45	9.37	0.14	9.37	0.14	9.37	0.31	9.82	0.64	9.82	0.20	9.82	0.48
11.68	0.56	11.68		11.68	0.21	10.66	0.24	10.66	0.18	10.66	0.36	11.10	0.51	11.10	0.34	11.10	0.12
12.94	0.62	12.94	0.43	12.94	0.88	11.96	0.28	11.96	0.23	11.96	0.56	12.38	0.78	12.38	0.49	12.38	0.71
14.20	0.70	14.20	0.52	14.20	0.93	13.25	0.38	13.25	0.32	13.25	0.65	13.65	1.01	13.65	0.72	13.65	0.95
15.46	0.95	15.46	0.72	15.46		14.54	0.57	14.54	0.36	14.54		14.93	1.14	14.93	0.79	14.93	
16.72	1.05	16.72	0.81	16.72	1.26	15.83	0.73	15.83	0.45	15.83	0.82	16.21	1.46	16.21	0.96	16.21	1.27
17.98	1.17	17.98	1.01	17.98	1.33	17.13	0.78	17.13	0.55	17.13	1.02	17.49	1.46	17.49	1.28	17.49	1.41
19.23	1.62	19.23	1.07	19.23	1.74	18.42	1.16	18.42	0.71	18.42	1.13	18.77	1.69	18.77	1.52	18.77	1.77
20.49	1.78	20.49		20.49	1.82	19.71	1.20	19.71		19.71	1.44	20.04	2.03	20.04		20.04	2.03
21.75	2.28	21.75	1.68	21.75	1.87	21.00	1.23	21.00	0.92	21.00	1.70	21.32	2.24	21.32	1.70	21.32	2.46
23.01		23.01	1.93	23.01	2.44	22.30		22.30	1.14	22.30	1.79	22.60		22.60	1.95	22.60	2.75
24.27	2.91	24.27	2.38	24.27	3.30	23.59	1.50	23.59	1.46	23.59	2.19	23.88	3.01	23.88	2.04	23.88	3.20
25.53	3.28	25.53	3.54	25.53	3.14	24.88	1.82	24.88	1.58	24.88	2.16	25.15	3.18	25.15	3.07	25.15	3.43
26.78	3.38	26.78	3.16	26.78	2.57	26.17	1.12	26.17	2.15	26.17	2.95	26.43	3.98	26.43	3.65	26.43	3.91
28.04	4.31	28.04	3.53	28.04	3.12	27.47	2.67	27.47	2.03	27.47	2.96	27.71	4.33	27.71	4.09	27.71	4.43
29.30	5.13	29.30	4.07	29.30	4.33	28.76	3.26	28.76	2.36	28.76	3.70	28.99	5.65	28.99	4.65	28.99	4.97
30.56	5.15	30.56	4.62	30.56	4.71	30.05	3.55	30.05	2.89	30.05	3.46	30.27	6.63	30.27	5.04	30.27	5.62
31.82	6.01	31.82	5.40	31.82		31.34	4.64	31.34	3.34	31.34		31.54	6.94	31.54	5.59	31.54	
33.08	4.05	33.08	4.17	33.08	4.52	32.64	2.81	32.64	2.48	32.64	2.81	32.82	4.60	32.82	4.92	32.82	5.26
34.34	5.25	34.34	4.37	34.34	4.97	33.93	3.45	33.93	2.82	33.93	3.20	34.10	5.26	34.10	5.72	34.10	5.99
35.59	5.50	35.59	4.67	35.59	5.58	35.22	4.08	35.22	3.29	35.22	3.73	35.38	5.76	35.38	6.24	35.38	6.62
36.85		36.85	5.54	36.85	5.78	36.51		36.51	3.58	36.51	3.94	36.65		36.65	7.22	36.65	6.89
38.11	6.73	38.11	6.32	38.11	5.51	37.81	4.55	37.81	4.33	37.81	4.49	37.93	6.34	37.93		37.93	8.38
39.37	6.92	39.37	7.13	39.37	6.51	39.10	6.32	39.10	4.95	39.10	5.50	39.21	7.38	39.21	9.35	39.21	8.52
40.63	8.20	40.63		40.63	7.05	40.39	8.71	40.39		40.39	6.06	40.49	6.82	40.49		40.49	9.86
41.89	7.78	41.89	8.86	41.89	8.06	41.68	8.38	41.68	7.12	41.68	6.65	41.77	9.77	41.77	11.25	41.77	10.51
43.15	10.32	43.15	10.79	43.15	8.99	42.98	8.57	42.98	7.62	42.98	7.63	43.04	10.32	43.04	12.95	43.04	12.26
44.40	11.55	44.40	11.46	44.40	9.89	44.27	9.29	44.27	9.13	44.27	8.91	44.32	11.21	44.32	15.45	44.32	14.71
45.66	11.32	45.66	12.26	45.66	10.75	45.56	10.34	45.56	9.54	45.56	10.85	45.60	11.16	45.60	16.37	45.60	15.15
46.92	9.08	46.92	12.56	46.92	9.99	46.85	11.20	46.85	9.83	46.85	10.10	46.88	12.48	46.88	15.59	46.88	15.80
48.18	11.67	48.18	12.89	48.18	11.12	48.15	11.28	48.15	10.94	48.15	10.62	48.15	13.07	48.15	17.03	48.15	15.70
49.44	11.23	49.44	12.57	49.44		49.44	10.77	49.44	10.88	49.44		49.43	9.56	49.43	17.58	49.43	
50.70	12.52	50.70	13.47	50.70	11.60	50.73	14.19	50.73	11.53	50.73	11.34	50.71	11.52	50.71	19.20	50.71	18.89

Table A.2: Calculated temperature (T) (°C) and measured respiration rate (Rs) (μg C g^{-1} h^{-1}) for a Horotiu, Te Kowhai and Te Rapa soil collected during autumn 2015.

	C20		Horotiu C21a		C35		C11		Te Kowhai C2a		D15		A10		Te Rapa D5		D7	
	T	Rs	T	Rs	T	Rs	T	Rs	T	Rs	T	Rs	T	Rs	T	Rs	T	Rs
1	1.92	-0.27	1.92		1.92	-0.18	3.24	0.05	3.24		3.24	-0.07	1.94	0.08	1.94		1.94	-0.11
2	3.10		3.10	-0.15	3.10	-0.10	4.32		4.32	-0.03	4.32	-0.07	3.07		3.07	-0.05	3.07	-0.05
3	4.28	-0.11	4.28	-0.10	4.28	-0.10	5.40	0.13	5.40	-0.03	5.40	-0.03	4.21	0.05	4.21	0.02	4.21	0.08
4	5.46	-0.10	5.46	-0.06	5.46	0.19	6.48	0.25	6.48	0.01	6.48	0.01	5.35	0.02	5.35	0.08	5.35	0.08
5	6.64	-0.02	6.64	-0.02	6.64	0.10	7.56	0.30	7.56	0.14	7.56	0.14	6.49	0.15	6.49	-0.14	6.49	0.14
6	7.82	-0.06	7.82	0.06	7.82	0.19	8.63	0.25	8.63	0.14	8.63	0.14	7.63	0.08	7.63	0.08	7.63	0.15
7	9.01	0.02	9.01	0.15	9.01	0.19	9.71	0.29	9.71	0.18	9.71	0.14	8.77	0.19	8.77	-0.08	8.77	0.21
8	10.19	0.11	10.19	0.23	10.19	0.27	10.79	0.33	10.79	0.31	10.79	0.32	9.91	0.19	9.91	0.11	9.91	0.21
9	11.37	0.19	11.37	0.28	11.37		11.87	0.46	11.87	0.54	11.87		11.05	0.36	11.05	0.18	11.05	
10	12.55	0.32	12.55	0.45	12.55	0.53	12.95	0.54	12.95	0.58	12.95	0.50	12.19	0.36	12.19	0.21	12.19	0.35
11	13.73	0.40	13.73	0.59	13.73	0.62	14.03	0.58	14.03	0.67	14.03	0.68	13.33	0.40	13.33	0.42	13.33	0.41
12	14.91	0.49	14.91	0.59	14.91	0.70	15.11	0.63	15.11	0.49	15.11	0.86	14.46	0.47	14.46	0.35	14.46	0.62
13	16.10	0.58	16.10		16.10	0.93	16.19	0.84	16.19		16.19	1.20	15.60	0.62	15.60		15.60	0.73
14	17.28	0.76	17.28	0.81	17.28	1.24	17.27	0.88	17.27	1.18	17.27	1.29	16.74	0.66	16.74	0.42	16.74	0.81
15	18.46		18.46	0.94	18.46	1.29	18.35		18.35	1.13	18.35	1.62	17.88		17.88	0.35	17.88	0.92
16	19.64	1.13	19.64	1.28	19.64	1.43	19.43	1.15	19.43	1.27	19.43	1.53	19.02	0.81	19.02	0.64	19.02	0.92
17	20.82	1.26	20.82	1.50	20.82	1.60	20.50	1.43	20.50	1.51	20.50	1.83	20.16	0.93	20.16	0.86	20.16	1.11
18	22.00	1.40	22.00	1.89	22.00	1.70	21.58	1.33	21.58	1.80	21.58	2.17	21.30	1.13	21.30	0.93	21.30	1.54
19	23.19	1.64	23.19	1.95	23.19	1.86	22.66	1.70	22.66	1.96	22.66		22.44	1.29	22.44		22.44	1.38
20	24.37	1.68	24.37	2.30	24.37	2.46	23.74	1.70	23.74	2.20	23.74	2.22	23.58	1.29	23.58	1.25	23.58	1.67
21	25.55	1.93	25.55	2.47	25.55	2.28	24.82	2.05	24.82	2.47	24.82	1.20	24.72	1.60	24.72	1.43	24.72	1.54
22	26.73	2.42	26.73	2.70	26.73		25.90	2.55	25.90	2.41	25.90		25.86	1.83	25.86	1.58	25.86	
23	27.91	2.72	27.91	3.11	27.91	3.00	26.98	2.63	26.98	3.00	26.98	3.20	26.99	2.28	26.99	1.76	26.99	2.21
24	29.09	3.09	29.09	3.84	29.09	3.47	28.06	2.86	28.06	3.41	28.06	3.67	28.13		28.13	2.08	28.13	2.53
25	30.28	3.30	30.28	4.00	30.28	3.71	29.14	3.35	29.14	2.62	29.14	3.94	29.27	2.58	29.27	2.30	29.27	2.36
26	31.46		31.46	4.38	31.46	4.65	30.22		30.22	3.91	30.22	4.20	30.41		30.41	2.50	30.41	3.12
27	32.64	4.24	32.64	5.15	32.64	5.12	31.30	3.88	31.30	4.74	31.30	4.44	31.55	3.16	31.55	2.80	31.55	2.98
28	33.82	4.48	33.82	5.43	33.82	5.13	32.37	4.49	32.37	4.53	32.37	5.28	32.69	3.19	32.69	3.08	32.69	4.08
29	35.00	5.13	35.00		35.00	5.22	33.45	4.56	33.45		33.45	5.71	33.83	3.63	33.83		33.83	4.28
30	36.18	5.69	36.18	5.78	36.18	5.55	34.53	3.24	34.53	5.30	34.53	6.48	34.97	4.28	34.97	3.82	34.97	5.00
31	37.37	6.18	37.37	7.63	37.37	6.45	35.61	5.73	35.61	6.32	35.61	9.12	36.11	1.59	36.11	4.49	36.11	5.29
32	38.55	8.04	38.55	8.53	38.55	7.06	36.69	5.91	36.69	6.66	36.69	7.76	37.25	4.83	37.25	5.07	37.25	5.97
33	39.73	7.76	39.73	9.26	39.73	8.34	37.77	6.89	37.77	7.52	37.77	8.97	38.39	6.32	38.39	6.34	38.39	6.96
34	40.91	8.85	40.91	10.03	40.91	8.41	38.85	7.61	38.85	8.69	38.85	9.25	39.52	6.50	39.52	3.53	39.52	7.82
35	42.09	9.65	42.09	11.39	42.09	9.51	39.93	8.68	39.93	9.81	39.93	12.63	40.66	6.31	40.66	7.90	40.66	9.26
36	43.27	10.77	43.27	11.88	43.27		41.01	10.35	41.01	7.55	41.01		41.80	6.43	41.80	8.45	41.80	
37	44.46	12.87	44.46	13.76	44.46	11.36	42.09	10.56	42.09	12.47	42.09	11.92	42.94	8.94	42.94	9.21	42.94	10.47
38	45.64		45.64	13.77	45.64	11.64	43.17		43.17	12.91	43.17	14.42	44.08		44.08	10.65	44.08	12.34
39	46.82	12.50	46.82	15.37	46.82	11.95	44.24	10.74	44.24	14.37	44.24	14.27	45.22	10.47	45.22	11.45	45.22	13.34
40	48.00	13.65	48.00	15.85	48.00	13.63	45.32	12.36	45.32	14.33	45.32	14.67	46.36	10.94	46.36	12.27	46.36	14.25
41	49.18	13.64	49.18		49.18	15.31	46.40	12.50	46.40		46.40	16.15	47.50	11.19	47.50		47.50	15.59
42	50.36	16.78	50.36	19.61	50.36	16.98	47.48	12.31	47.48	17.06	47.48	14.20	48.64	10.09	48.64	15.81	48.64	17.04

Table A.3: Calculated temperature (T) (°C) and measured respiration rate (Rs) (μg C g^{-1} h^{-1}) for a Horotiu, Te Kowhai and Te Rapa soil collected during winter 2015.

	Horotiu								Te Kowhai								Te Rapa							
C20		C21a		C35		C11		C2a		D15		A10		D5		D7								
T	Rs	T	Rs	T	Rs	T	Rs	T	Rs	T	Rs	T	Rs	T	Rs	T	Rs							
1.99	-0.21	1.99		1.99	-0.14	2.13	0.09	2.13		2.13	-0.08	1.95	0.04	1.95		1.95	-0.12							
3.15		3.15	-0.08	3.15	-0.11	3.28		3.28	-0.06	3.28	-0.02	3.11		3.11	0.01	3.11	-0.05							
4.31	-0.08	4.31	-0.18	4.31	-0.02	4.43	0.15	4.43	-0.03	4.43	0.01	4.26	-0.08	4.26	0.01	4.26	0.00							
5.47	-0.04	5.47	-0.04	5.47	-0.01	5.58	0.17	5.58	0.01	5.58	0.04	5.42	0.10	5.42	0.07	5.42	0.01							
6.63	0.01	6.63	0.05	6.63	-0.30	6.72	0.24	6.72	0.04	6.72	0.07	6.58	0.15	6.58	0.07	6.58	0.07							
7.78	-0.01	7.78	0.11	7.78	0.10	7.87	0.24	7.87	0.10	7.87	0.23	7.73	0.24	7.73	0.12	7.73	0.08							
8.94	0.05	8.94	0.11	8.94	-0.07	9.02	0.39	9.02	0.14	9.02	0.25	8.89	0.28	8.89	-0.09	8.89	0.12							
10.10	0.23	10.10	0.26	10.10	0.11	10.17	0.37	10.17	0.20	10.17	0.36	10.05	0.30	10.05	0.23	10.05	0.21							
11.26	0.21	11.26	0.31	11.26		11.32	0.49	11.32	0.28	11.32		11.21	0.35	11.21	0.34	11.21								
12.42	0.33	12.42	0.41	12.42	0.33	12.46	0.54	12.46	0.34	12.46	0.36	12.36	0.43	12.36	0.44	12.36	-0.20							
13.58	0.51	13.58	0.50	13.58	0.49	13.61	0.55	13.61	0.41	13.61	0.36	13.52	0.46	13.52	0.45	13.52	0.45							
14.74	0.54	14.74	0.53	14.74	0.47	14.76	0.70	14.76	0.59	14.76	0.79	14.68	0.32	14.68	0.54	14.68	0.46							
15.90	0.88	15.90		15.90	0.55	15.91	0.82	15.91		15.91	0.98	15.84	0.52	15.84		15.84	0.69							
17.06	0.74	17.06	0.64	17.06	0.68	17.05	0.91	17.05	0.65	17.05	1.28	16.99	0.35	16.99	0.71	16.99	0.79							
18.22		18.22	0.87	18.22	0.74	18.20		18.20	0.78	18.20	1.44	18.15		18.15	0.74	18.15	0.84							
19.37	1.06	19.37	0.89	19.37	0.85	19.35	1.06	19.35	0.89	19.35	1.57	19.31	0.80	19.31	0.86	19.31	0.90							
20.53	1.29	20.53	1.20	20.53	1.34	20.50	1.27	20.50	1.12	20.50	1.95	20.47	0.85	20.47	1.03	20.47	0.85							
21.69	1.57	21.69	1.33	21.69	1.14	21.64	1.19	21.64	1.25	21.64	2.11	21.62	1.05	21.62	1.16	21.62	1.16							
22.85	1.65	22.85	1.63	22.85	1.41	22.79	1.54	22.79	1.80	22.79	2.22	22.78	1.18	22.78	1.37	22.78	0.83							
24.01	1.87	24.01	1.75	24.01	1.53	23.94	1.92	23.94	1.80	23.94	2.47	23.94	1.29	23.94	1.48	23.94	1.42							
25.17	2.06	25.17	2.27	25.17	1.75	25.09	1.95	25.09	1.94	25.09	2.84	25.10	0.69	25.10	1.00	25.10	1.79							
26.33	2.47	26.33	2.33	26.33		26.24	2.15	26.24	2.16	26.24		26.25	1.77	26.25	1.76	26.25								
27.49	3.09	27.49	2.78	27.49	2.24	27.38	2.65	27.38	2.48	27.38	2.90	27.41	0.97	27.41	2.25	27.41	2.14							
28.65	3.09	28.65	3.63	28.65	2.65	28.53	2.81	28.53	2.67	28.53	4.04	28.57	1.39	28.57	2.66	28.57	2.48							
29.80	3.74	29.80	3.44	29.80	3.16	29.68	2.79	29.68	2.69	29.68	4.49	29.73	2.49	29.73	2.63	29.73	3.30							
30.96		30.96	4.20	30.96	2.80	30.83		30.83	3.51	30.83	4.91	30.88		30.88	3.40	30.88	3.54							
32.12	3.61	32.12	3.82	32.12	3.58	31.97	3.71	31.97	3.98	31.97	5.30	32.04	3.02	32.04	3.57	32.04	3.71							
33.28	5.54	33.28	4.24	33.28	3.81	33.12	4.13	33.12	4.57	33.12	6.35	33.20	3.30	33.20	4.10	33.20	4.02							
34.44	3.08	34.44		34.44	4.92	34.27	4.24	34.27		34.27	7.05	34.36	4.04	34.36		34.36	4.36							
35.60	4.15	35.60	5.19	35.60	4.65	35.42	5.01	35.42	4.88	35.42	8.62	35.51	4.08	35.51	1.96	35.51	5.01							
36.76	5.86	36.76	6.82	36.76	5.45	36.57	5.36	36.57	5.78	36.57	8.50	36.67	4.91	36.67	5.50	36.67	5.54							
37.92	8.28	37.92	7.10	37.92	6.05	37.71	5.93	37.71	6.63	37.71	9.14	37.83	5.33	37.83	6.52	37.83	6.12							
39.08	6.86	39.08	8.90	39.08	6.76	38.86	6.49	38.86	7.30	38.86	10.01	38.98	6.36	38.98	5.96	38.98	6.76							
40.23	9.68	40.23	6.49	40.23	7.77	40.01	7.11	40.01	8.15	40.01	12.25	40.14	6.37	40.14	8.25	40.14	8.73							
41.39	12.09	41.39	10.68	41.39	8.97	41.16	7.56	41.16	9.78	41.16	13.28	41.30	8.17	41.30	9.26	41.30	9.96							
42.55	12.31	42.55	12.20	42.55		42.30	9.41	42.30	10.37	42.30		42.46	8.50	42.46	10.39	42.46								
43.71	13.15	43.71	8.52	43.71	9.83	43.45	9.16	43.45	11.59	43.45	14.15	43.61	9.43	43.61	10.17	43.61	12.47							
44.87		44.87	13.07	44.87	10.90	44.60		44.60	12.51	44.60	14.72	44.77		44.77	11.73	44.77	12.62							
46.03	15.27	46.03	13.89	46.03	11.76	45.75	11.20	45.75	10.73	45.75	17.42	45.93	10.99	45.93	13.15	45.93	14.66							
47.19	13.21	47.19	14.45	47.19	11.27	46.90	12.44	46.90	12.74	46.90	17.84	47.09	10.66	47.09	13.97	47.09	16.32							
48.35	15.75	48.35		48.35	12.71	48.04	13.00	48.04		48.04	18.37	48.24	10.51	48.24		48.24	18.64							
49.51	17.74	49.51	14.15	49.51	12.85	49.19	13.69	49.19	15.65	49.19	19.15	49.40	11.08	49.40	21.70	49.40	21.31							
50.67	21.28	50.67	17.87	50.67	17.23	50.34	14.71	50.34	20.59	50.34	20.45	50.56	10.54	50.56	23.13	50.56								

Table A.4: Calculated temperature (T) (°C) and measured respiration rate (Rs) (μg C g^{-1} h^{-1}) for a Horotiu, Te Kowhai and Te Rapa soil collected during spring 2015.

Horotiu									Te Kowhai						Te Rapa					
C20		C21a		C35		C11		C2a		D15		A10		D5		D7				
T	Rs	T	Rs	T	Rs	T	Rs	T	Rs	T	Rs	T	Rs	T	Rs	T	Rs			
2.70	-0.23	2.70		2.70	-0.20	2.11	-0.02	2.11	-0.03	2.11	-0.12	2.44	-0.02	2.44		2.44				
3.88		3.88	-0.11	3.88	-0.17	3.35		3.35		3.35	-0.09	3.63		3.63	-0.02	3.63	-0.07			
5.07	-0.20	5.07	-0.08	5.07	-0.13	4.58	0.04	4.58	0.00	4.58	-0.41	4.81	-0.02	4.81	-0.03	4.81	0.01			
6.25	-0.11	6.25	-0.08	6.25	-0.05	5.81	0.08	5.81	0.08	5.81	0.05	6.00	0.02	6.00	0.03	6.00	0.06			
7.44		7.44	0.00	7.44	0.02	7.04	-0.11	7.04	0.09	7.04	0.13	7.19	0.03	7.19	0.09	7.19	0.09			
8.63	0.05	8.63	-0.53	8.63	0.05	8.28	0.20	8.28	0.12	8.28	0.14	8.38	0.06	8.38	0.09	8.38	0.17			
9.81	0.06	9.81	0.03	9.81	0.18	9.51	0.26	9.51	0.25	9.51	0.32	9.57	0.16	9.57	0.17	9.57	0.26			
11.00	0.14	11.00	0.15	11.00	0.19	10.74	0.38	10.74	0.27	10.74	0.27	10.75	0.18	10.75	0.22	10.75	0.31			
12.18	0.24	12.18	0.15	12.18		11.98	0.53	11.98	0.43	11.98		11.94	0.29	11.94	0.28	11.94				
13.37	0.35	13.37	0.26	13.37	0.47	13.21	0.56	13.21	0.51	13.21	0.56	13.13	0.30	13.13	0.42	13.13	0.65			
14.55	0.41	14.55	0.39	14.55	0.50	14.44	0.81	14.44	0.68	14.44	0.68	14.32	0.45	14.32	0.55	14.32	0.64			
15.74	0.52	15.74	0.52	15.74	0.58	15.68	0.78	15.68	0.74	15.68	0.69	15.51	0.42	15.51	0.55	15.51	0.90			
16.93	0.73	16.93		16.93	0.75	16.91	1.07	16.91		16.91	0.96	16.69	0.60	16.69		16.69	1.00			
18.11	0.92	18.11	0.55	18.11	0.99	18.14	0.98	18.14	0.94	18.14	0.99	17.88	0.68	17.88	0.90	17.88	0.61			
19.30		19.30	0.76	19.30	1.00	19.38		19.38	0.95	19.38	1.28	19.07		19.07	0.96	19.07	1.37			
20.48	1.20	20.48	1.25	20.48	1.34	20.61	1.51	20.61	1.34	20.61	1.47	20.26	0.87	20.26	1.15	20.26	1.63			
21.67	1.42	21.67	1.06	21.67	1.55	21.84	1.81	21.84	1.55	21.84	1.51	21.45	0.94	21.45	1.45	21.45	1.74			
22.85	1.55	22.85	1.41	22.85	1.75	23.08	1.97	23.08	1.69	23.08	1.86	22.63	1.19	22.63	1.69	22.63	1.90			
24.04	1.82	24.04	1.62	24.04	1.82	24.31	2.19	24.31	2.19	24.31	2.08	23.82	1.35	23.82	2.01	23.82	2.56			
25.23	2.14	25.23	1.83	25.23	2.19	25.54	2.54	25.54	2.30	25.54	2.79	25.01	1.46	25.01	2.04	25.01	2.49			
26.41	2.36	26.41	2.21	26.41	0.28	26.78	2.63	26.78	2.35	26.78	2.63	26.20	0.36	26.20	2.53	26.20	2.65			
27.60	2.21	27.60	2.37	27.60		28.01	2.61	28.01	2.97	28.01		27.39	2.16	27.39	2.01	27.39				
28.78	1.87	28.78	2.70	28.78	2.94	29.24	2.83	29.24	3.01	29.24	3.32	28.57	2.11	28.57	3.27	28.57	3.48			
29.97	3.26	29.97	2.80	29.97	3.38	30.47	3.44	30.47	3.31	30.47	3.43	29.76	2.54	29.76	3.44	29.76	3.58			
31.15	4.32	31.15	3.40	31.15	3.76	31.71	3.84	31.71	3.41	31.71	3.87	30.95	2.70	30.95	3.82	30.95	2.76			
32.34		32.34	2.58	32.34	3.98	32.94		32.94	4.12	32.94	4.96	32.14		32.14	4.42	32.14	5.02			
33.53	4.93	33.53	4.31	33.53	4.82	34.17	4.66	34.17	4.29	34.17	5.35	33.33	3.14	33.33	0.97	33.33	5.40			
34.71	5.25	34.71	4.80	34.71	5.06	35.41	4.84	35.41	5.28	35.41	6.14	34.51	3.76	34.51	5.34	34.51	5.72			
35.90	5.91	35.90		35.90	5.65	36.64	5.23	36.64		36.64	6.19	35.70	3.97	35.70		35.70	7.10			
37.08	6.92	37.08	5.61	37.08	6.17	37.87	6.02	37.87	5.84	37.87	7.35	36.89	4.82	36.89	6.42	36.89	7.44			
38.27	7.22	38.27	5.94	38.27	6.94	39.11	5.87	39.11	7.50	39.11	7.56	38.08	5.41	38.08	7.04	38.08	9.93			
39.45	9.31	39.45	7.26	39.45	8.17	40.34	7.16	40.34	8.66	40.34	9.93	39.27	6.45	39.27	8.93	39.27	9.27			
40.64	10.20	40.64	8.59	40.64	8.72	41.57	8.29	41.57	9.25	41.57	10.01	40.46	7.21	40.46	9.75	40.46	11.62			
41.83	10.88	41.83	9.67	41.83	9.74	42.81	7.61	42.81	11.56	42.81	11.53	41.64	7.74	41.64	11.50	41.64	13.01			
43.01	13.02	43.01	9.81	43.01	10.90	44.04	11.67	44.04	12.28	44.04	13.79	42.83	8.86	42.83	13.34	42.83	14.58			
44.20	13.91	44.20	11.95	44.20		45.27	11.04	45.27	12.92	45.27		44.02	9.38	44.02		44.02				
45.38	15.12	45.38	13.33	45.38	12.72	46.51	12.06	46.51		46.51	15.39	45.21	10.63	45.21	16.53	45.21	17.19			
46.57		46.57	14.16	46.57	12.71	47.74		47.74	3.14	47.74	16.97	46.40		46.40	15.76	46.40	8.57			
47.75	15.40	47.75	14.18	47.75	13.07	48.97	12.88	48.97	13.93	48.97	16.83	47.58	10.57	47.58	16.88	47.58	20.12			
48.94	17.16	48.94	15.07	48.94	14.30	50.21	13.83	50.21	16.10	50.21	16.82	48.77	11.20	48.77	14.07	48.77	22.94			
50.13	19.55	50.13		50.13	16.29	51.44	15.30	51.44		51.44	18.88	49.96	12.13	49.96		49.96	25.26			

Table A 5: Calculated temperature (T) (°C) and measured respiration rate (Rs) (µg C g^{-1} h^{-1}) for a Horotiu soil before pre-incubation (Time Zero)

T0							
a		b		c		d	
T	Rs	T	Rs	T	Rs	T	Rs
4.27	-0.11	4.27	-0.05	5.93	0.13	5.93	0.25
5.55	-0.05	5.55	0.00	7.18	0.19	7.18	0.37
6.83	0.05	6.83	0.10	8.43	0.24	8.43	0.36
8.12	0.00	8.12	0.16	9.68	0.29	9.68	0.47
9.40	0.10	9.40	0.21	10.93	0.45	10.93	0.80
10.69	0.16	10.69	0.21	12.18	0.45	12.18	0.70
11.97	0.37	11.97	0.52	13.44	0.76	13.44	0.86
13.25	0.32	13.25	0.47	14.69	0.83	14.69	1.14
14.54	0.46	14.54	0.62	15.94	0.99	15.94	1.37
15.82	0.58	15.82	0.73	17.19	1.19	17.19	1.47
17.10	0.74	17.10	0.89	18.44	1.40	18.44	1.94
18.39	0.84	18.39	1.00	19.69	1.62	19.69	2.13
19.67	0.94	19.67	0.88	20.94	2.35	20.94	2.97
20.96	1.27	20.96	1.26	22.20	2.57	22.20	2.09
22.24	1.48	22.24	1.26	23.45	2.48	23.45	2.91
23.52	1.35	23.52	1.66	24.70	3.00	24.70	3.34
24.81	1.61	24.81	1.81	25.95	3.20	25.95	3.86
26.09		26.09	1.96	27.20	3.61	27.20	3.99
27.37	2.33	27.37	1.42	28.45	4.94	28.45	4.66
28.66	2.46	28.66	2.73	29.71	4.04	29.71	4.76
29.94	3.01	29.94	3.09	30.96	5.07	30.96	5.21
31.23	2.96	31.23	3.00	32.21	5.13	32.21	5.39
32.51	4.99	32.51	5.24	33.46	8.24	33.46	9.41
33.79	5.15	33.79	6.25	34.71	12.80	34.71	10.07
35.08	6.28	35.08	5.19	35.96	10.39	35.96	10.76
36.36	6.72	36.36	7.48	37.21	10.02	37.21	12.44
37.64	8.06	37.64	7.91	38.47	13.39	38.47	13.23
38.93	7.73	38.93	8.94	39.72	12.72	39.72	14.30
40.21	9.04	40.21	10.29	40.97	14.68	40.97	15.80
41.49	10.07	41.49	10.41	42.22	16.55	42.22	16.51
42.78	11.19	42.78	11.18	43.47	16.64	43.47	17.57
44.06	11.76	44.06	12.37	44.72	17.49	44.72	19.40
45.35	12.06	45.35	12.82	45.97	18.46	45.97	19.60
46.63	13.06	46.63	13.36	47.23	18.68	47.23	18.76
47.91	13.42	47.91	13.18	48.48	17.88	48.48	18.39
49.20	12.52	49.20	13.46	49.73	17.96	49.73	19.12
50.48	12.79	50.48	13.66	50.98	17.34	50.98	21.05

Table A.6: Calculated temperature (T) (°C) and measured respiration rate (Rs) (μg C g^{-1} h^{-1}) for a Horotiu soil after 2-months of pre-incubation at 35, 20, 10 and 4°C (2 replicates per incubation temperature) (Time One).

Time One																
35 °C				20 °C			10 °C			4 °C						
T	Rs	T	Rs	T	Rs	T	Rs	T	Rs	T	Rs	T	Rs	T	Rs	T	Rs
2.70		2.70	-0.20	2.11	-0.02	2.11	-0.12	2.44	-0.02	2.44		2.44		2.44		2.44	
3.88	-0.11	3.88	-0.17	3.35		3.35	-0.09	3.63		3.63	-0.02	3.63	-0.02	3.63	-0.02	3.63	-0.07
5.07	-0.08	5.07	-0.13	4.58	0.04	4.58	-0.03	4.58	-0.41	4.81	-0.02	4.81	-0.03	4.81	-0.03	4.81	0.01
6.25	-0.08	6.25	-0.05	5.81	0.08	5.81	0.00	5.81	0.05	6.00	0.02	6.00	0.03	6.00	0.03	6.00	0.06
7.44	0.00	7.44	0.02	7.04	-0.11	7.04	0.08	7.04	0.13	7.19	0.03	7.19	0.09	7.19	0.09	7.19	0.09
8.63	-0.53	8.63	0.05	8.28	0.20	8.28	0.12	8.28	0.14	8.38	0.06	8.38	0.09	8.38	0.09	8.38	0.17
9.81	0.03	9.81	0.18	9.51	0.26	9.51	0.25	9.51	0.32	9.57	0.16	9.57	0.17	9.57	0.22	9.57	0.26
11.00	0.15	11.00	0.19	10.74	0.38	10.74	0.27	10.74	0.27	10.75	0.18	10.75	0.22	10.75	0.28	10.75	0.31
12.18	0.15	12.18		11.98	0.53	11.98	0.43	11.98		11.94	0.29	11.94	0.28	11.94	0.42	11.94	
13.37	0.26	13.37	0.47	13.21	0.56	13.21	0.51	13.21	0.56	13.13	0.30	13.13	0.42	13.13	0.55	13.13	0.65
14.55	0.39	14.55	0.50	14.44	0.81	14.44	0.68	14.44	0.68	14.32	0.45	14.32	0.55	14.32	0.55	14.32	0.64
15.74	0.52	15.74	0.58	15.68	0.78	15.68	0.74	15.68	0.69	15.51	0.42	15.51	0.55	15.51	0.55	15.51	0.90
16.93		16.93	0.75	16.91	1.07	16.91		16.91	0.96	16.69	0.60	16.69		16.69		16.69	1.00
18.11	0.55	18.11	0.99	18.14	0.98	18.14	0.94	18.14	0.99	17.88	0.68	17.88	0.90	17.88	0.96	17.88	0.61
19.30	0.76	19.30	1.00	19.38		19.38	0.95	19.38	1.28	19.07		19.07	0.96	19.07	1.15	19.07	1.37
20.48	1.25	20.48	1.34	20.61	1.51	20.61	1.34	20.61	1.47	20.26	0.87	20.26	1.15	20.26	1.45	20.26	1.63
21.67	1.06	21.67	1.55	21.84	1.81	21.84	1.55	21.84	1.51	21.45	0.94	21.45	1.45	21.45	1.69	21.45	1.74
22.85	1.41	22.85	1.75	23.08	1.97	23.08	1.69	23.08	1.86	22.63	1.19	22.63	1.69	22.63	2.01	22.63	1.90
24.04	1.62	24.04	1.82	24.31	2.19	24.31	2.19	24.31	2.08	23.82	1.35	23.82	2.01	23.82	2.04	23.82	2.56
25.23	1.83	25.23	2.19	25.54	2.54	25.54	2.30	25.54	2.79	25.01	1.46	25.01	2.04	25.01	2.53	25.01	2.49
26.41	2.21	26.41	0.28	26.78	2.63	26.78	2.35	26.78	2.63	26.20	0.36	26.20	2.53	26.20	2.01	26.20	2.65
27.60	2.37	27.60		28.01	2.61	28.01	2.97	28.01		27.39	2.16	27.39	2.01	27.39	3.27	27.39	
28.78	2.70	28.78	2.94	29.24	2.83	29.24	3.01	29.24	3.32	28.57	2.11	28.57	3.27	28.57	3.44	28.57	3.48
29.97	2.80	29.97	3.38	30.47	3.44	30.47	3.31	30.47	3.43	29.76	2.54	29.76	3.44	29.76	3.82	29.76	3.58
31.15	3.40	31.15	3.76	31.71	3.84	31.71	3.41	31.71	3.87	30.95	2.70	30.95	3.82	30.95	4.42	30.95	2.76
32.34	2.58	32.34	3.98	32.94		32.94	4.12	32.94	4.96	32.14		32.14	4.42	32.14	0.97	32.14	5.02
33.53	4.31	33.53	4.82	34.17	4.66	34.17	4.29	34.17	5.35	33.33	3.14	33.33	0.97	33.33	5.34	33.33	5.40
34.71	4.80	34.71	5.06	35.41	4.84	35.41	5.28	35.41	6.14	34.51	3.76	34.51	5.34	34.51		34.51	5.72
35.90		35.90	5.65	36.64	5.23	36.64		36.64	6.19	35.70	3.97	35.70		35.70	6.42	35.70	7.10
37.08	5.61	37.08	6.17	37.87	6.02	37.87	5.84	37.87	7.35	36.89	4.82	36.89	6.42	36.89	7.04	36.89	7.44
38.27	5.94	38.27	6.94	39.11	5.87	39.11	7.50	39.11	7.56	38.08	5.41	38.08	7.04	38.08	8.93	38.08	9.93
39.45	7.26	39.45	8.17	40.34	7.16	40.34	8.66	40.34	9.93	39.27	6.45	39.27	8.93	39.27	9.75	39.27	9.27
40.64	8.59	40.64	8.72	41.57	8.29	41.57	9.25	41.57	10.01	40.46	7.21	40.46	9.75	40.46	11.50	40.46	11.62
41.83	9.67	41.83	9.74	42.81	7.61	42.81	11.56	42.81	11.53	41.64	7.74	41.64	11.50	41.64	13.34	41.64	13.01
43.01	9.81	43.01	10.90	44.04	11.67	44.04	12.28	44.04	13.79	42.83	8.86	42.83	13.34	42.83		42.83	14.58
44.20	11.95	44.20		45.27	11.04	45.27	12.92	45.27		44.02	9.38	44.02		44.02		44.02	
45.38	13.33	45.38	12.72	46.51	12.06	46.51		46.51	15.39	45.21	10.63	45.21	16.53	45.21		45.21	17.19
46.57	14.16	46.57	12.71	47.74		47.74	3.14	47.74	16.97	46.40		46.40	15.76	46.40		46.40	8.57
47.75	14.18	47.75	13.07	48.97	12.88	48.97	13.93	48.97	16.83	47.58	10.57	47.58	16.88	47.58		47.58	20.12
48.94	15.07	48.94	14.30	50.21	13.83	50.21	16.10	50.21	16.82	48.77	11.20	48.77	14.07	48.77		48.77	22.94
50.13		50.13	16.29	51.44	15.30	51.44		51.44	18.88	49.96	12.13	49.96		49.96		49.96	25.26

Table A.7: Calculated temperature (T) (°C) and measured respiration rate (Rs) (μg C g^{-1} h^{-1}) for a Horotiu soil after 4-months of pre-incubation at 35, 20, 10 and 4°C (2 replicates per incubation temperature) (Time Two).

Time Two																			
35 °C				20 °C			10 °C			4 °C									
T	Rs	T	Rs	T	Rs	T	Rs	T	Rs	T	Rs	T	Rs	T	Rs	T	Rs	T	Rs
1.91	-0.43	1.91	-0.40	1.62	-0.04	1.62	0.07	1.88	0.04	1.88	0.21	1.96	-0.17	1.96	-0.17	1.96	-0.17	1.96	0.14
3.04		3.04	-0.40	2.76		2.76	0.18	3.02		3.02	0.15	3.10		3.10		3.10		3.10	0.37
4.18	-0.37	4.18	-0.40	3.90	0.01	3.90	0.12	4.16	0.04	4.16	0.10	4.23	-0.06	4.23	-0.06	4.23	-0.06	4.23	-0.14
5.32	-0.26	5.32	-0.30	5.05	0.30	5.05	0.23	5.30	0.10	5.30	0.26	5.37	-0.06	5.37	-0.06	5.37	-0.06	5.37	0.36
6.46	-0.26	6.46	-0.19	6.19	0.13	6.19	0.29	6.44	0.16	6.44	0.20	6.51	0.06	6.51	0.06	6.51	0.06	6.51	0.42
7.60	-0.20	7.60	-0.18	7.34	0.13	7.34	0.35	7.58	0.27	7.58	0.04	7.65	0.17	7.65	0.17	7.65	0.17	7.65	0.52
8.73	-0.26	8.73	-0.24	8.48	0.07	8.48	0.39	8.72	0.38	8.72	0.32	8.79	0.23	8.79	0.23	8.79	0.23	8.79	0.64
9.87	0.09	9.87	-0.19	9.62	0.19	9.62	0.51	9.86	0.27	9.86	0.49	9.93	0.28	9.93	0.28	9.93	0.28	9.93	0.82
11.01	0.09	11.01	-0.08	10.77	0.24	10.77	0.56	11.00	0.39	11.00	0.48	11.07	0.35	11.07	0.35	11.07	0.35	11.07	0.74
12.15	0.14	12.15	-0.08	11.91	0.24	11.91	0.56	12.14	0.50	12.14	0.69	12.20	0.29	12.20	0.29	12.20	0.29	12.20	0.65
13.29	0.26	13.29	-0.03	13.06	0.36	13.06	0.72	13.28	0.33	13.28	0.65	13.34	0.45	13.34	0.45	13.34	0.45	13.34	0.82
14.42	0.03	14.42	-0.03	14.20	0.47	14.20	0.62	14.42	0.73	14.42	0.85	14.48	0.63	14.48	0.63	14.48	0.63	14.48	1.03
15.56	0.25	15.56	0.13	15.34	0.57	15.34	0.82	15.56	0.60	15.56	0.78	15.62	0.74	15.62	0.74	15.62	0.74	15.62	1.14
16.70	0.25	16.70	0.24	16.49	0.58	16.49	0.88	16.70	0.95	16.70	1.14	16.76	0.68	16.76	0.68	16.76	0.68	16.76	1.43
17.84		17.84		17.63		17.63		17.84		17.84		17.90		17.90		17.90		17.90	
18.98	0.31	18.98	0.40	18.78	0.69	18.78	0.99	18.98	1.35	18.98	1.20	19.04	0.85	19.04	0.85	19.04	0.85	19.04	1.81
20.11	0.37	20.11	0.40	19.92	0.81	19.92	1.25	20.12	1.41	20.12	1.40	20.17	1.14	20.17	1.14	20.17	1.14	20.17	1.89
21.25	0.42	21.25	0.40	21.06	0.96	21.06	1.33	21.26	1.55	21.26	1.67	21.31	1.37	21.31	1.37	21.31	1.37	21.31	1.80
22.39	0.48	22.39	0.44	22.21	1.05	22.21	1.61	22.40	1.41	22.40	1.71	22.45	1.30	22.45	1.30	22.45	1.30	22.45	2.22
23.53	0.52	23.53	0.45	23.35	1.26	23.35	1.47	23.54	1.84	23.54	1.51	23.59	1.51	23.59	1.51	23.59	1.51	23.59	2.64
24.66	0.64	24.66	0.45	24.50	1.35	24.50	1.61	24.68	2.06	24.68	1.78	24.73	1.25	24.73	1.25	24.73	1.25	24.73	2.65
25.80	0.69	25.80	0.61	25.64	1.48	25.64	1.72	25.82	2.20	25.82	2.04	25.87	1.86	25.87	1.86	25.87	1.86	25.87	2.55
26.94	0.81	26.94	0.66	26.78	1.69	26.78	1.94	26.96	2.07	26.96	2.04	27.01	2.41	27.01	2.41	27.01	2.41	27.01	2.34
28.08	1.03	28.08	0.86	27.93	1.85	27.93	2.25	28.10	2.28	28.10	2.54	28.14	2.57	28.14	2.57	28.14	2.57	28.14	3.13
29.22	1.27	29.22	0.92	29.07	2.11	29.07	2.49	29.24	2.38	29.24	2.67	29.28	2.96	29.28	2.96	29.28	2.96	29.28	3.10
30.35		30.35	1.01	30.22		30.22	2.64	30.38		30.38	3.04	30.42		30.42		30.42		30.42	3.82
31.49	1.40	31.49	1.09	31.36	2.21	31.36	2.85	31.52	2.51	31.52	3.05	31.56	3.27	31.56	3.27	31.56	3.27	31.56	3.93
32.63	1.61	32.63	1.37	32.50	2.83	32.50		32.66	3.43	32.66	2.82	32.70	3.56	32.70	3.56	32.70	3.56	32.70	
33.77	1.71	33.77		33.65	2.86	33.65	3.13	33.80	3.70	33.80		33.84	3.42	33.84	3.42	33.84	3.42	33.84	
34.91	1.85	34.91	1.35	34.79	3.12	34.79	3.71	34.94	3.81	34.94	3.73	34.98	4.39	34.98	4.39	34.98	4.39	34.98	4.29
36.04	2.16	36.04	1.62	35.94	2.33	35.94	3.83	36.08	4.23	36.08	4.41	36.11		36.11		36.11		36.11	5.05
37.18	2.23	37.18	2.10	37.08	3.85	37.08	4.33	37.22	4.20	37.22	4.47	37.25	4.87	37.25	4.87	37.25	4.87	37.25	4.74
38.32	2.42	38.32	2.17	38.22	3.99	38.22	4.41	38.36	5.02	38.36	4.91	38.39	5.12	38.39	5.12	38.39	5.12	38.39	5.72
39.46	2.89	39.46	2.35	39.37	3.84	39.37	5.41	39.50	5.29	39.50	5.69	39.53	4.88	39.53	4.88	39.53	4.88	39.53	6.41
40.60	3.01	40.60	2.31	40.51	4.91	40.51	5.16	40.64	6.39	40.64	5.35	40.67	6.06	40.67	6.06	40.67	6.06	40.67	6.48
41.73	3.24	41.73	2.65	41.66	5.46	41.66	5.74	41.78	6.91	41.78	6.12	41.81	7.11	41.81	7.11	41.81	7.11	41.81	7.12
42.87	3.40	42.87	3.13	42.80	6.10	42.80	6.32	42.92	7.09	42.92	6.89	42.95	7.17	42.95	7.17	42.95	7.17	42.95	8.19
44.01		44.01	2.90	43.94		43.94	6.91	44.06		44.06	7.37	44.09		44.09		44.09		44.09	7.34
45.15	3.58	45.15	3.37	45.09	6.61	45.09	6.51	45.20	7.27	45.20		45.22	8.44	45.22	8.44	45.22	8.44	45.22	7.82
46.29	3.60	46.29	3.34	46.23	7.09	46.23	6.58	46.34	6.35	46.34	7.25	46.36	8.57	46.36	8.57	46.36	8.57	46.36	7.28
47.42	3.61	47.42		47.38	6.58	47.38	6.25	47.48	8.12	47.48		47.50	8.77	47.50	8.77	47.50	8.77	47.50	
48.56	3.45	48.56	3.25	48.52	6.25	48.52	6.63	48.62	7.87	48.62	7.58	48.64	8.71	48.64	8.71	48.64	8.71	48.64	8.63
49.70	3.60	49.70	3.16	49.66	7.26	49.66	7.08	49.76	8.42	49.76	8.89	49.78	9.33	49.78	9.33	49.78	9.33	49.78	8.77
50.84	3.47	50.84	3.40	50.81	7.76	50.81	7.92	50.90	8.13	50.90	8.15	50.92	8.77	50.92	8.77	50.92	8.77	50.92	10.68

Table A.8: Calculated temperature (T) (°C) and measured respiration rate (Rs) (μg C g^{-1} h^{-1}) for a Horotiu soil after 6-months of pre-incubation at 35, 20, 10 and 4°C (2 replicates per incubation temperature) (Time Three).

Time Three																
35 °C				20 °C				10 °C				4 °C				
T	Rs	T	Rs	T	Rs	T	Rs	T	Rs	T	Rs	T	Rs	T	Rs	
1.31	-0.06	1.31		1.87	0.01	1.87		1.93	-0.01	1.93		1.85	-0.08	1.85		
2.45		2.45	-0.20	3.01		3.01	-0.08	3.10		3.10	-0.07	3.01		3.01	0.02	
3.59	-0.03	3.59	-0.13	4.15	-0.02	4.15	-0.02	4.26	0.01	4.26	-0.01	4.17	-0.02	4.17	-0.20	
4.73	0.04	4.73	0.08	5.29	0.00	5.29	0.00	5.42	-0.04	5.42	0.06	5.32	-0.03	5.32	0.06	
5.86	0.04	5.86	0.08	6.43	0.03	6.43	-0.04	6.58	-0.04	6.58	0.06	6.48	0.09	6.48	0.14	
7.00	0.08	7.00	0.08	7.57	0.04	7.57	-0.02	7.74	0.06	7.74	0.12	7.64	0.14	7.64	0.22	
8.14	0.12	8.14	0.12	8.71	0.11	8.71	-0.02	8.90	0.11	8.90	0.19	8.80	-0.23	8.80	0.20	
9.28	0.08	9.28	0.05	9.85	0.11	9.85	0.04	10.06	0.09	10.06	0.21	9.96	0.14	9.96	0.26	
10.42	0.19	10.42	0.08	10.99	0.17	10.99	0.09	11.22	0.12	11.22	0.29	11.12	0.09	11.12	0.28	
11.56	0.15	11.56	0.19	12.12	0.17	12.12	0.11	12.39	0.19	12.39	0.32	12.27	0.38	12.27	0.33	
12.69	0.15	12.69	0.27	13.26	-0.02	13.26	0.10	13.55	0.24	13.55	0.21	13.43	0.12	13.43	0.41	
13.83	0.23	13.83	0.38	14.40	0.27	14.40	0.11	14.71	0.32	14.71	0.36	14.59	0.33	14.59	0.39	
14.97	0.19	14.97		15.54	0.24	15.54		15.87	0.41	15.87		15.75	0.44	15.75		
16.11	0.23	16.11	0.31	16.68	0.07	16.68	0.17	17.03	0.44	17.03	0.39	16.91	0.51	16.91	0.22	
17.25		17.25	0.38	17.82		17.82	0.11	18.19		18.19	0.53	18.06		18.06	0.38	
18.38	0.26	18.38	0.38	18.96	0.11	18.96	0.30	19.35	0.53	19.35	0.49	19.22	0.66	19.22	0.59	
19.52	0.34	19.52	0.46	20.10	0.38	20.10	0.36	20.51	0.60	20.51	0.73	20.38	0.67	20.38	0.71	
20.66	0.38	20.66	0.50	21.24	0.48	21.24	0.36	21.67	0.72	21.67	0.80	21.54	0.79	21.54	0.79	
21.80	0.45	21.80	0.53	22.38	0.12	22.38	0.43	22.84	0.81	22.84	0.87	22.70	0.95	22.70	0.91	
22.94	0.53	22.94	0.61	23.52	0.52	23.52	0.55	24.00	0.92	24.00	0.98	23.85	1.20	23.85	0.86	
24.08	0.53	24.08	0.54	24.65	0.62	24.65	0.56	25.16	1.17	25.16	1.08	25.01	1.21	25.01	1.16	
25.21	0.49	25.21	0.23	25.79	0.73	25.79	0.57	26.32	1.10	26.32	1.19	26.17	1.40	26.17	1.35	
26.35	0.37	26.35	0.70	26.93	0.70	26.93	0.69	27.48	1.29	27.48	1.41	27.33	1.53	27.33	1.30	
27.49	0.64	27.49	0.77	28.07	0.84	28.07	0.81	28.64	1.59	28.64	1.57	28.49	1.67	28.49	1.57	
28.63	0.68	28.63	0.78	29.21	0.90	29.21	0.86	29.80	2.08	29.80	1.67	29.64	1.87	29.64	2.18	
29.77		29.77	0.54	30.35		30.35	0.97	30.96		30.96	2.07	30.80		30.80	2.21	
30.91	0.00	30.91	0.93	31.49	1.19	31.49	1.19	32.13	1.36	32.13	2.14	31.96	2.21	31.96	1.45	
32.04	0.72	32.04	0.73	32.63	1.33	32.63		33.29	2.41	33.29	2.43	33.12	2.72	33.12	2.79	
33.18	0.84	33.18		33.77	1.56	33.77		34.45	2.80	34.45		34.28	2.57	34.28		
34.32	0.83	34.32	1.18	34.91	1.67	34.91	1.31	35.61	2.63	35.61	2.87	35.44	3.23	35.44	2.74	
35.46	1.05	35.46	1.34	36.05	1.84	36.05	1.77	36.77	3.27	36.77	3.62	36.59	2.77	36.59	3.58	
36.60	1.16	36.60	1.48	37.19	1.70	37.19	2.17	37.93	3.22	37.93	4.01	37.75	4.22	37.75	4.15	
37.74	1.34	37.74	1.58	38.32	2.64	38.32	2.25	39.09	4.10	39.09	4.40	38.91	4.73	38.91	4.54	
38.87	1.34	38.87	1.53	39.46	2.72	39.46	2.61	40.25	3.95	40.25	4.70	40.07	5.15	40.07	5.29	
40.01	1.66	40.01	1.82	40.60	3.15	40.60	3.01	41.41	4.52	41.41	5.09	41.23	5.03	41.23	5.23	
41.15	1.49	41.15	2.07	41.74	3.59	41.74	3.40	42.58	5.42	42.58	1.45	42.38	4.25	42.38	6.24	
42.29	1.50	42.29	2.23	42.88	3.57	42.88	4.13	43.74	5.02	43.74	6.01	43.54	6.49	43.54	6.02	
43.43		43.43	2.49	44.02		44.02	3.60	44.90		44.90	6.47	44.70		44.70	6.88	
44.57	2.40	44.57	2.81	45.16	3.73	45.16	4.62	46.06	5.14	46.06	6.66	45.86	6.67	45.86	5.31	
45.70	2.59	45.70	3.01	46.30	4.77	46.30	4.46	47.22	4.65	47.22	6.81	47.02	7.21	47.02	7.29	
46.84	2.94	46.84		47.44	4.76	47.44		48.38	6.81	48.38		48.17	6.97	48.17		
47.98	2.87	47.98	3.42	48.58	3.19	48.58	4.88	49.54	5.84	49.54	8.25	49.33	7.39	49.33	7.30	
49.12	2.48	49.12	2.85	49.72	4.76	49.72	5.01	50.70	7.33	50.70	8.52	50.49	6.83	50.49	7.86	
50.26	2.40	50.26	2.82	50.85	5.51	50.85	6.20	51.87	8.71	51.87	10.22	51.65	9.13	51.65	10.24	

Table A.9: Calculated temperature (T) (°C) and measured respiration rate (Rs) (μg C g^{-1} h^{-1}) for a Horotiu soil after 8-months of pre-incubation at 35, 20, 10 and 4°C (2 replicates per incubation temperature) (Time Four).

Time Four																
35 °C				20 °C			10 °C			4 °C						
T	Rs	T	Rs	T	Rs	T	Rs	T	Rs	T	Rs	T	Rs	T	Rs	T	Rs
2.37	-0.16	2.37		2.58	-0.13	2.58		3.01	-0.01	3.01		3.76	0.01	3.76			
3.60		3.60	-0.01	3.81		3.81	-0.18	4.22		4.22	-0.03	4.97		4.97	-0.07		
4.83	-0.14	4.83	-0.03	5.03	-0.07	5.03	-0.20	5.44	0.02	5.44	-0.02	6.17	0.04	6.17	-0.02		
6.06	-0.07	6.06	0.01	6.26	-0.10	6.26	-0.13	6.65	0.01	6.65	-0.01	7.38	0.08	7.38	0.01		
7.28	-0.04	7.28	0.10	7.49	-0.02	7.49	-0.07	7.87	-0.05	7.87	0.00	8.58		8.58	0.02		
8.51	-0.03	8.51	0.09	8.71	0.06	8.71	-0.02	9.08	0.08	9.08	0.03	9.79	0.23	9.79	0.12		
9.74	-0.17	9.74	0.14	9.94	0.10	9.94	0.06	10.30	0.12	10.30	0.04	10.99	0.29	10.99	0.23		
10.97	-0.24	10.97	0.19	11.16	0.12	11.16	0.06	11.51	0.18	11.51	0.07	12.20	0.36	12.20	0.27		
12.20	0.03	12.20	0.28	12.39	0.10	12.39	0.12	12.73	0.27	12.73	0.07	13.40	0.36	13.40	0.28		
13.43	0.10	13.43	0.35	13.62	0.20	13.62	0.20	13.94	0.27	13.94	0.07	14.61	0.41	14.61	0.44		
14.66	0.10	14.66	0.34	14.84	0.27	14.84	0.32	15.16	0.35	15.16	0.14	15.81	0.55	15.81	0.48		
15.89	0.22	15.89	0.35	16.07	0.38	16.07	0.38	16.37	0.34	16.37	0.13	17.02	0.55	17.02	0.58		
17.12	0.32	17.12		17.30	0.35	17.30		17.58	0.43	17.58		18.22	0.64	18.22			
18.35	0.34	18.35	0.39	18.52	0.36	18.52	0.38	18.80	0.52	18.80	0.15	19.43	0.76	19.43	0.63		
19.58		19.58	0.40	19.75		19.75	0.37	20.01		20.01	0.21	20.63		20.63	0.70		
20.81	0.20	20.81	0.41	20.97	0.50	20.97	0.52	21.23	0.60	21.23	0.21	21.84	0.76	21.84	0.73		
22.04	0.16	22.04	0.50	22.20	0.56	22.20	0.59	22.44	0.75	22.44	0.34	23.04	1.07	23.04	0.86		
23.27	0.28	23.27	0.67	23.43	0.64	23.43	0.64	23.66	0.79	23.66	0.36	24.25	1.15	24.25	0.95		
24.50	0.37	24.50	0.75	24.65	0.76	24.65	0.59	24.87	0.86	24.87	0.44	25.45	1.36	25.45	1.06		
25.73	0.46	25.73	0.50	25.88	0.77	25.88	0.66	26.09	0.91	26.09	0.47	26.66	1.39	26.66	1.16		
26.96	0.43	26.96	0.90	27.11	0.92	27.11	0.76	27.30	1.09	27.30	0.58	27.86	1.55	27.86	1.38		
28.19	0.52	28.19	0.83	28.33	0.92	28.33	0.87	28.51	1.19	28.51	0.59	29.07	1.79	29.07	1.59		
29.42	0.50	29.42	0.83	29.56	1.14	29.56	0.87	29.73	1.33	29.73	0.71	30.27	1.75	30.27	1.80		
30.65	0.62	30.65	0.96	30.78	1.26	30.78	1.09	30.94	1.52	30.94	0.33	31.48	2.19	31.48	1.94		
31.88	0.76	31.88	0.97	32.01	1.43	32.01	1.35	32.16	1.61	32.16	1.00	32.68	2.46	32.68	2.46		
33.11		33.11	1.14	33.24		33.24	1.45	33.37		33.37	1.26	33.89		33.89	2.54		
34.34	0.92	34.34	1.22	34.46	1.86	34.46	1.77	34.59	2.29	34.59	1.41	35.09	3.05	35.09	3.02		
35.57	1.03	35.57	0.97	35.69	1.78	35.69	1.85	35.80	2.45	35.80	1.59	36.30	3.66	36.30	3.29		
36.80	0.83	36.80		36.91	2.14	36.91		37.02	2.86	37.02		37.50	3.70	37.50			
38.03	1.28	38.03	1.40	38.14	2.47	38.14	2.06	38.23	3.49	38.23	1.98	38.71	4.11	38.71	3.60		
39.26	1.30	39.26	1.74	39.37	2.76	39.37	2.88	39.45	3.59	39.45	2.55	39.91	4.63	39.91	4.57		
40.49	1.40	40.49	1.93	40.59	3.15	40.59	3.19	40.66	4.02	40.66	2.90	41.12	5.40	41.12	5.13		
41.72	1.79	41.72	2.05	41.82	3.17	41.82	3.47	41.87	4.59	41.87	3.29	42.32	6.00	42.32	3.30		
42.95	1.95	42.95	2.13	43.05	3.91	43.05	3.86	43.09	5.21	43.09	3.85	43.53	6.40	43.53	6.13		
44.18	2.10	44.18	2.45	44.27	4.05	44.27	4.18	44.30	5.91	44.30	4.19	44.73	6.79	44.73	6.41		
45.41	2.42	45.41	2.81	45.50	4.34	45.50	4.09	45.52	5.76	45.52	5.09	45.94	6.82	45.94	6.67		
46.64	2.52	46.64	2.72	46.72	3.86	46.72	4.58	46.73	5.96	46.73	4.70	47.14	6.99	47.14	7.22		
47.87		47.87	2.81	47.95		47.95	4.44	47.95		47.95	4.93	48.35		48.35	6.96		
49.10	2.58	49.10	2.80	49.18	4.64	49.18	4.62	49.16	3.69	49.16	5.32	49.55	7.82	49.55	6.78		
50.33	2.29	50.33	1.90	50.40	4.84	50.40	4.94	50.38	7.63	50.38	5.21	50.76	8.83	50.76	9.11		

Table A.10: Calculated temperature (T) (°C) and measured respiration rate (Rs) (μg C g^{-1} h^{-1}) for a Horotiu soil after 10-months of pre-incubation at 35, 20, 10 and 4°C (2 replicates per incubation temperature) (Time Five).

Time Five																
35 °C				20 °C			10 °C			4 °C						
T	Rs	T	Rs	T	Rs	T	Rs	T	Rs	T	Rs	T	Rs	T	Rs	T	Rs
4.14	-0.24	4.14		4.14	-0.23	-0.19	2.37		2.37	-0.10	2.54	-0.23	2.54			
5.30		5.30	-0.21	5.30	-0.23		3.56	-0.10	3.56	-0.06	3.72		3.72	-0.18		
6.45	-0.25	6.45	-0.06	6.45	-0.18	-0.19	4.75	-0.31	4.75	-0.01	4.91	-0.20	4.91	-0.28		
7.61	-0.22	7.61	-0.09	7.61	-0.15	-0.13	5.95	-0.02	5.95	0.01	6.10	-0.14	6.10	-0.12		
8.76	-0.17	8.76	0.06	8.76	-0.09	-0.10	7.14	0.03	7.14	0.08	7.29	-0.08	7.29	-0.05		
9.92	-0.18	9.92	-0.06	9.92	0.00	-0.05	8.33	0.03	8.33	0.13	8.47	-0.01	8.47	-0.01		
11.07	-0.12	11.07	0.06	11.07	0.11	-0.01	9.52	0.08	9.52	0.19	9.66	0.04	9.66	0.01		
12.23	-0.07	12.23	0.14	12.23	0.04	-0.01	10.72	0.08	10.72	0.17	10.85	0.10	10.85	0.10		
13.38	-0.06	13.38	0.22	13.38		0.00	11.91	0.13	11.91		12.04	0.14	12.04	0.15		
14.54	-0.02	14.54	0.16	14.54	0.28	0.03	13.10	0.14	13.10	0.34	13.22	0.27	13.22	0.20		
15.69	-0.02	15.69	0.34	15.69	0.39	0.09	14.29	0.23	14.29	0.39	14.41	0.33	14.41	0.32		
16.85	0.05	16.85	0.39	16.85	0.41	0.17	15.49	0.35	15.49	0.38	15.60	0.39	15.60	0.34		
18.00	0.08	18.00		18.00	0.42	0.22	16.68		16.68	0.00	16.79	0.45	16.79			
19.16	0.14	19.16	0.48	19.16	0.00	0.19	17.87	0.37	17.87	0.54	17.97	0.51	17.97	0.44		
20.31		20.31	0.57	20.31	0.11		19.06	0.47	19.06	0.71	19.16		19.16	0.57		
21.47	0.17	21.47	0.49	21.47	0.60	0.29	20.25	0.53	20.25	0.76	20.35	0.70	20.35	0.87		
22.62	0.23	22.62	0.55	22.62	0.61	0.22	21.45	0.61	21.45	0.74	21.54	0.79	21.54	0.94		
23.78	0.25	23.78	0.63	23.78	0.69	0.27	22.64	0.67	22.64	0.80	22.72	0.95	22.72	1.13		
24.93	0.35	24.93	0.54	24.93	0.86	0.28	23.83	0.74	23.83	0.97	23.91	0.99	23.91	1.22		
26.09	0.38	26.09	0.73	26.09	1.01	0.37	25.02	0.89	25.02	1.17	25.10	1.15	25.10	1.07		
27.24	0.05	27.24	0.30	27.24	0.87	0.42	26.22	0.87	26.22	1.06	26.29	1.26	26.29	0.99		
28.40	0.47	28.40	0.80	29.55	1.07	0.45	27.41	0.92	27.41		27.47	1.63	27.47	0.88		
29.55	0.54	29.55	0.06	30.71	1.19	0.52	28.60	0.97	28.60	1.12	28.66	1.68	28.66	1.53		
30.71	0.57	30.71	0.93	31.86	1.40	0.61	29.79	1.08	29.79	1.44	29.85	1.91	29.85	1.94		
31.86	0.73	31.86	1.05	33.02	1.47	0.65	30.99	1.34	30.99	1.11	31.04	2.07	31.04	2.21		
33.02		33.02	1.23	34.18	1.69	0.87	32.18	1.53	32.18	1.80	32.22		32.22	2.49		
34.18	0.79	34.18	1.67	35.33	1.72	0.99	33.37	1.66	33.37	1.82	33.41	2.67	33.41	2.65		
35.33	0.91	35.33	1.47	36.49	1.91	1.12	34.56	1.99	34.56	2.38	34.60	2.84	34.60	2.75		
36.49	0.91	36.49		37.64	2.32	1.38	35.76		35.76	2.51	35.79	3.10	35.79			
37.64	1.21	37.64		38.80	2.62	1.56	36.95	2.30	36.95	2.39	36.97	3.63	36.97	3.40		
38.80	1.17	38.80	1.82	39.95	3.24	1.58	38.14	2.77	38.14	3.08	38.16	4.36	38.16	3.60		
39.95	1.36	39.95	1.77	41.11	2.09	1.97	39.33	3.35	39.33	3.59	39.35	3.76	39.35	4.78		
41.11	1.58	41.11	1.84	42.26	3.60	2.32	40.53	3.58	40.53	3.91	40.54	5.04	40.54	4.99		
42.26	1.59	42.26	1.01	43.42	4.18	2.64	41.72	3.98	41.72	4.43	41.72	5.52	41.72	5.51		
43.42	1.78	43.42	2.04	44.57		2.90	42.91	4.55	42.91	4.80	42.91	6.30	42.91	5.90		
44.57	1.95	44.57	2.72	45.73	4.10	3.27	44.10	4.64	44.10		44.10	5.85	44.10	5.76		
45.73	2.33	45.73	2.83	46.88	2.07		45.30	5.08	45.30	4.74	45.29	6.89	45.29	6.52		
46.88		46.88	1.94	48.04	4.63	3.49	46.49	5.25	46.49	5.56	46.47		46.47	6.70		
48.04	2.49	48.04	3.16	49.19	4.68	3.63	47.68	5.27	47.68	4.86	47.66	4.21	47.66	7.44		
49.19	2.17	49.19		50.35	4.40	3.61	48.87	5.82	48.87	4.34	48.85	6.62	48.85	9.79		
50.35	2.29	50.35					50.07		50.07	6.61	50.04	7.57	50.04			

Table A.11: Calculated temperature (T) (°C) and measured respiration rate (Rs) (μg C g^{-1} h^{-1}) for a Horotiu soil wetted up to 7 different moisture contents (20, 03, 40, 50, 60, 70 and 80%).

Horotiu

20%		30%		40%		50%		60%		70%		80%	
T	Rs	T	Rs	T	Rs	T	Rs	T	Rs	T	Rs	T	Rs
2.04	-0.22	2.04		2.04	-0.11	2.45	0.00	2.43	0.01	2.43		2.43	0.57
3.11		3.11	-0.16	3.11	-0.11	3.52		3.49		3.49	0.65	3.49	0.62
4.19	-0.16	4.19	-0.11	4.19	-0.06	4.58	0.00	4.56	0.30	4.56	0.86	4.56	0.80
5.26	-0.16	5.26	-0.16	5.26	-0.06	5.64	0.13	5.63	0.38	5.63	0.72	5.63	0.82
6.33	-0.16	6.33	-0.05	6.33	0.11	6.70	0.13	6.69	0.41	6.69	0.81	6.69	0.85
7.40	0.00	7.40	-0.05	7.40	0.06	7.77	0.18	7.76	0.32	7.76	0.97	7.76	1.00
8.48	-0.05	8.48	0.00	8.48	0.17	8.83	0.28	8.82	0.59	8.82	1.53	8.82	1.51
9.55	0.00	9.55	0.05	9.55	0.17	9.89	0.35	9.89	0.60	9.89	1.58	9.89	1.57
10.62	0.03	10.62	0.05			10.95	0.34	10.96	0.67	10.96	1.52	10.96	
11.70	0.05	11.70	0.16	0.34		12.02	0.48	12.02	0.79	12.02	2.15	12.02	1.34
12.77	0.16	12.77	0.16	0.28		13.08	0.55	13.09	0.76	13.09	2.05	13.09	2.21
13.84	0.16	13.84	0.27	0.56		14.14	0.84	14.16	1.14	14.16	1.55	14.16	2.08
14.91	0.26	14.91		0.62		15.20	0.82	15.22	1.25	15.22		15.22	1.96
15.99	0.26	15.99	0.32	0.65		16.27	0.98	16.29	1.45	16.29	1.55	16.29	2.69
17.06		17.06	0.48	0.74		17.33		17.35		17.35	1.02	17.35	2.20
18.13	0.52	18.13	0.58	0.90		18.39	1.06	18.42	2.04	18.42	3.14	18.42	3.02
19.21	0.58	19.21	0.69	0.95		19.45	1.30	19.49	1.99	19.49	2.50	19.49	2.62
20.28	0.62	20.28	0.72	1.06		20.52	1.35	20.55	2.26	20.55	2.61	20.55	3.55
21.35	0.68	21.35	0.85	1.19		21.58	1.50	21.62	2.44	21.62	2.17	21.62	3.13
22.43	0.73	22.43	1.01	1.53		22.64	1.43	22.69	2.51	22.69	2.35	22.69	3.59
23.50	0.98	23.50	1.04	1.41		23.70	1.89	23.75	2.82	23.75	1.69	23.75	2.37
24.57	1.01	24.57	1.32			24.77	2.00	24.82	3.03	24.82	2.74	24.82	
25.64	2.21	25.64	2.31	2.67		25.83	3.63	25.88	5.27	25.88	5.87	25.88	6.93
26.72	2.80	26.72	2.50	3.09		26.89	4.28	26.95	5.80	26.95	6.10	26.95	7.20
27.79	2.59	27.79	2.91	3.44		27.95	4.73	28.02	5.83	28.02	6.74	28.02	9.55
28.86		28.86	3.10	3.87		29.02		29.08		29.08	6.19	29.08	6.09
29.94	2.72	29.94	3.43	4.27		30.08	4.89	30.15	6.21	30.15	7.47	30.15	9.45
31.01	3.41	31.01	3.64	5.15		31.14	5.74	31.21	7.01	31.21	8.01	31.21	10.36
32.08	4.25	32.08		5.05		32.20	6.03	32.28	7.36	32.28		32.28	7.07
33.15	4.44	33.15	4.50	5.35		33.27	7.17	33.35	8.37	33.35	6.16	33.35	6.24
34.23	5.17	34.23	5.25	6.38		34.33	7.38	34.41	8.41	34.41	5.66	34.41	6.98
35.30	4.83	35.30	5.62	6.67		35.39	7.68	35.48	7.38	35.48	7.27	35.48	11.06
36.37	5.66	36.37	6.32	7.64		36.45	10.19	36.55	9.23	36.55	6.38	36.55	8.37
37.45	6.72	37.45	6.88	7.47		37.52	9.07	37.61	9.35	37.61	5.21	37.61	11.33
38.52	7.69	38.52	7.46	8.50		38.58	10.17	38.68	9.66	38.68	9.67	38.68	7.83
39.59	7.13	39.59	8.20			39.64	9.77	39.74	10.76	39.74	7.95	39.74	
40.66	9.43	40.66	8.84	9.40		40.70	10.90	40.81	10.78	40.81	11.18	40.81	15.29
41.74		41.74	10.42	10.21		41.77		41.88		41.88	10.18	41.88	15.18
42.81	11.18	42.81	10.79	11.16		42.83	11.91	42.94	12.08	42.94	12.34	42.94	15.42
43.88	12.24	43.88	11.97	12.43		43.89	13.35	44.01	13.03	44.01	14.87	44.01	14.00
44.96	12.94	44.96		13.31		44.95	14.18	45.08	12.03	45.08		45.08	15.44
46.03	14.29	46.03	12.81	13.07		46.02	14.05	46.14	13.78	46.14	15.91	46.14	13.81
47.10	14.58	47.10	13.12	12.60		47.08	14.69	47.21	14.10	47.21	10.13	47.21	14.50
48.17	14.00	48.17	12.73			48.14	4.22	48.27	13.43	48.27	15.45	48.27	

Table A.12: Calculated temperature (T) (°C) and measured respiration rate (Rs) (μg C g^{-1} h^{-1}) for a Te Kowhai soil wetted up to 7 different moisture contents (20, 03, 40, 50, 60, 70 and 80%).

Te Kowhai														
20%		30%		40%		50%		60%		70%		80%	
T	Rs	T	Rs	T	Rs	T	Rs	T	Rs	T	Rs	T	Rs
2.00	-0.10	2.00		2.00	0.17	2.00	-0.10	2.00		2.00	0.46	2.00	
3.07		3.07	-0.01	3.07	0.35	3.07		3.07	0.05	3.07		3.07	1.09
4.15	-0.01	4.15	0.08	4.15	0.44	4.14	0.05	4.14	0.10	4.14	0.74	4.14	1.03
5.22	0.03	5.22	0.17	5.22	0.58	5.21	0.10	5.21	0.26	5.21	0.64	5.21	1.31
6.29	-0.01	6.29	0.12	6.29	0.64	6.28	0.05	6.28	0.26	6.28	1.91	6.28	1.48
7.36	0.21	7.36	0.16	7.36	0.58	7.35	0.10	7.35	0.31	7.35	1.42	7.35	1.42
8.43	0.25	8.43	0.29	8.43	0.72	8.42	0.15	8.42	0.41	8.42	1.31	8.42	1.29
9.51	0.25	9.51	0.30	9.51	0.73	9.49	0.20	9.49	0.61	9.49	1.77	9.49	1.77
10.58	0.16	10.58	0.47	10.58		10.56	0.20	10.56	0.72	10.56	1.97	10.56	2.08
11.65	0.21	11.65	0.51	11.65	0.91	11.63	0.25	11.63	0.76	11.63	2.20	11.63	1.95
12.72	0.42	12.72	0.74	12.72	0.99	12.70	0.50	12.70	0.98	12.70	2.26	12.70	1.65
13.79	0.34	13.79	0.86	13.79	1.09	13.77	0.49	13.77	1.08	13.77	2.54	13.77	1.94
14.87	0.46	14.87		14.87	1.12	14.84	0.64	14.84		14.84	2.89	14.83	
15.94	0.42	15.94	0.86	15.94	1.22	15.91	0.78	15.91	0.98	15.91	3.06	15.90	2.03
17.01		17.01	0.92	17.01	1.27	16.98		16.98	1.03	16.98		16.97	2.70
18.08	0.67	18.08	0.95	18.08	1.27	18.05	1.05	18.05	1.43	18.05	3.83	18.04	1.98
19.16	0.77	19.16	0.92	19.16	1.07	19.12	1.33	19.12	1.80	19.12	3.44	19.11	2.52
20.23	0.85	20.23	1.16	20.23	1.80	20.19	1.53	20.19	2.20	20.19	4.23	20.18	2.84
21.30	0.93	21.30	1.34	21.30	1.65	21.26	1.47	21.26	2.10	21.26	4.66	21.25	2.40
22.37	0.94	22.37	1.55	22.37	2.00	22.33	1.65	22.33	2.31	22.33	4.35	22.32	2.57
23.44	1.13	23.44	1.61	23.44	2.06	23.40	1.68	23.40	2.48	23.40	4.96	23.39	2.95
24.52	1.19	24.52	1.76	24.52		24.47	1.85	24.47	2.68	24.47	5.11	24.46	4.06
25.59	1.32	25.59	1.90	25.59	2.36	25.54	2.03	25.54	2.82	25.54	4.49	25.53	3.42
26.66	1.46	26.66	2.11	26.66	2.72	26.61	2.28	26.61	3.38	26.61	5.74	26.60	3.35
27.73	1.75	27.73	2.16	27.73	2.61	27.68	2.56	27.68	3.49	27.68	5.52	27.67	3.66
28.80		28.80	2.36	28.80	2.92	28.75		28.75	3.57	28.75		28.74	3.78
29.88	2.02	29.88	2.52	29.88	3.09	29.82	3.06	29.82	3.82	29.82	6.51	29.81	6.36
30.95	2.36	30.95	2.86	30.95	3.34	30.89	3.26	30.89	4.28	30.89	6.61	30.88	3.85
32.02	2.68	32.02		32.02	3.75	31.96	3.65	31.96		31.96	7.94	31.95	
33.09	2.87	33.09	3.59	33.09	3.93	33.03	3.77	33.03	5.09	33.03	7.38	33.02	4.51
34.16	2.65	34.16	3.97	34.16	4.25	34.10	4.18	34.10	5.23	34.10	8.07	34.09	5.15
35.24	3.34	35.24	4.33	35.24	4.82	35.17	4.45	35.17	5.68	35.17	9.21	35.16	5.42
36.31	3.73	36.31	4.74	36.31	5.46	36.24	5.09	36.24	6.09	36.24	9.23	36.23	4.81
37.38	4.71	37.38	5.27	37.38	5.69	37.30	5.72	37.30	6.22	37.30	8.48	37.30	4.32
38.45	5.01	38.45	5.84	38.45	6.36	38.37	6.23	38.37	6.96	38.37	9.86	38.37	5.18
39.53	5.55	39.53	6.53	39.53		39.44	6.83	39.44	7.59	39.44	9.19	39.44	5.38
40.60	6.16	40.60	7.53	40.60	7.57	40.51	6.53	40.51	7.88	40.51	10.83	40.51	6.18
41.67		41.67	8.11	41.67	8.78	41.58		41.58	8.81	41.58		41.58	6.56
42.74	8.19	42.74	8.79	42.74	8.88	42.65	8.29	42.65	8.64	42.65	11.18	42.65	6.35
43.81	8.20	43.81	9.28	43.81	10.07	43.72	9.63	43.72	10.03	43.72	11.17	43.72	5.89
44.89	8.99	44.89		44.89	10.61	44.79	10.06	44.79		44.79	11.89	44.79	
45.96	9.57	45.96	11.23	45.96	10.26	45.86	10.47	45.86	10.98	45.86	12.75	45.86	6.26
47.03	9.82	47.03	10.99	47.03	10.91	46.93	10.47	46.93	11.19	46.93	12.08	46.93	6.35
48.10	5.08	48.10	10.63	48.10		48.00	10.34	48.00	12.03	48.00	3.61	48.00	6.95

Table A.13: Calculated temperature (T) (°C) and measured respiration rate (Rs) (µg C g^{-1} h^{-1}) for a Te Rapa soil wetted up to 7 different moisture contents (20, 03, 40, 50, 60, 70 and 80%).

Te Rapa																									
20%		30%		40%		50%		60%		70%		80%													
T	Rs	T	Rs	T	Rs	T	Rs	T	Rs	T	Rs	T	Rs												
3.46	0.13	3.46		3.46	0.14	4.39	0.24	4.39		4.09	0.20	4.09													
4.50		4.50	0.21	4.50	0.27	5.41		5.41	0.19	5.12		5.12	0.58												
5.54	0.09	5.54	0.13	5.54	0.31	6.44	0.29	6.44	0.35	6.15	0.32	6.15	1.07												
6.58	0.13	6.58	0.26	6.58	0.31	7.46	0.46	7.46	0.47	7.17	0.43	7.17	1.69												
7.62	0.21	7.62	0.22	7.62	0.36	8.49	0.35	8.49	0.58	8.20	0.37	8.20	1.43												
8.66	0.21	8.66	0.39	8.66	0.44	9.51	0.45	9.51	0.62	9.23	0.55	9.23	1.64												
9.70	0.25	9.70	0.39	9.70	0.53	10.54	0.57	10.54	0.79	10.26	0.78	10.26	2.15												
10.73	0.25	10.73	0.47	10.73	0.63	11.56	0.56	11.56	1.10	11.29	0.89	11.29	1.77												
11.77	0.34	11.77	0.48	11.77		12.58	0.67	12.58	1.12	12.31	1.00	12.31	1.96												
12.81	0.33	12.81	0.48	12.81	0.98	13.61	0.90	13.61	1.21	13.34	1.29	13.34	2.53												
13.85	0.46	13.85	0.75	13.85	0.84	14.63	0.99	14.63	1.33	14.37	1.67	14.37	1.98												
14.89	0.46	14.89	0.72	14.89	0.93	15.66	1.19	15.66	1.76	15.40	1.61	15.40	2.07												
15.93	0.66	15.93		15.93	0.97	16.68	1.25	16.68		16.43	2.12	16.43													
16.97	0.66	16.97	0.79	16.97	0.98	17.71	1.24	17.71	1.77	17.45	2.27	17.45	2.85												
18.01		18.01	0.86	18.01	1.28	18.73		18.73	1.88	18.48		18.48	2.02												
19.05	0.86	19.05	1.66	19.05	1.47	19.75	1.65	19.75	2.08	19.51	2.56	19.51	2.27												
20.09	0.95	20.09	1.51	20.09	1.71	20.78	1.92	20.78	2.31	20.54	3.07	20.54	3.16												
21.13	0.95	21.13	1.25	21.13	1.75	21.80	2.17	21.80	2.29	21.57	2.79	21.57	3.06												
22.17	1.20	22.17	1.69	22.17	1.79	22.83	2.06	22.83	2.30	22.59	3.38	22.59	3.25												
23.21	1.27	23.21	1.78	23.21	2.21	23.85	1.70	23.85	2.99	23.62	3.43	23.62	3.33												
24.25	1.47	24.25	1.83	24.25	2.18	24.88	2.38	24.88	3.18	24.65	3.99	24.65	4.61												
25.29	1.60	25.29	2.29	25.29		25.90	2.69	25.90	3.28	25.68	4.02	25.68	3.93												
26.33	1.78	26.33	2.04	26.33	1.76	26.92	1.28	26.92	3.05	26.71	4.37	26.71	4.60												
27.37	1.77	27.37	2.56	27.37	2.78	27.95	3.02	27.95	3.69	27.74	4.19	27.74	4.86												
28.41	2.04	28.41	2.56	28.41	3.06	28.97	3.25	28.97	3.85	28.76	5.37	28.76	4.03												
29.45		29.45	2.91	29.45	3.46	30.00		30.00	3.99	29.79		29.79	4.87												
30.49	2.27	30.49	2.82	30.49	3.67	31.02	3.76	31.02	4.80	30.82	5.83	30.82	4.66												
31.53	2.50	31.53	3.41	31.53	4.00	32.05	3.93	32.05	4.68	31.85	6.38	31.85	5.04												
32.57	2.83	32.57		32.57	3.81	33.07	4.41	33.07		32.88	6.47	32.88													
33.61	3.44	33.61	4.19	33.61	4.38	34.09	4.55	34.09	5.10	33.90	7.20	33.90	3.58												
34.65	3.75	34.65	4.39	34.65	4.78	35.12	4.70	35.12	5.36	34.93	8.00	34.93	6.89												
35.69	3.68	35.69	4.67	35.69	5.55	36.14	4.89	36.14	6.09	35.96	7.75	35.96	5.89												
36.73	4.36	36.73	5.05	36.73	5.64	37.17	6.15	37.17	6.73	36.99	8.28	36.99	5.90												
37.77	4.40	37.77	5.55	37.77	5.42	38.19	6.29	38.19	6.76	38.02	6.51	38.02	6.44												
38.81	5.18	38.81	6.13	38.81	6.47	39.22	6.87	39.22	7.53	39.04	7.66	39.04	8.24												
39.85	6.01	39.85	6.76	39.85	7.29	40.24	7.55	40.24	8.30	40.07	7.93	40.07	8.04												
40.89	6.25	40.89	7.47	40.89	7.29	41.26	7.41	41.26	8.87	41.10	9.00	41.10	8.71												
41.93		41.93	7.94	41.93	7.29	42.29		42.29	9.46	42.13		42.13	7.44												
42.97	7.31	42.97	8.45	42.97	8.16	43.31	8.81	43.31	10.12	43.16	10.11	43.16	8.59												
44.01	8.43	44.01	8.50	44.01	8.75	44.34	9.58	44.34	10.94	44.18	10.43	44.18	7.35												
45.04	8.57	45.04		45.04	8.60	45.36	7.35	45.36		45.21	10.00	45.21													
46.08	8.74	46.08	9.43	46.08	9.31	46.39	9.42	46.39	11.67	46.24	10.40	46.24	8.02												
47.12	9.12	47.12	9.31	47.12	9.11	47.41	9.82	47.41	10.44	47.27	11.33	47.27	10.68												
48.16	9.27	48.16	10.13	48.16		48.43	8.22	48.43	10.78	48.30	11.12	48.30	11.10												