Temperature and Moisture Sensitivity of Soil Microbial Respiration in Adjacent Irrigated and Non-Irrigated Soils

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Abstract

Irrigation is a commonly used management practice that is crucial for increasing plant growth and production, especially in areas prone to drought such as the Canterbury region of the South Island, New Zealand. Historically, irrigation was thought to increase soil carbon content due to increased production, however recent studies have shown that irrigation causes a loss of soil carbon (C). One possible mechanism for the C loss is an increase in soil microbial respiration under irrigation. The added soil moisture under irrigation releases microbial moisture limitations and enables soil microbes to access more C, therefore increasing respiration and decreasing soil C content. Soil microbial respiration also fluctuates seasonally. Irrigation changes the inherent seasonal effect by increasing soil moisture content during the hottest part of the year, therefore increasing soil microbial respiration rates.

In this thesis soil samples were collected from 13 paired irrigated and non-irrigated sites in Canterbury and two sites at Rangiriri in the Waikato region of the North Island. The sites in Canterbury were sampled once while the sites at Rangiriri were used for a seasonal analysis and were sampled twice. Soil samples were wet to five different moisture contents and incubated on a temperature gradient block for five hours. MMRT curves were then fitted to the respiration data obtained from incubation and the temperature optima (T_{opt}), temperature inflection point (T_{inf}) and change in heat capacity ($\Delta C_p^{\ddagger}$) were calculated. The absolute respiration rates at 10°C (R10) and 20°C (R20) were also calculated.

Irrigation had a significant effect on soil microbial respiration in the Canterbury soils but not the Rangiriri soils. In Canterbury, the T_{opt} and T_{inf} were higher in the irrigated soils by 8.8°C and 7.6°C respectively while the R10 and R20 were both nearly 50% higher in the non-irrigated soils and all differences were statistically significant. There was no difference between treatments in any of the parameters measured in the Rangiriri soils.

The difference in temperature sensitivity and absolute respiration rate in the Canterbury soils was thought to be due to the soil microbes under irrigation decomposing less readily available soil C which has a higher temperature sensitivity and leads to a reduced respiration rate. Another possible explanation for the differences in temperature sensitivity and absolute respiration rate was that there had been a shift in soil microbial community structure between the two treatments and this should be further investigated.

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Chapter 1.

Introduction

1.1 Background

Soil microbial respiration is a critical part of both the soil cycle of carbon (C) stocks and the global C cycle. The amount of organic C in soil is a result of the balance between photosynthesis, which adds C to the soil through plant detritus, and respiration which removes C from the soil through the production of carbon dioxide (CO_2) (Janzen, 2004). This balance is easily disrupted, especially through anthropogenic influences such as the processes involved in agricultural systems. Fertiliser application, harvest, drainage and irrigation are all agricultural management practices that affect the soil C cycle. A recent study by Mudge *et al.* (2017) showed that over 34 sites throughout New Zealand there was nearly 7 t ha^{-1} less C in irrigated soils than in non-irrigated soils. This is a significant difference in C that could be explained by a number of mechanisms including: non-irrigated soils having greater root biomass than irrigated soils; by C leaching from irrigated soil due to the increase in water being applied; by an increase in microbial activity induced by the addition of water or a combination of mechanisms (Mudge *et al.*, 2017). The way that irrigation effects microbial activity is not well known and in a warming world where increasing food demands will lead to increased use of irrigation it is imperative to understand how microbial ecosystems will respond to changes in soil moisture and temperature.

One proxy for microbial activity is soil microbial respiration. The rate of soil microbial respiration is controlled by three main factors: C substrate availability, moisture and temperature (Davidson & Janssens, 2006; Conant *et al.*, 2011). The type of C available to microbes will affect their activity as some types of C, such as plant detritus that has large amounts of lignin, require more energy to break down (Davidson & Janssens, 2006). The amount of moisture in the soil is important in two ways, if there is too little moisture microbes can become dehydrated and their enzyme activity becomes inhibited so they struggle to decompose organic C (Cook & Orchard, 2008). If there is too much moisture present and the soil has become saturated, microbes cannot diffuse oxygen across their cell walls (Cook & Orchard, 2008).

The most important factor controlling soil microbial respiration is temperature. In general, soil microbial respiration rates increase with temperature, to a point- the

temperature optima- after which respiration rates begin to decline. Temperature affects respiration in two ways, intrinsically and extrinsically. Intrinsic temperature effects on microbes directly affect the enzyme kinetics of the microbes while extrinsic effects indirectly affect microbes by influencing C availability from the soil (Davidson & Janssens, 2006).

Seasonality also influences soil microbial respiration (Suseela *et al.*, 2012). Some studies have shown that as temperature increases during summer, so do respiration rates, while the opposite happens in winter (Suseela *et al.*, 2012). Other studies have shown that the temperature increase during summer increases decomposition rates which results in a reduction of readily available, labile C causing reduced respiration rates (Davidson *et al.*, 2000; Kirschbaum, 2013). Soil microbes may also alter their temperature response with season by tuning their metabolism in response to changes in soil temperature (Robinson *et al.*, 2017). Irrigation changes the effect of seasonality by increasing soil moisture content at the warmest time of the year, so determining how irrigation effects soil microbial respiration, especially as seasons change is important.

There have been numerous mathematical equations developed to model soil microbial respiration. Most of these models are based on the Arrhenius equation developed by Arrhenius (1889). This model shows that reaction rate increases exponentially with temperature, which is true for chemical reactions but is not logical for biogeochemical reactions such as microbial respiration. Another model developed by Lloyd and Taylor (1994) is similar to the Arrhenius equation but accounts for the activation energy that microbes need to overcome in order to begin breaking down organic C. This causes the slope of the curve produced by the Lloyd & Taylor equation to be less steep than the curve produced by the Arrhenius equation but the curve continues to increase with temperature. Macromolecular rate theory (MMRT) is a model developed recently that accounts for a temperature optima (T_{opt}), a point at which respiration rates peak and begin to decline (Hobbs *et al.*, 2013; Schipper *et al.*, 2014). Another important piece of information can be derived from MMRT, the temperature inflection point (T_{inf}). This is the temperature at which the respiration curve is steepest and the temperature at which respiration is most sensitive to changes in temperature (Hobbs, *et al.,* 2013; Schipper *et al.,* 2014). These two pieces of information can be used to describe the temperature response of microbial communities. In a warming climate, it is important to know how microbial communities will respond to increases in temperature and the T_{opt} and T_{inf} are crucial in understanding this.

As well as modelling respiration, there have been many methods developed to measure respiration at varying temperatures. Historically these methods involved long periods of incubation where microbes adapted to the change in conditions or incubation at a few different temperatures which made curve fitting difficult. Robinson *et al.* (2017) developed a method of measuring respiration over a range of temperatures in a short, five-hour incubation, therefore minimising the likelihood of adaptation. These methods were used in this thesis.

While the effect of irrigation on soil microbial respiration is not well understood, the few studies that have been undertaken have found that irrigation increases the respiration rate of soil microbes (Sainju *et al.*, 2008; Condron *et al.*, 2014; Smith & Brye, 2014; Trost *et al.*, 2014; Gong *et al.*, 2015),. Moisture also influences microbial community structure as different types of microbes are more resilient to moisture deficits than others (Manzoni *et al.*, 2012; Ma *et al.*, 2015). Microbes in irrigated soil may also adapt to having more moisture at warmer times of the year which could constrain microbes in non-irrigated soil.

This research will compare the temperature sensitivity of microbial respiration between irrigated and non-irrigated soils taken from 13 sites throughout the Canterbury region of the South Island, New Zealand, and from two sites on a farm in Rangiriri in the Waikato region of the North Island, New Zealand, in order to determine whether there is any difference in temperature and moisture sensitivity between the two treatments. Five moisture contents were used in this analysis to mimic a range of moistures the soil could experience throughout the year.

1.2 Aims and Objectives

The overarching aim of this thesis was to investigate whether there is a difference in microbial response to moisture and temperature between irrigated and non-irrigated soil. There are were main objectives for this study:

- To determine differences in temperature and moisture sensitivity of soil microbes between adjacent irrigated and non-irrigated soil.
- To determine whether temperature and moisture sensitivity changed seasonally and with soil type.

The key hypotheses for this thesis were:

- There would be a difference in temperature sensitivity between the irrigated and non-irrigated soils.
- There would be a seasonal effect on the temperature sensitivity of microbial respiration.
- There would be a difference in sensitivity between soil types.

1.3 Thesis layout

Chapter 2 is a review of the literature surrounding irrigation, the factors that affect soil microbial respiration and some of the models used to measure and predict respiration rates and temperature sensitivity.

Chapter 3 contains detailed general methodology for soil sampling, method development, laboratory methods and model fitting.

Chapter 4 presents the data from Canterbury and the discussion relating to the temperature and moisture sensitivity of the Canterbury soils. This chapter has been set out in the format of a paper so that it can be adapted for publication in a peer-reviewed journal. Due to this, there is some repetition from chapters 1-3.

Chapter 5 presents the data from the Rangiriri study and the discussion relating to the seasonal temperature and moisture sensitivity of the Rangiriri soils. This chapter is also set out in the format of a paper and due to this, there is some repetition from chapters 1-3.

Chapter 6 will summarise the main conclusions found in this study and propose ideas for future research.

Chapter 2.

Literature Review

2.1 Irrigation

Irrigation is an important management practice used in farming to increase production. Globally, irrigation is responsible for 40% of food production (UNESCO, 2015) and in New Zealand there is around 740,000 hectares of irrigated land. Over half of this irrigated land is in the Canterbury region of the South Island, New Zealand, where there is over 440,000 hectares of irrigated land, 230,000 of which are dairy farms (Ministry for Primary Industries, 2014). Irrigation is a crucial management practice in the Canterbury region particularly, due to low rainfall (500-700 mm per year) (NIWA, 2012), and free draining, gravelly soils that are dominant in the region (Landcare Research, 2010).

As the world's population is predicted to increase by 2 billion people before 2050 (UNESCO, 2015), the demand for food will increase. Appropriate management of irrigation will become more important to maintain and increase production to meet the demand for additional food.

Historically irrigation was thought to increase soil carbon (C) due to the increased production that occurs following irrigation (Rixon, 1966). Recent studies have shown however, that increased production from irrigation does not result in an increase in soil C (Schipper *et al.*, 2013; Condron *et al.*, 2014). Mudge *et al.* (2017) found that for 34 paired irrigated/non-irrigated sites throughout New Zealand, there was significantly less C in the irrigated soils by nearly 7 t C ha^{-1} in the top 30 cm of soil. Exactly why there is a significant C loss is unknown. One possible mechanism for this C loss is an increase in microbial activity induced by the increased moisture availability from irrigation.

Microbial respiration tends to increase under irrigation respiration (Sainju *et al.*, 2008; Condron *et al.*, 2014; Smith & Brye, 2014; Trost *et al.*, 2014; Gong *et al.*, 2015) due to the increase in moisture enabling microbes to access more organic soil C and releasing microbes of moisture limitations in otherwise dry soils. Rainfall affects soil in a similar way to irrigation by influencing soil moisture content and in studies where rainfall was modified by the use of rainout shelters, respiration decreased with declining

precipitation and increased periods between rainfall events (Harper *et al.*, 2005; Nakano *et al.*, 2008; Talmon *et al.*, 2011).

An increase in microbial activity means an increase in CO_2 production from soil which, as irrigation use increases, could have a significant effect on not only the soil C cycle, but the entire global C cycle.

2.2 Global Carbon Cycle

The global C cycle is an incredibly important biogeochemical cycle that has a large influence on planet earth, in particular the climate. The global C cycle describes the exchange of C between four pools: atmospheric C (CO_2), biota- vegetation and other organisms, soil organic matter and the ocean. Of these four pools, the ocean is the largest, containing 38 000 Pg C, this is followed by the soil organic matter pool which contains 1500 Pg C (Smith, 2008). These four pools of C interact and C is cycled between them (Figure 2.1).

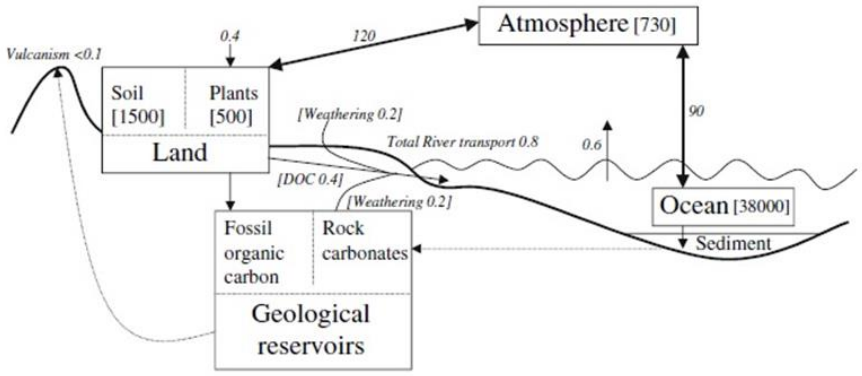


Figure 2.1 Global carbon cycle from Smith (2008) (Pg C)

Atmospheric C is fixed by plants during photosynthesis and converted into organic C which is then turned into soil organic matter through plant detritus and root exudates (Smith, 2008). A proportion of this soil organic matter is then converted back into atmospheric CO_2 by soil microorganisms that decompose organic matter in the soil. Microorganisms use organic C as an energy source and emit CO_2 as they respire (soil microbial respiration).

The global C cycle is incredibly sensitive to changes in flux from any of the four C pools. Currently, the cycle is being disrupted by anthropogenic influences, especially increased atmospheric CO_2 which is increasing in concentration at a rate of 3.3 Pg yr^{-1} (Lal, 2004).

2.3 Soil Carbon Cycle

Organic C is added to the soil through plant and root detritus and is lost from the soil through the production of CO_2 and methane (CH_4) as well as leaching of both dissolved and particulate C compounds (Davidson & Janssens, 2006). There are various pools of organic C (Figure 2.2). These soil C pools can be defined by their mean residence time and how easily accessible the C is to microorganisms in the soil. Davidson and Janssens (2006) describe the three soil C pools based on the Century and RothC models; the fast/ microbial pool, the slow/humified pool and the passive/inert pool. The soil C pool described as 'fast' or 'microbial' is temperature sensitive, has a short residence time and consists of plant detritus that is low in lignin and high in nitrogen (N), such as leaf and root detritus. This pool of soil organic C is easily accessible to soil microorganisms and turns over quickly. At the opposite end of the soil C availability scale the pool of C is described as 'passive' or 'inert' (Figure 2.2). This soil C pool has a long residence time and consists of plant detritus that is high in lignin and low in N, such as tree trunks. The 'passive' or 'inert' C pool is less readily available to soil microbes and takes considerably longer to cycle back to atmospheric CO_2 . This pool of C can also be referred to as recalcitrant C.

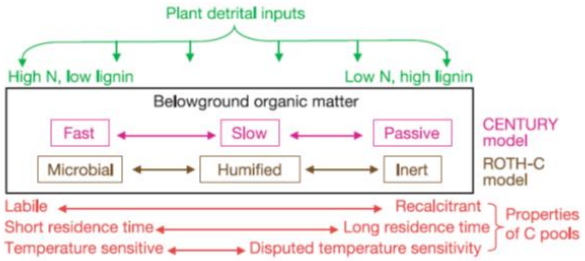


Figure 2.2. Pools of organic soil C as defined by the Century and RothC models (Davidson & Janssens, 2006)

The amount of C in soil is ultimately determined by the balance between photosynthesis and respiration. Microbes in the soil decompose organic C and produce CO_2 and CH_4. Both photosynthesis and respiration are chemical processes that occur within plants and microbes respectively. These chemical processes can be either directly (proximate) or indirectly (distal) influenced by a number of factors, which in turn influences the amount of C in the soil. Distal factors that affect soil C are land management practices such as tillage, harvest and irrigation. The type of tillage undertaken can influence soil C concentration, for example, conventional tillage reduces the amount of organic C in the soil due to soil disaggregation and the exposure of otherwise occluded C to decomposition by soil microbes. Harvest removes C from the soil as aboveground plant matter is removed, therefore reducing both root mass and plant detritus inputs into soil (Nave *et al.*, 2010). As mentioned in section 2.1,

irrigation has been shown to reduce soil C (Mudge *et al.*, 2017) and the mechanisms behind this C loss are not clear. The proximate factors directly influence microbial function and include temperature and moisture (Schipper *et al.*, 2014). In this research soil respiration (CO_2 respired) at different temperatures and soil moisture contents were used to investigate the temperature sensitivity of soil respiration and how temperature and moisture interact to control biochemical processes. The controls of soil microbial respiration are discussed in section 2.4.

2.4 Controls of Soil Microbial Respiration

Soil microbial respiration is a sensitive process that can be influenced by several different factors such as C substrate availability, moisture and temperature. These three factors all influence the chemical reactions taking place inside soil microbes. As well as affecting individual microbes, other factors influence microbial community size and composition, such as tillage and moisture content.

2.4.1 Carbon Substrate Availability

Microbes in soil decompose organic C and use it as an energy source. As mentioned in section 2.3, there are different pools of C in the soil, some more readily available to microbes than others (Davidson & Janssens, 2006). C substrate availability can be influenced by the structure of C present in the soil and also by environmental constraints. Temperature effects on soil microbial respiration are closely linked with C substrate availability and these will be discussed in more detail below.

Plant detritus that is low in lignin is easily accessible for microbes to decompose (Figure 2.2). Lignin is one of the three components that make up wood and is responsible for wood hardness. Microbes in the soil have difficulty decomposing lignin due to its complex structure and the nature of the chemical bonds in lignin molecules (Bi, 2016). Lignin forms compact covalent bonds with other molecules that make it difficult for microbial enzymes to penetrate plant cell walls (Bi, 2016). Plant matter with high lignin has a higher activation energy than plant matter with low lignin, meaning that it takes more energy for the microbes to begin to break molecules down (Conant *et al.*, 2011).

Environmental constraints that influence C substrate availability and therefore soil respiration can take the form of either physical or chemical constraints (Davidson & Janssens, 2006). C can be physically excluded from microbes by becoming protected inside soil aggregates where oxygen concentrations may be low and where microbial enzymes cannot physically access (Davidson & Janssens, 2006).

Chemical occlusion of soil C occurs when C becomes adsorbed to the surface of soil minerals and soil microbes cannot break the covalent or electrostatic bonds between the C and mineral surface (Davidson & Janssens, 2006). Once chemically protected the organic C can no longer be decomposed due to the large amount of energy required to break chemical bonds (Oades, 1988).

2.4.2 Moisture

As soil moisture increases, so does microbial respiration (Cook & Orchard, 2008). When a dry soil becomes wet, the addition of water causes a sudden, large increase in CO_2 production, commonly known as the 'Birch Effect' (Jarvis *et al.*, 2007; Unger *et al.*, 2010). The amount of CO_2 produced then levels out over time (Cook & Orchard, 2008). As the moisture levels in soil decrease, the water in soil pores drains and the film of water that covers soil aggregates thins (Stark & Firestone, 1995). The film of water around the aggregates also becomes more tightly bound to the aggregate making it more difficult for the microbes to access water. When the microbes become water restricted they become dehydrated which inhibits their enzyme activity (Stark & Firestone, 1995). Limited enzyme activity means that the microbes cannot decompose organic matter which can result in death and in a decrease in CO_2 production (Stark & Firestone, 1995). There will also be a point where the soil becomes waterlogged and there is insufficient oxygen available for the microbes to decompose organic matter (Clark & Gilmour, 1983). The important controls of soil moisture content are inputs from rainfall and irrigation (Section 2.1) and losses through drainage and evaporation.

Moisture is also a determining factor of microbial community composition (Drenovsky *et al.*, 2004; Ma *et al.*, 2015) and rainfall manipulation is an effective way to measure how differences in rainfall amount and increased periods between rainfall events influence microbial community composition (Canarini *et al.*, 2016; Zhao *et al.*, 2016). In general, microbes living in soil can be sorted into three groups; soil fauna, bacteria and fungi. Soil fauna can be further divided into soft- and hard-bodied fauna and bacteria can be divided into gram-positive bacteria and gram-negative bacteria. As moisture content changes, the microbes that are active in the soil also change. Fungi are able to be active in both wet and dry soil due to their long hyphae which enable them to access water that would be inaccessible to other microbes and fungi: bacteria ratios are higher in soils with a lower moisture content (Zeglin *et al.*, 2013; Ma *et al.*, 2015). The type of bacteria found in soil also changes with moisture content, in wet soils gram-positive bacteria are dominant while in dry soils, gram-negative bacteria are more prevalent (Ma, *et al.,* 2015).

2.4.3 Temperature

One of the most researched controls of microbial respiration is temperature. As with any chemical reaction, changes in temperature will cause changes in reaction rates. Generally, as temperature increases, so will respiration rates through increased decomposition. An increase in decomposition and respiration means an increase in CO_2 production which poses a serious problem with rising global temperatures (Conant *et al.*, 2011).

Temperature affects microbial respiration in two ways; intrinsically and extrinsically. Intrinsic factors directly affect microbial enzyme kinetics which determine how microbes metabolise C in the soil (Davidson & Janssens, 2006; Schipper *et al.*, 2014). Extrinsic factors indirectly affect microbes through factors that cause organic C to become occluded from microbes through physical and chemical constraints (Davidson & Janssens, 2006).

As mentioned above, intrinsic effects directly affect microbial enzymes involved in the breakdown of soil C. Intrinsic effects refer to the direct response of biochemical reactions in the absence of any other limitations. Soil microbes can only assimilate soluble C that is low in lignin and high in N (Conant, *et al.,* 2011). Plants produce C in various forms that can undergo further breakdown to form C that has an aromatic structure, large molecular weight or C that is insoluble- the recalcitrant pool of C (Figure 2.2). (Sollins *et al.*, 1996; Davidson & Janssens, 2006). These forms of C must then be broken down by extracellular enzymes produced by microbes until they are completely available to the microbes. This breakdown requires a higher activation energy than the breakdown of more labile C and so the decomposition rates of recalcitrant C will be considerably lower than those of less complex, labile C (Davidson & Janssens, 2006). Higher activation energies will, in turn cause the decomposition of the recalcitrant C to have higher temperature sensitivity.

Extrinsic effects refer to the temperature controlling other factors that can alter substrate supply and environmental constraints such as chemical and physical protection, drought, freeze/thaw and flooding. The response of respiration to temperature under these restraints may be much lower than the intrinsic sensitivity of the substrate (Davidson & Janssens, 2006). These factors are discussed in more detail in sections 2.4.1 and 2.4.2.

2.5 Modelling Microbial Respiration

In a changing climate is it imperative to understand how microbial communities will respond to increasing global temperatures and various other environmental stressors such as flooding, drought or application of irrigation waters. Many different theoretical frameworks have been developed in order to model and predict soil microbial respiration and three of these models; the Arrhenius equation, the Lloyd & Taylor equation and the Macromolecular Rate Theory (MMRT) will be discussed in further detail.

2.5.1 Arrhenius

First developed in 1889, the Arrhenius model is one of the simplest explanations of the relationship between temperature and chemical reaction rate. Arrhenius noted that for a reaction to take place, the reactants must first gain a minimum amount of energy to create products; the activation energy (E_A) (Arrhenius, 1889) and so developed the following equation:

$$k = Ae^{-E_A/(RT)}$$

Where k is a rate constant, T is the absolute temperature measured in Kelvin, A is a pre-exponential factor, R is the universal gas constant and E_A is the activation energy for reaction (Arrhenius, 1889). The Arrhenius equation produces reaction rate curves that continue to increase exponentially with temperature (Figure 2.3) due to the fact that as temperature increases, more energy is available to overcome E_A and so more reaction can occur.

The Q_{10} describes the increase in reaction rate for every 10°C. For biological reactions, it is widely accepted that reaction rates double with every 10°C increase in temperature which results in a Q_{10} of 2. The Arrhenius equation describes that at a temperature between 0°C and 30°C and with an activation energy of 50 kJ mol^{-1}, the Q_{10} does equal 2. However, the Arrhenius equation also predicts that the Q_{10} of chemical reactions decreases with increasing temperature due to a relative increase in molecules with sufficient energy to react (Arrhenius, 1889; Davidson & Janssens, 2006).

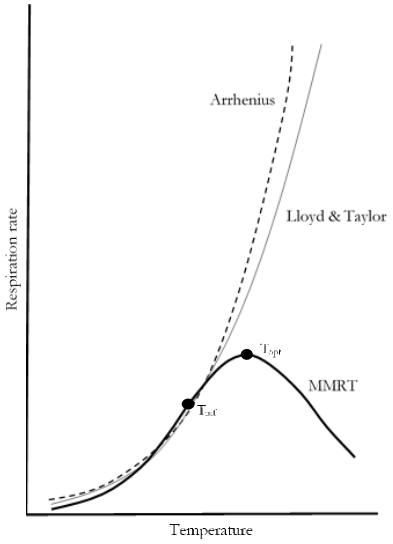


Figure 2.3. Example of Arrhenius, Lloyd & Taylor and MMRT curves. T_{inf} and T_{opt} points are shown on MMRT curve

When applied to microbial respiration, the Arrhenius equation shows that when microbes decompose more recalcitrant C, the E_A is higher as the C is more difficult to decompose and so will have higher temperature sensitivity (Davidson & Janssens, 2006; Sierra, 2012).

2.5.2 Lloyd & Taylor

While the Arrhenius model works well in a chemical setting, it is not always logical in a biological setting. Lloyd and Taylor (1994) acknowledged this and developed a model, based on the Arrhenius equation, that accounted for the fact that soil microbial respiration is influenced by an ever-changing population made up of many different organisms which may have different temperature sensitivities (Figure 2.3). The Lloyd & Taylor equation, shown below, acknowledges that E_A is not constant as described by Arrhenius but decreases with increasing temperature (Lloyd & Taylor, 1994).

$$R = R_{10} e^{E_0\left(\frac{1}{283.15-T_0}-\frac{1}{T-T_0}\right)} = R_{10} e^{308.56\left(\frac{1}{56.02}-\frac{1}{T-227.13}\right)}$$

Where E_0 is 308.56 K and T_0 is 227 K.

This equation is used to calculate respiration at 10°C and fits respiration data well for a range of different soil ecosystems. There is one key factor that both the Arrhenius and Lloyd & Taylor models do not account for, an upper limit to respiration rates known as the temperature optima (T_{opt}) (Figure 2.3).

2.5.3 Macromolecular Rate Theory

Macromolecular Rate Theory (MMRT) is a theoretical framework recently developed by Hobbs *et al.* (2013) and Schipper *et al.* (2014) that accounts for an optimum temperature after which respiration rates begin to decline (Figure 2.3). In the past this decline has been attributed to enzyme denaturation, however enzyme denaturation occurs at far higher temperature than those soil microbes would be exposed to in the field. The decline in respiration rate seen after T_{opt} is instead caused by the heat capacity (C_p) of microbial enzymes (Hobbs *et al.*, 2013; Schipper *et al.*, 2014)

Heat capacity is defined as the temperature dependence of Gibbs Free Energy (G) (Schipper, *et al.,* 2014). As MMRT is an expansion of the Arrhenius equation, the E_A found in both the Arrhenius and Lloyd & Taylor models is substituted by the change in Gibbs free energy between the ground and transition state which can be calculated as the difference between the change in enthalpy for the reaction ($\Delta H^{\ddagger}$) and the change in entropy for the reaction ($\Delta S^{\ddagger}$) (Schipper, *et al.,* 2014). The $\Delta C_p^{\ddagger}$, which is the

difference between the heat capacity in the ground state and the heat capacity in the transition state, defines the temperature dependence of $\Delta G^{\ddagger}$ and so describes the temperature dependence of the reaction rate as shown in the following equation:

$$\ln(k) = \ln\left(\frac{k_B T}{h}\right) - \frac{\left[\Delta H^{\ddagger}_{T_0} + \Delta C^{\ddagger}_p (T - T_0)\right]}{RT} + \frac{\left[\Delta S^{\ddagger}_{T_0} + \Delta C^{\ddagger}_p \ln(T/T_0)\right]}{R}$$

From MMRT, two important pieces of information can be calculated; the temperature optima (T_{opt}), the point on the respiration curve where respiration begins to decrease, and the temperature inflection point (T_{inf}) which is the steepest point on the curve and the temperature at which respiration is most sensitive to changes in temperature (Figure 2.3).

2.6 Determining Temperature and Moisture Sensitivity

The methods used in this thesis were developed by Robinson, *et al.* (2017) to rapidly measure soil respiration over a range of temperatures. Previous incubation trials have highlighted a number of limitations and concerns. The length of incubation is an important factor that can influence the results of incubation trials (Hamdi *et al.*, 2013). Many studies have shown that the type of organic C being decomposed by the microbe's changes throughout a long incubation period (Rey & Jarvis, 2006; Conant *et al.*, 2008; Hamdi *et al.*, 2013). The type of organic C influences respiration as the decomposition of more labile C is less sensitive to temperature than recalcitrant, less readily available C (Conant *et al.*, 2008). Microbes are likely to use the more labile C early in the incubation so towards the end of a long-term incubation recalcitrant C would be available which would then reduce respiration rates.

Soil microbial populations can also adapt to increased temperature if left to incubate over a long period of time (Fissore *et al.*, 2009; Cusack *et al.*, 2012). Thermal adaptation would cause inaccurate results as respiration decreases as the microbes adapt (Bradford *et al.*, 2010) which could be mistaken for respiration decreasing due to temperature. As well as adapting to changes in temperature over long periods, microbial communities can change composition in response to changes in moisture content as different types of microbes are able to be active in different environmental conditions (Drenovsky, *et al.,* 2010).

These problems were reduced in the methods developed by Robinson, *et al.* (2017) by incubating the soil for 5 hours. A five-hour incubation is long enough to obtain

detailed respiration data but not long enough for microbes to adapt to the change in temperature, change community composition, or to use all available labile C.

Another common issue with soil respiration studies is that respiration is only measured at a few temperatures (Bradford *et al.*, 2010; Haddix *et al.*, 2011) which causes issues when fitting curves such as MMRT as just about any curve can fit data with only a few points. This problem is solved in the methods used by incubating soil on a temperature gradient block which ranges in temperature from 5°C to 60°C. Using the temperature gradient block gives at least 20 data points per moisture content per treatment which enables MMRT curves to be fitted accurately.

2.7 Seasonality

Seasonality effects soil microbial activity through changes in both temperature and moisture content. During summer, temperatures are warmer and there is less rainfall. This results in a decrease in soil moisture content through increased evapotranspiration and decreased soil water inputs. Increased temperature in summer results in an increase in microbial respiration (Suseela *et al.*, 2012). In winter, there is increased rainfall and cooler temperatures along with other factors such as freeze/thaw processes which inhibit C substrate availability and moisture availability (Conant, *et al.,* 2011) all of which result in a reduced respiration rate. Other studies, however, found that seasonal changes in temperature cause changes in the amount of labile C available (Davidson *et al.*, 2000; Kirschbaum, 2013). As temperature increases, so do decomposition rates which causes a reduction in readily decomposable labile C and a reduction in respiration rate. Robinson, *et al.* (2017) also found that temperature sensitivity of microbial respiration is partially dependant on the season when samples were collected. They also found that microbes may potentially alter their temperature response with season by tuning their metabolism in response to changes in soil temperature.

Irrigation interferes with the seasonal cycle by increasing soil moisture content at the warmest time of the year and releasing the moisture limitation of microbes in dry soils. Suseela, *et al.* (2012) found that drought conditions reduced microbial respiration rates and temperature sensitivity, especially in autumn and spring.

2.8 Research Needs

The effect that irrigation has on soil C cycling is unclear and has not been extensively investigated. Mudge, *et al* (2017) discovered that over 34 paired irrigated and non-irrigated sites throughout New Zealand there was a significant loss of soil C under irrigation and hypothesised that one reason for this loss was an increase in microbial activity induced by the added moisture from irrigation.

Soil microbial respiration was used as a proxy for microbial activity and paired irrigated and non-irrigated soils from Canterbury and the Waikato were incubated across a temperature gradient of 5°C to 60°C and at five moisture contents (80%MWHC, 65%MWHC, 50%MWHC, 35%MWHC, 20%MWHC). The data obtained was used to calculate T_{opt}, T_{inf}, $\Delta C_p^{\ddagger}$ and the respiration rates at 10°C (R10) and 20°C (R20) which were then compared between treatments and moisture contents to determine if there was any significant difference in microbial activity between irrigated and non-irrigated soils and whether an increase in microbial activity corresponds to the loss of soil C in soils under irrigation.

Chapter 3.

Methods

3.1 Method Overview

This chapter describes the general methodology used to carry out this investigation. Soil samples were collected from 13 sites in the Canterbury region of the South Island, New Zealand and two sites at Rangiriri in the Waikato region of the North Island, New Zealand. These samples were collected from irrigated areas and adjacent non-irrigated areas at each site. Prior to analysis the soils were moisture adjusted and left to pre-incubate for 24 hours at 20°C. Following pre-incubation, the samples were analysed using the methods described by Robinson *et al.* (2017). The respiration rates for this analysis were used to fit the MMRT curve to in order to calculate the T_{opt} and T_{inf} points. Chapter 4 presents the results and discussion relating to the temperature and moisture sensitivity of the soils from the Canterbury region while Chapter 5 gives an account of the Rangiriri seasonal sampling.

3.2 Sampling Methods

Soil samples were taken in the field using a bucket sampler. The bucket sampler was pushed into the soil and pulled out again, taking a small core from the top 10 cm of soil. Following collection, the samples were placed in a chilly bin with ice and transported back to Waikato University where they were sieved to 2 mm before being transferred to the fridge for storage at 4°C. Sample collection was done differently at Canterbury and Rangiriri so sampling methods for each are described in Chapter 4 and Chapter 5 respectively.

3.3 Method Development

In the method developed by Robinson *et al.* (2017), the soil was moisture adjusted and left to pre-incubate at 20°C before being analysed on the temperature gradient block. The soils were left to settle for a week as there is generally a 'flush' of CO_2 produced from the soil following the addition of moisture. This is known as the "Birch Effect". Leaving the samples to adjust for this period reduces the influence of this increase in CO_2 following wetting.

This research aimed to determine the effect that irrigation has on CO_2 production which meant that the effect of pre-incubation length on CO_2 production needed to be determined. In order to do this, two Canterbury sites were chosen (sites 16 and 24) that had large differences in moisture contents between irrigated and non-irrigated treatments and pre-incubation trials were carried out.

To determine the appropriate pre-incubation length of time soil from site 24 was used and the first trial consisted of three repetitions of each treatment. Three bags containing 30 g of soil were weighed out and moisture adjusted to 65% MWHC (method outlined in section 3.6.2) with 3.24 g of water added to the irrigated soil and 11.96 g of water added to the non-irrigated soil.

Following moisture adjustment, the soils were left to pre-incubate for three different lengths of time; 24 hours, 72 hours and one week. After pre-incubation, the soils were analysed using the methods outlined in the general methodology section (section 3.5) but using eight tubes across the block for trial one and 12 tubes across the block for trial two. MMRT curves were then fitted to the data and statistical analysis was carried out. It was determined that there was very little difference between the 24 and 72 hour pre-incubations but that that one-week pre-incubation yielded lower CO_2 concentrations. It was then decided that the 24-hour pre-incubation was the most appropriate pre-incubation period due to practicality and time restraints, so the 24-hour pre-incubation was used for the main analysis.

During the pre-incubation trials, it was noted that respiration data above 52°C was variable and unreliable. Robinson, *et al.* (2017) also found that respiration data above 50°C was variable and therefore constrained their analysis to below 50°C. It is likely that there are unknown biogeochemical reactions occurring above 50°C that interfere with microbial respiration rate measurement. Soil in the field is highly unlikely to reach such high temperatures and so the decision was made to exclude respiration data above 52°C in this study.

3.4 Temperature Gradient Block Description

The key piece of laboratory equipment used for this research was the temperature gradient block (Figure 3.2). A common limitation to soil microbial research is the inability to measure respiration over a range of temperatures which results in insufficient data to accurately fit respiration curves. The temperature gradient block overcame these limitations by allowing analysis of soil microbial respiration over a large temperature gradient, in this case from 2°C to 60°C increasing in approximately 1°C increments. The temperature gradient block was constructed from aluminium and had three rows of 44, 20 mm holes drilled at 10 mm intervals. A water bath was connected at one end which pumped antifreeze through the end of the block to cool it. At the other end of the block a heater was connected which heated the block. The combination of the cold-water bath and the heater created a temperature gradient throughout the block. There were temperature thermistors located in seven holes across the block which measured temperatures throughout incubation which ensured the temperature gradient was linear and stayed stable for the incubation period. The top of the block was covered with Perspex glass and the sides of the block were insulated with polystyrene to help maintain stability in the temperature gradient.

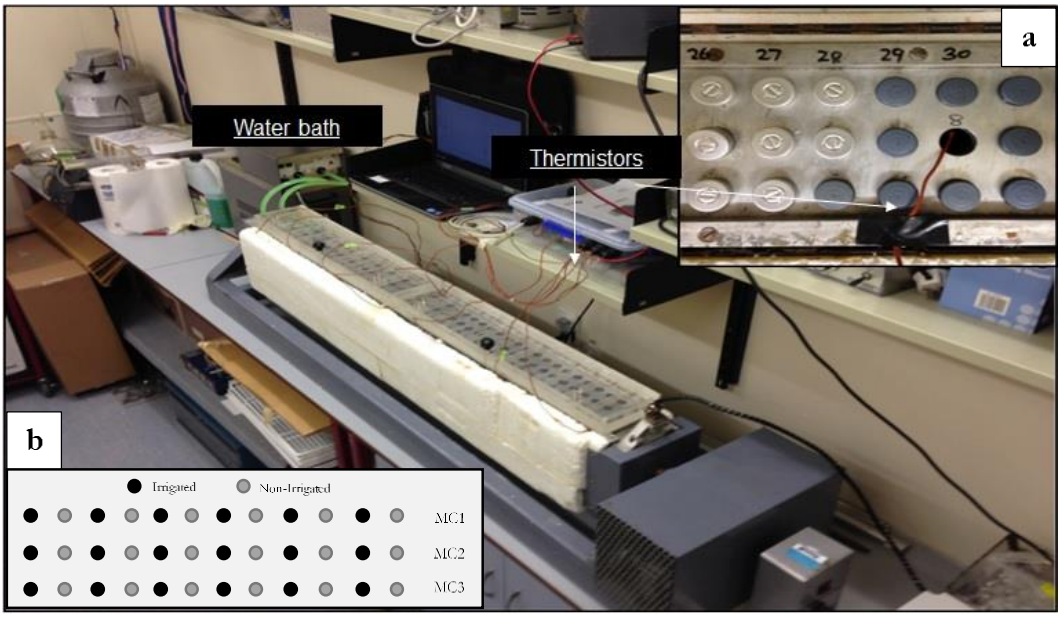


Figure 3.1. Temperature gradient block. Inset a: Hungate tubes and thermistors. Inset b: Layout of tubes in the temperature gradient block per treatment and moisture content. Image modified from Robinson (2016).

3.5 General Methodology

Prior to analysis, soil samples were sieved and the field moisture content and maximum water holding capacity were determined following methods outlined below

3.5.1 Sieving

Within 24 hours of collection the soil was sieved to 2 mm to remove any large particles, stones or plant matter. The sieved soil was then placed in a plastic bag and stored at 4°C until it was analysed.

3.5.2 Field Moisture Content

To determine the field moisture content of the soil a small aluminium pie tin was weighed empty and the weight was noted. A teaspoon of sieved soil was then placed in the pie tin and the tin and soil were reweighed. The tin and soil were then placed in an oven set to 105°C overnight. The following morning the tin and soil were weighed again and the field moisture content was calculated using the following calculation:

$$MC = \frac{(tin + fresh) - (tin + dry)}{(tin + dry) - tin}$$

Where tin is the weight of the tin, fresh is the weight of the soil before drying, dry is the weight of the soil following drying and MC is the moisture content. The soil was weighed in the tin before and after drying.

The moisture factor was then determined using the following equation:

$$MF = 1 + \frac{MC}{100}$$

Where MC is the moisture content and MF is the moisture factor.

3.5.3 Maximum Water Holding Capacity

To calculate the maximum water holding capacity (MWHC) of the soil, glass funnels were set up with rubber tubes attached to their stems. A small amount of cotton wool was placed in the opening of the neck of each funnel which was then dampened with distilled water. Sieved soil was then placed on top of the glass wool and gently compacted into place. The soil was then thoroughly wet with distilled water with the rubber tube on the stem of the funnel left open. Once the water had drained through the soil the rubber tubes were blocked using a bulldog clip and the soil was saturated

with distilled water again. Water was added until there was standing water on top of the soil and the stem of the funnel was filled with water. The top of the funnel was then covered with tin foil to prevent evaporation and the soil was left to soak overnight. The following day the moisture content of the soil was determined using the methods outlined in section 3.5.2. The MWHC of the soil is the moisture content at which the soil is saturated and cannot hold any more water.

3.5.4 Moisture Adjustment and Pre-Incubation

Prior to respiration analysis the soils were moisture adjusted and pre-incubated for 24 hours at 20°C (Section 3.3). The moisture contents were calculated as a percentage of the maximum water holding capacity (%MWHC) and there were five moisture contents used for this analysis: 80%MWHC, 65%MWHC. 50%MWHC, 35%MWHC and 20%MWHC. This ensured a wide range of moistures that the soil was likely to experience throughout the year as well as a wide range of temperatures.

The way in which the soil moisture was adjusted depended on the field moisture content of the soil and the %MWHC being prepared. If the soil had a field moisture content of less than the %MWHC being prepared, then the soil had water added to it but if the field moisture content was higher than the %MWHC being prepared then the soil needed to be dried down. The MWHC and field moisture content were calculated using the methods in section 3.5.2.

If the soil required water to be added to reach the desired %MWHC then 75 g of soil was weighed into a plastic bag in a container on the scales. The scales were then set to zero and water was added using a spray bottle until the correct amount of water had been added. The calculation for the amount of water needed is shown below:

$$\left(\frac{weight\ of\ soil}{field\ MF \times target\ MF}\right)$$

Where the 'weight of soil' is the amount weighed out to wet up, so 75 g, the 'field MF' is the field moisture factor of the soil and the 'target MF' is the moisture factor of the desired %MWHC being prepared. Once the soil was wet to the desired %MWHC the plastic bag was sealed with non-absorbent cotton wool to prevent moisture loss and placed in a temperature controlled room at 20°C for 24 hours to pre-incubate.

If the soil needed to dry to reach the desired %MWHC then the following formula was used to calculate the amount of soil needed to reach the desired %MWHC once the soil has dried to 75 g:

$$\left(\frac{weight\ of\ soil}{field\ MF} \times target\ MF\right) - weight\ of\ soil$$

Where the weight of soil is the weight the soil once it had dried to the right %MWHC, 75 g, the field MF is the field MF of the soil and the target MF is the moisture factor of the desired %MWHC. The soil was then left to dry at 4°C until it had dried to the desired %MWHC and was then placed in a plastic bag which was sealed with non-absorbent cotton wool in order to prevent moisture loss. The drying of the soil was timed so that the soil was dry in time to be pre-incubated for 24 hours at 20°C in a temperature controlled room before being run on the temperature gradient block.

3.5.5 Temperature Gradient Block Methods

The temperature gradient block for incubating soil (Figure 3.1) was turned on the night before analysis to ensure a stable temperature gradient between 5°C at the cold end and 60°C at the hot end. Thermistors were evenly spaced along the block to measure and record 7 temperatures which allowed for calculation of a discrete temperature at each tube hole.

A laptop was connected to the block to record thermistor temperatures and temperature gradient across the block.

Following pre-incubation soil samples were weighed into labelled Hungate tubes at 3 g (± 0.05 g) per tube. There were 20 tubes of irrigated soil and 20 tubes of non-irrigated soil per moisture content. The temperature gradient block had 132 holes in total with seven of the holes containing thermistors which meant that up to three moisture contents could be incubated on the block at once.

Once the soil was weighed out, the tubes and soil were transported to the temperature gradient block. The tubes were capped using a rubber stopper and an aluminium cap that was crimped around the top of the tube in order to prevent gas leakage. The tubes were capped individually immediately prior to being placed in the block. Three empty tubes were capped and placed in the block as blanks.

Irrigated tubes from one moisture content were placed in the top row of the block first, starting at the cold end of the block and the time noted when the first tube was

placed. A space was left between each of the irrigated tubes and the non-irrigated tubes from the same moisture content were placed in the gaps which meant the tubes were placed with treatments alternating (Figure 3.1). This was then repeated for each moisture content on each row of the block. Once all the tubes had been placed the Perspex lid was closed tightly to prevent fluctuations in temperature and the tubes were left to incubate for five hours from when the first tube was placed

3.5.6 Infrared Gas Analyser Methods

After five hours of incubation the headspace of each tube was sampled for CO_2 using labelled 1 mL insulin syringes. Gas samples were taken in the order that they were originally placed. The syringe was then inserted into a large rubber bung for transport to the infra-red gas analyser (IRGA). Robinson *et al.* (2017) determined that this was a sufficient way to transport the syringes and no leakage occurred. This was repeated for each tube with individual syringes.

A standard curve was produced for the IRGA using a 1% CO_2 standard before and after the gas samples were injected. The insulin syringes were inserted into the IRGA in the same order sampled from the block and the CO_2 concentrations from each tube were read by the computer as peaks heights in mV.

3.5.7 Respiration calculations

Respiration rates (Rs) were calculated using the peak heights produced in MATLAB. Rs was determined using the following calculation:

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

Where Rs is the respiration rate in μL CO_2 g soil^{-1} hr^{-1}, H_s is the peak height of the sample (mV), V_i is the injection volume (mL), H_{st} is the height of the standard (mV), H_b is the peak height of the blank (mV), S is the CO_2 concentration of the standard, either 1% or 2% CO_2 per mL of gas, V is the headspace volume of the Hungate tube (mL), ODW is the oven dry weight of the soil being analysed (g) and t is the length of time the soil was incubated for (hr). This calculation was completed individually for each sample and resulted in respiration curves for the temperature gradient.

3.6 Macromolecular Rate Theory Methods

Respiration data was fitted with Macromolecular Rate Theory (MMRT) and the temperature gradient data measured by the thermistors on the block. The temperature optima (T_{opt}) and temperature inflection point (T_{inf}) were calculated using the calculations below:

$$T_{opt} = \frac{\left(\Delta H^{\ddagger} - \left(\Delta C_p^{\ddagger} \times 280\right)\right)}{\left(\left(-\Delta C_p^{\ddagger}\right) - 8314\right)} \qquad T_{inf} = \frac{T_{opt}}{1 + \left(2.883 \div \left(\sqrt{-\Delta C_p^{\ddagger}}\right)\right)}$$

Where $\Delta H^{\ddagger}$ is the change in enthalpy and $\Delta C_p^{\ddagger}$ is the change in heat capacity.

Bootstrapping was also carried out during the fitting of MMRT curves. The curve was fitted 1000 times and the best fit was used to calculate T_{opt} and T_{inf}. If there was no fit, then the bootstrapping was increased to 5000. If there was still no fit, the data was noted as a non- fit.

3.7 Statistical Analysis

Statistical differences T_{opt}, T_{inf}, $\Delta C_p^{\ddagger}$, R10 and R20 between irrigated and non-irrigated treatments, soil type and length of irrigation were tested using standard two-way analysis of variance (ANOVA) methods.

Chapter 4.

Temperature and Moisture Sensitivity Soil Microbial Respiration in Adjacent Irrigated and Non-Irrigated Soil

4.1 Abstract

Irrigation is a crucial management practice used to increase plant growth and production, especially in areas of low rainfall such as the Canterbury region of the South Island, New Zealand. Recent studies have shown, however, that irrigation causes a significant reduction in soil C stocks. One possible mechanism for this loss is an increase in microbial activity due to the added moisture from irrigation. Here, I analysed soils collected from adjacent irrigated and non-irrigated areas at 13 sites throughout the Canterbury region to determine if there was a difference in temperature and moisture sensitivity of microbial respiration between the two treatments.

Soils were wet to five different moisture contents and incubated for five hours on a temperature gradient block (~5 to 60°C) to assess the pattern of respiration over various temperatures and moisture contents. The respiration data collected following incubation was fitted with MMRT to calculate T_{opt} and T_{inf} and the absolute respiration rates at 10°C (R10) and 20°C (R20) were also calculated. The T_{opt} and T_{inf} were, on average, 8.8°C and 7.6°C higher in the irrigated soil respectively while the non-irrigated soil had a higher R10 by 43% and a higher R20 by 48%. All differences were statistically significant, therefore irrigation had a significant effect on soil microbial activity. It is likely that the reduced C stocks in irrigated soils resulted in soil microbes accessing more difficult to decompose, recalcitrant soil C, resulting in reduced respiration rates in irrigated soil. A change in microbial community composition is a likely explanation for the differences in T_{opt}, T_{inf}, R10 and R20 as soil moisture content is a major controlling factor of soil microbial community composition.

4.2 Introduction

Soil microbial respiration is a critical part of both the soil and global carbon (C) cycles. The amount of organic C in soil is a result of the balance between photosynthesis and respiration (Davidson & Janssens, 2006). This balance is easily disrupted, especially through anthropogenic influences such as agricultural management practises, e.g. irrigation. Irrigation increases production by controlling water uptake by roots at critical growing periods or during times of unreliable natural rainfall in semi-arid regions. While irrigation increases production, the effects of irrigation on the soil C cycle is not well known. Historically, irrigation was thought to increase soil C, however, recent studies have shown that irrigation causes a loss of soil C (Schipper *et al.*, 2013; Condron *et al.*, 2014). Mudge, *et al.* (2017) found that at 34 sites throughout New Zealand there was nearly 7 t ha^{-1} less C in irrigated soils than in non-irrigated soils. One of the main mechanisms postulated for the difference in soil C was a shift in microbial activity induced by the addition of water from irrigation during periods when moisture would normally limit microbial activity (Mudge, *et al.* 2017).

The effect of irrigation on microbial activity and specifically the cycling of soil C is also not well known and in a warming world where increasing food demands will lead to increased use of irrigation it is imperative to understand how microbial communities will respond to changes in soil moisture and temperature. Microbial activity is controlled by three main factors: C substrate availability, moisture and temperature (Davidson & Janssens, 2006). Some types of C are more readily available to microbes than others (Sollins, *et al.* 1996, Davidson & Janssens, 2006, Conant, *et al.* 2011). Soil C is often categorised as being in three different pools which are defined by how easily they are accessed by soil microbes. The labile C pool is the most readily available pool of C and is made up of plant detritus that is low in lignin and high in nitrogen (N). The labile C pool is thought to be the least affected by physical or chemical protection. The second pool of C is the passive pool which is an intermediate pool of C that is less readily available to microbes and the third is the recalcitrant pool of C which is made up of plant detritus that the most physically or chemically protected and therefore the C is not readily available to soil microbes (Davidson & Janssens, 2006).

Soil moisture content controls C decomposition in two ways; if there is too little moisture microbes can become dehydrated and if there is too much moisture present then the soil becomes saturated, reducing oxygen supply. Soil moisture is also

important for control of C substrate availability as moisture films on soil aggregates are important for allowing soil C to diffuse to organisms (Cook, *et al.* 2008).

While the effect of irrigation on soil microbial respiration is not well understood, the few studies that have been undertaken have found that irrigation generally increases the respiration rates of soil microbes (Sainju *et al.*, 2008; Condron *et al.*, 2014; Smith & Brye, 2014; Trost *et al.*, 2014; Gong *et al.*, 2015). Moisture also influences microbial community structure as some types of microbes are more resilient to moisture deficits than others (Manzoni *et al.*, 2012; Ma *et al.*, 2015). Microbes in irrigated soil may also adapt to having more moisture at warmer times of the year when dry conditions would constrain microbes in non-irrigated soils.

The other key controlling factor of soil microbial respiration is temperature. Temperature change affects microbes both directly, through physiological changes relating to enzyme kinetics and a microbes' ability to metabolise C; and indirectly through factors that affect the availability of organic C to microbes e.g. physical and chemical occlusion (Davidson & Janssens, 2006). In general, soil microbial respiration rates increase with temperature, to a point, known as the temperature optima, after which respiration rates begin to decline. Our ability to predict how increased irrigation will affect soil C stocks depends on understanding the temperature dependence of respiration.

Many different models have been developed to model temperature dependence of microbial respiration including the Arrhenius equation, Lloyd and Taylor model and more recently Macromolecular Rate Theory (MMRT) (Figure 4.1). MMRT is a theoretical framework developed recently by Schipper, *et al.* (2014), based on thermodynamics (Hobbs *et al.*, 2013), that explicitly includes a temperature optima (T_{opt}), a point at which respiration rates peak and begin to decline. Other important pieces of information can be derived from MMRT such as the temperature inflection point (T_{inf}) and the change in heat capacity ($\Delta C_p^{\ddagger}$). The T_{inf} is the temperature at which the respiration curve is steepest and the temperature at which respiration is most sensitive to changes in temperature.

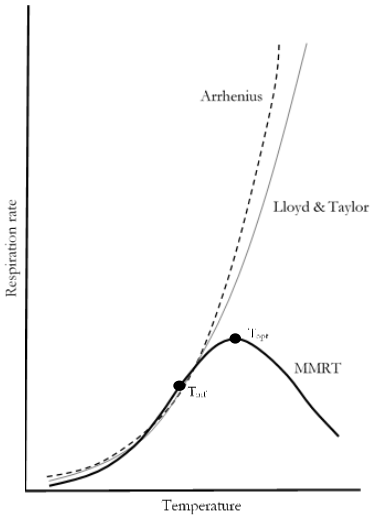


Figure 4.1 Example of Arrhenius, Lloyd & Taylor and MMRT curves. T_{inf} and T_{opt} points are shown on MMRT curve

The $\Delta C_p^{\ddagger}$ is the change in heat capacity during enzymic reactions and relates to the temperature dependence of enzyme reactions (Hobbs *et al.*, 2013; Schipper *et al.*, 2014).

Here I compared the temperature sensitivity of microbial respiration of soil collected from 13 paired irrigated and non-irrigated sites in the Canterbury region. The aim was to determine whether there was any difference in the temperature and moisture sensitivity of respiration between the two treatments. The methods used were developed by Robinson, *et al.* (2017) and this research builds on these proven methods. It was hypothesised that the loss of soil C under irrigation shown by Mudge, *et al.* (2017) can be attributed to differences in temperature and moisture sensitivity of microbial respiration; whereby the moisture limitation is removed in hotter periods in the irrigated soils and microbes are able to be active for longer periods resulting in a decline in soil C stocks.

4.3 Methods

4.3.1 Site Description

Soils were collected from 13 paired irrigated and dryland pasture sites in the Canterbury region. (Figure 4.2, Table 4.1). Four soil types were sampled; Pallic, Gley, Recent and Brown soils. The length of irrigation varied from 3 years to 20 years (Table 4.1). The sites were sampled in November of 2015 after irrigation had commenced. All sites were grazed by dairy cattle. The mean annual temperature for the sites ranged between 10.0°C and 11.9°C and the mean annual precipitation was between 632 mm and 1002 mm (Mudge *et al.*, 2017).

Figure 4.2 Map of New Zealand adapted from Mudge, *et al.* (2017) showing sites in the Canterbury region of the South Island, New Zealand

Each paired site consisted of an irrigated and unirrigated area within the same paddock. Soils were sampled from two replicate 10 m by 10 m sampling areas randomly located within each treatment area. A bucket sampler was used to take 25 soil cores (0-0.1 m) from within each 10 m by 10 m sampling area with the cores then bulked into one sample. Following collection, the samples were sieved to 2 mm. Soils from the two replicate 10 m by 10 m sampling areas at each paired site were then subsampled, combined into one sample and stored at 4°C until required.

4.3.2 General Methodology

The methods used to measure microbial respiration were developed by Robinson, *et al.* (2017) and are summarised below.

Prior to respiration analysis the soils were moisture adjusted and pre-incubated for 24 hours at 20°C (Chapter 3, Section 5). The moisture contents were calculated as a percentage of the maximum water holding capacity (%MWHC) and there were five moisture contents used for this analysis: 80%MWHC, 65%MWHC. 50%MWHC,

35%MWHC and 20%MWHC. This ensured a wide range of moistures that the soil was likely to experience throughout the year as well as a wide range of temperatures.

The temperature gradient block for incubating soil (Figure 4.2) was turned on the night before analysis to ensure a stable temperature gradient between 5°C at the cold end and 60°C at the hot end. Thermistors were evenly spaced along the block to measure and record 7 temperatures which allowed for calculation of a discrete temperature at each tube hole.

A laptop was connected to the block to record thermistor temperatures and temperature gradient across the block.

Following pre-incubation soil samples were weighed into labelled Hungate tubes at 3 g (± 0.05 g) per tube. There were 20 tubes of irrigated soil and 20 tubes of non-irrigated soil per moisture content. The temperature gradient block had 132 holes in total with seven of the holes containing thermistors which meant that up to three moisture contents could be incubated on the block at once.

Once the soil was weighed out, the tubes and soil were transported to the temperature gradient block. The tubes were capped using a rubber stopper and an aluminium cap that was crimped around the top of the tube in order to prevent gas leakage. The tubes were capped individually immediately prior to being placed in the block.

Table 4.1. Soil type and irrigation length (IL) of 13 paired irrigated and non-irrigated sites from the Canterbury region of the South Island, New Zealand from Mudge, *et al.* (2017).

Site	Soil Type	IL (Years)
Site 1 I	Pallic	20
Site 1 NI	Pallic	-
Site 2 I	Recent	6
Site 2 NI	Recent	-
Site 4 I	Gley	10
Site 4 NI	Gley	-
Site 6 I	Brown	10
Site 6 NI	Brown	-
Site 7 I	Brown	15
Site 7 NI	Brown	-
Site 9 I	Brown	20
Site 9 NI	Brown	-
Site 10 I	Recent	12
Site 10 NI	Recent	-
Site 11 I	Recent	12
Site 11 NI	Recent	-
Site 13 I	Gley	3
Site 13 NI	Gley	-
Site 15 I	Recent	5
Site 15 NI	Recent	-
Site 16 I	Pallic	9
Site 16 NI	Pallic	-
Site 18 I	Pallic	10
Site 18 NI	Pallic	-
Site 24 I	Recent	19
Site 24 NI	Recent	-

Irrigated tubes from one moisture content were placed in the top row of the block first, starting at the cold end of the block and the time noted when the first tube was placed. A space was left between each of the irrigated tubes and the non-irrigated tubes from the same moisture content were placed in the gaps which meant the tubes were placed with treatments alternating (Figure 4.3). This was then repeated for each moisture content on each row of the block. Once all the tubes had been placed the

Perspex lid was closed tightly to prevent fluctuations in temperature and the tubes were left to incubate for five hours from when the first tube was placed.

After five hours of incubation the headspace of each tube was sampled for CO_2 using labelled 1 mL insulin syringes. Gas samples were taken in the order that they were originally placed. The syringe was then inserted into a large rubber bung for transport to the infra-red gas analyser (IRGA). Robinson *et al*, (2017) determined that this was a sufficient way to transport the syringes and no leakage occurred. This was repeated for each tube with individual syringes.

A standard curve was produced for the IRGA using a 1% CO_2 standard before and after the gas samples were injected. The insulin syringes were inserted into the IRGA in the same order sampled from the block and the CO_2 concentrations from each tube were read by the computer as peaks heights in mV.

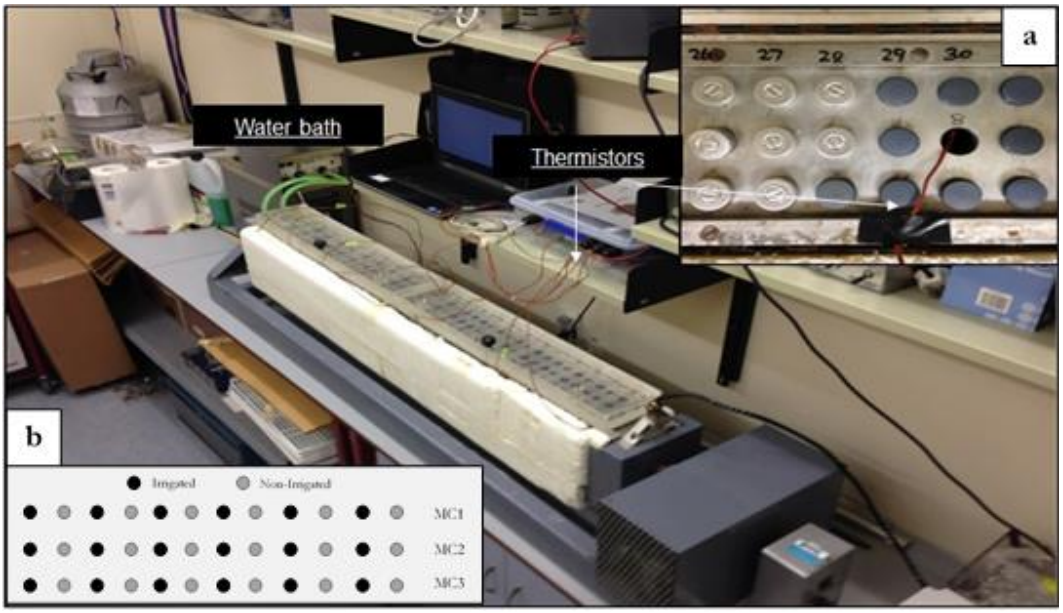


Figure 4.3 Temperature gradient block. Inset a: Hungate tubes and thermistors. Inset b: Layout of tubes in the temperature gradient block per treatment and moisture content. Image modified from Robinson, (2016)

4.3.3 Data Analysis

Respiration rates (Rs) were calculated using the peak heights produced in MATLAB. Rs was determined using the following calculation:

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

Where Rs is the respiration rate in μL CO_2 g $soil^{-1}$ hr^{-1}, H_s is the peak height of the sample (mV), V_i is the injection volume (mL), H_{st} is the height of the standard (mV), H_b is the peak height of the blank (mV), S is the CO_2 concentration of the standard, either 1% or 2% CO_2 per mL of gas, V is the headspace volume of the Hungate tube (mL), ODW is the oven dry weight of the soil being analysed (g) and t is the length of time the soil was incubated for (hr). This calculation was completed individually for each sample and resulted in respiration curves for the temperature gradient. Data analysis was undertaken on respiration data to 52°C (Chapter 3, Section 3).

Rs data was fitted using MMRT and the temperature gradient data from the block obtained from the seven thermistors in the block which recorded temperature throughout incubation. The temperature optima (T_{opt}) and temperature inflection point (T_{inf}) were calculated using the calculations below:

$$T_{opt} = \frac{\left(\Delta H^{\ddagger} - \left(\Delta C_p^{\ddagger} \times 280\right)\right)}{\left(\left(-\Delta C_p^{\ddagger}\right) - 8314\right)} \qquad T_{inf} = \frac{T_{opt}}{1 + \left(2.883 \div \left(\sqrt{-\Delta C_p^{\ddagger}}\right)\right)}$$

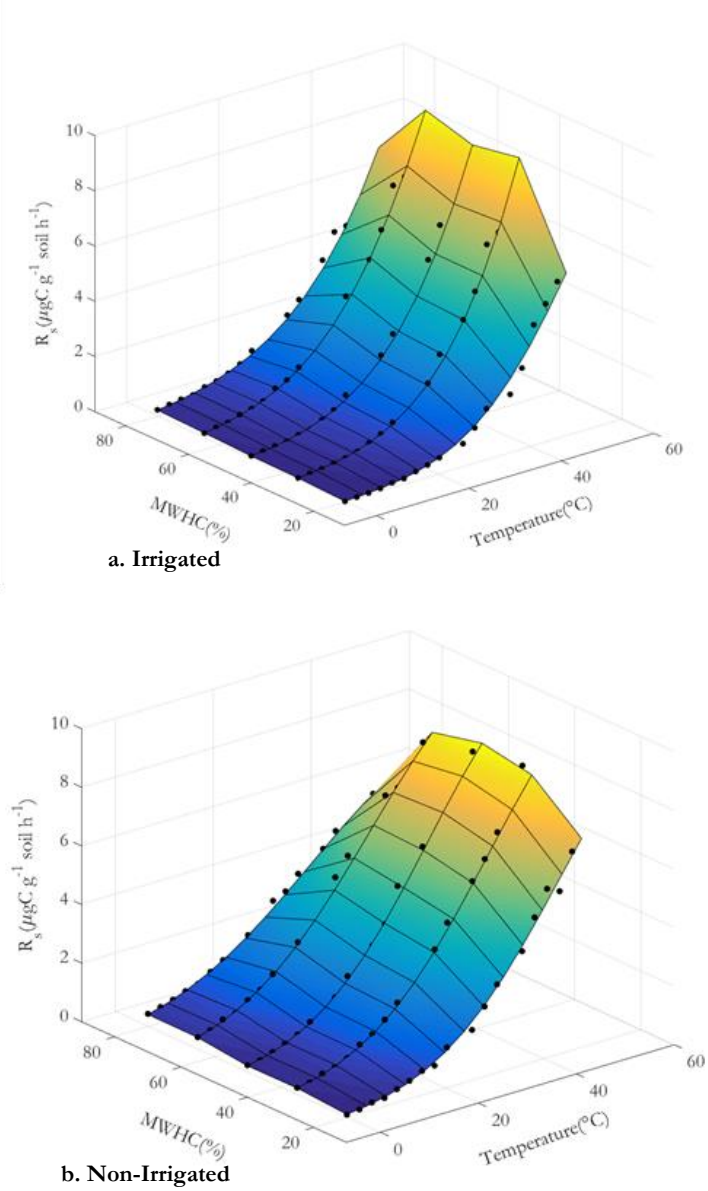
Where $\Delta H^{\ddagger}$ is the change in enthalpy and $\Delta C_p^{\ddagger}$ is the change in heat capacity.

Bootstrapping was also carried out during the fitting of MMRT curves. The curve was fitted 1000 times and the best fit was used to calculate T_{opt} and T_{inf}. If there was no fit, then the bootstrapping was increased to 5000. If there was still no fit, the data was noted as a non- fit. Following MMRT curve fitting, 3-D temperature-moisture webs were drawn. To create these webs, MMRT was fitted to the temperature response at each moisture content and then a surface created by a loess interpolation was fitted between curves at each moisture contentR10 and R20 were calculated using the respiration rate at the closest temperature to 10°C and 20°C as there was some very slight temperature fluctuation on the block. Statistical differences T_{opt}, T_{inf}, $\Delta C_p^{\ddagger}$, R10 and R20 between irrigated and non-irrigated treatments, moisture content, soil type and length of irrigation were tested using standard two-way analysis of variance (ANOVA) methods.

4.4 Results

4.4.1 Microbial Respiration

A 3-D web to visualise the average effect of temperature on respiration rates at different moisture contents is shown for irrigated and non-irrigated soil across 13 Canterbury sites (Figure 4.4 a and b). Respiration rate increased with temperature and varied with soil moisture content. Respiration rates were low at low temperatures and steadily increased with increasing temperature. Respiration is generally lowest at 20%MWHC and 80%MWHC.



a. Irrigated

b. Non-Irrigated

Figure 4.4 Averaged respiration response (Rs) to increasing temperature for 13 paired irrigated (a) and non-irrigated (b) soils set at different moisture contents (%MWHC). MMRT was fitted to each temperature response curve and the surface created by a loess interpolation between curves.

In order to demonstrate differences in overall respiration rates and curvature of MMRT fit, one moisture content (65% MWHC) was selected for display (Figure 4.5, Figure 4.6). Figure 4.6 shows the same data as Figure 4.5 but replotted using the natural log scale (ln) to highlight differences in curve shape at low temperatures. Graphs of the remaining four moisture contents can be found in the appendices.

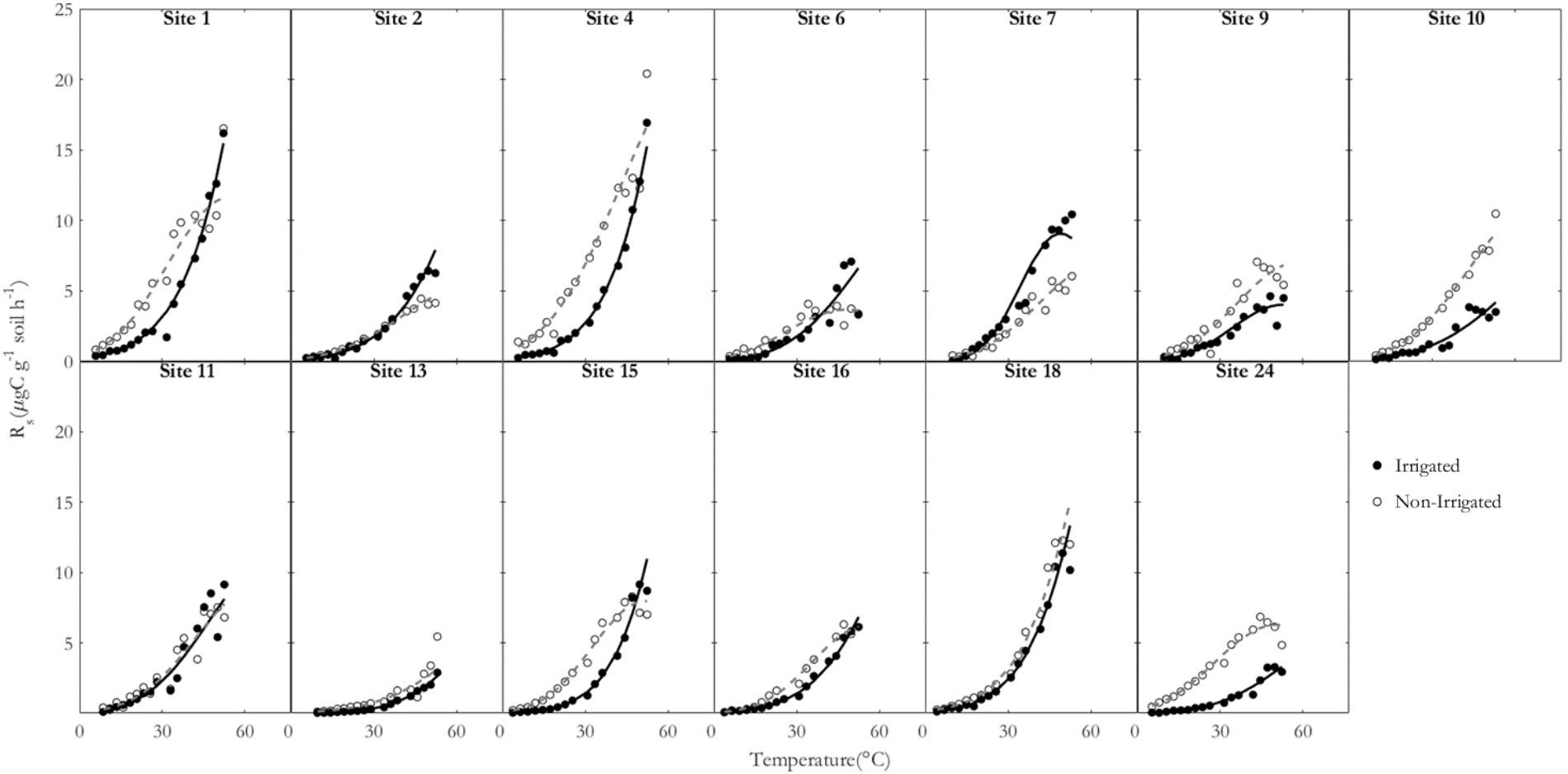


Figure 4.5 Example of respiration rates in response to temperature at 65 %MWHC for the 13 irrigated and non-irrigated Canterbury sites fitted with the MMRT model. Sites are labelled in accordance with the original Canterbury sites from Mudge, *et al.* (2017)

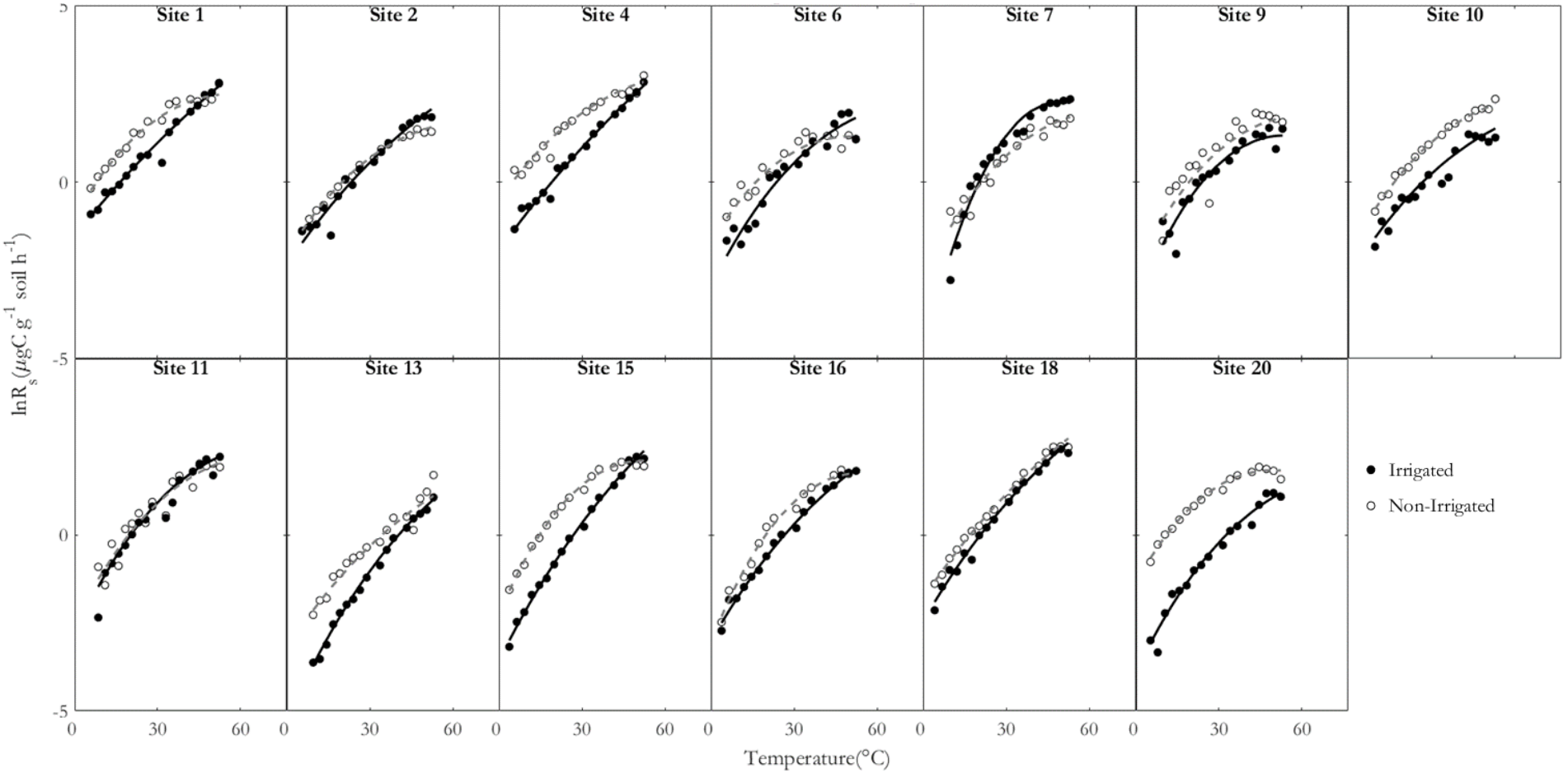


Figure 4.6 Example of the natural log of respiration rates in response to temperature at 65 %MWHC for the 13 irrigated and non-irrigated Canterbury sites fitted with the MMRT model. The sites are labelled in accordance with the original Canterbury sites from Mudge, *et al.* (2017)

T_{opt} ranged from 47.7°C to 89.3°C in the irrigated soil and from 38.37°C to 89.0°C in the non-irrigated soil throughout all the sites and moisture contents (Table 4.2). The T_{inf} ranged from 33.2°C to 59.7°C in the irrigated soil and from 22.5°C to 56.1°C in the non-irrigated soil (Table 4.2)

Table 4.2 T_{opt} (°C), T_{inf} (°C), $\Delta C_p^{\ddagger}$, R10 (µg C g^{-1} hr^{-1}) and R20 (µg C g^{-1} hr^{-1}) values for 13 paired irrigated and non-irrigated sites in the Canterbury region of the South Island, New Zealand. Sites are labelled in accordance with the original Canterbury sites from Mudge, et al. (2017)

		T_{opt}		T_{inf}		$\Delta C_p^{\ddagger}$		R10		R20	
Site	%MWHC	I	NI	I	NI	I	NI	I	NI	I	NI
1	20	83.084	80.502	58.982	52.482	-1801.050	-1324.911	0.052	0.310	0.317	1.128
	35	78.758	76.691	54.704	50.079	-1797.054	-1421.914	0.149	0.447	0.859	1.595
	50	71.333	67.996	48.437	44.155	-1892.537	-1709.687	0.203	0.421	0.727	1.622
	65	78.578	68.306	51.877	40.118	-1391.846	-1215.681	0.301	0.447	1.082	1.067
	80	81.899	79.771	50.882	47.334	-1075.829	-993.756	0.213	0.394	0.642	0.763
2	20	70.380	86.105	49.942	49.942	-2349.380	-1238.921	0.143	0.122	0.329	0.356
	35	89.273	68.210	59.668	43.664	-1245.977	-1613.746	0.232	0.266	0.544	0.932
	50	85.931	83.229	53.644	49.664	-1028.326	-936.659	0.418	0.373	0.827	0.869
	65	78.578	68.306	51.877	40.118	-1391.846	-1215.681	0.301	0.447	1.082	1.067
	80	81.899	79.771	50.882	47.334	-1075.829	-993.756	0.213	0.394	0.642	0.763
4	20	69.032	66.245	48.425	48.425	-2306.105	-2532.945	-0.088	0.147	0.296	0.671
	35	83.756	61.112	56.666	37.920	-1439.459	-1742.723	0.256	0.701	0.886	2.183
	50	86.831	64.721	58.173	40.477	-1319.191	-1624.814	0.285	0.756	0.985	2.372
	65	70.696	66.850	48.509	38.524	-1996.781	-1201.410	0.498	1.630	1.480	4.287
	80	81.212	70.643	52.166	37.164	-1244.793	-841.954	0.605	1.312	1.239	3.459
6	20	77.914	70.632	53.387	53.387	-1714.534	-1307.586	0.023	0.290	0.134	0.926
	35	73.805	53.009	49.953	33.805	-1760.782	-2374.899	0.128	0.230	0.193	0.808
	50	69.735	41.740	44.521	28.391	-1536.084	-4808.056	0.254	0.350	0.813	1.612
	65	66.624	48.287	43.082	27.418	-1697.158	-1959.356	0.171	0.920	1.138	1.201
	80	74.610	67.442	44.742	31.623	-1127.765	-751.821	0.399	0.527	0.749	0.904
7	20	-	-	-	-	-	-	-	-	-	-
	35	66.834	76.506	41.296	47.556	-1473.498	-1228.723	0.244	0.171	0.602	0.427
	50	66.046	58.030	41.726	36.906	-1632.223	-2028.891	0.169	0.185	0.603	0.520
	65	47.687	62.620	33.241	38.808	-4108.613	-1667.540	0.062	0.435	1.662	1.106
	80	69.597	48.238	42.945	30.367	-1365.434	-2626.091	0.503	0.000	0.853	0.644
9	20	80.479	68.596	54.135	54.135	-1507.032	-1812.837	0.054	0.085	0.227	0.361
	35	80.570	55.844	53.874	35.578	-1458.641	-2186.613	0.084	0.291	0.259	0.732
	50	68.835	58.692	45.542	36.276	-1811.061	-1829.089	0.092	0.273	0.315	0.794
	65	56.559	49.919	35.457	29.410	-2062.941	-2051.075	0.329	0.189	0.985	1.601
	80	77.876	48.096	45.991	27.852	-1017.960	-2097.138	0.338	0.746	0.572	1.065

Site	%MWHC	T_{opt}		T_{inf}		$\Delta C_p^{\ddagger}$		R10		R20	
		I	NI	I	NI	I	NI	I	NI	I	NI
10	20	-	-	-	-	-	-	-	-	-	-
	35	83.133	53.839	53.470	31.715	-1173.221	-1799.522	0.149	0.476	0.179	1.258
	50	75.663	70.493	46.295	43.067	-1175.122	-1335.624	0.263	0.484	0.204	1.304
	65	75.302	72.062	44.626	42.378	-1103.055	-1134.671	0.160	0.434	0.612	1.516
	80	76.589	61.802	45.950	37.216	-1082.543	-1532.331	-0.054	0.638	0.456	1.197
11	20	-	-	-	-	-	-	-	-	-	-
	35	49.246	63.495	36.246	39.008	-5137.428	-1567.885	0.201	0.360	0.631	1.145
	50	82.261	58.372	52.755	34.968	-1200.895	-1675.865	0.258	0.456	0.830	1.328
	65	64.873	61.842	41.117	38.244	-1742.689	-1719.013	0.338	0.239	1.011	1.375
	80	58.599	55.326	40.744	30.891	-2514.619	-1493.922	0.358	0.720	0.658	1.692
13	20	63.247	82.725	46.596	46.596	-3376.113	-1874.488	-0.047	-0.038	-0.021	0.076
	35	63.448	78.642	45.521	51.874	-2983.298	-1447.652	-0.015	0.116	0.161	0.537
	50	76.600	63.880	53.082	42.323	-1832.717	-2048.043	-0.022	0.087	0.211	0.638
	65	85.740	69.088	57.848	41.388	-1397.781	-1247.816	0.027	0.103	0.138	0.448
	80	72.153	57.731	50.058	35.925	-1978.787	-1913.213	0.121	0.070	0.274	0.344
15	20	58.735	64.579	35.895	35.895	-1831.780	-1374.072	0.263	0.649	0.815	1.656
	35	84.345	67.762	55.083	39.896	-1239.900	-1239.425	0.392	0.870	0.937	2.578
	50	82.794	63.785	57.328	41.624	-1626.201	-1911.831	0.052	0.250	0.199	1.024
	65	84.350	51.972	58.666	32.761	-1598.496	-2375.409	0.111	0.426	0.431	1.760
	80	82.435	55.446	55.340	33.166	-1381.419	-1803.513	0.133	0.445	0.397	1.554
16	20	81.441	50.322	54.870	54.870	-1479.724	-4060.330	0.083	0.033	0.375	0.201
	35	70.485	57.530	48.941	37.958	-2125.437	-2356.414	0.036	0.178	0.345	0.460
	50	67.888	79.395	43.118	50.193	-1587.273	-1214.303	0.154	0.273	0.493	0.623
	65	85.977	68.022	56.160	43.130	-1202.221	-1582.328	0.164	0.302	0.544	0.785
	80	85.843	81.418	55.067	53.403	-1431.273	-1315.854	0.049	0.105	0.136	0.242
18	20	82.720	66.827	55.947	55.947	-1463.040	-1891.609	0.130	0.127	0.565	0.657
	35	55.619	58.240	39.128	40.433	-3258.485	-2823.120	0.173	0.099	0.633	0.541
	50	56.107	60.310	37.629	40.562	-2654.413	-2364.253	0.069	0.123	0.450	0.886
	65	86.654	88.928	56.891	56.055	-1168.411	-1021.148	0.368	0.513	0.985	1.283
	80	73.780	84.679	45.823	51.129	-1275.040	-944.328	0.447	0.593	1.247	1.519
24	20	60.985	51.221	41.515	41.515	-2453.152	-1946.303	0.035	0.675	0.162	2.180
	35	67.309	38.371	44.217	22.485	-1816.853	-3095.613	0.082	1.067	0.389	2.680
	50	72.618	51.382	48.669	29.650	-1761.655	-1850.549	0.075	1.047	0.324	2.308
	65	73.093	50.677	47.314	27.690	-1495.240	-1634.837	0.108	1.006	0.364	2.268
	80	81.794	83.067	52.133	52.591	-1190.535	-1129.207	0.118	0.934	0.296	2.535

Overall, when averaged across all sites and moisture contents, the T_{opt} for the irrigated soil was 8.8°C higher than the non-irrigated soil and the T_{inf} was 7.6°C higher in the irrigated soil and both differences were significant (p=<0.001) (Table 4.3). Analysis of variance of T_{opt} and T_{inf} for irrigated and non-irrigated soils revealed that both were significantly higher in the irrigated soils and the effect was not dependant on the moisture content of the incubated soil (Table 4.3).

Both R10 and R20 were dependant on both soil moisture content and whether the soils were irrigated or not. The respiration rates were 43% higher at 10°C and 48% higher at 20°C in the non-irrigated soil than in the irrigated soil which was significant (Table 4.2, Table 4.3.). R10 and R20 also varied with moisture content, with rates generally increasing with increasing moisture content (p=<0.001) (Table 4.3). There was no significant relationship between moisture or irrigation and $\Delta C_p^{\ddagger}$ (change in heat capacity). There was a slight relationship between length of irrigation and T_{opt} and T_{inf} but due to the lack of data available on irrigation length no conclusions could be drawn from this relationship.

Table 4.3 P-values from ANOVA of average T_{opt}, T_{inf}, $\Delta C_p^{\ddagger}$, R10 and R20 values from 13 paired irrigated and non-irrigated sites in the Canterbury region of the South Island, New Zealand.

Factor	T_{opt}	T_{inf}	ΔC_p	R10	R20
Moisture	0.687	0.410	0.013	<0.001	<0.001
Irrigation	<0.001	<0.001	0.956	0.009	0.006
Moisture*Irrigation	0.809	0.753	0.655	0.549	0.700
Soil Type	ND*	ND*	0.598	0.809	0.319
Length of Irrigation	0.038	0.018	0.981	0.394	0.427

ND = Not determined

4.4.2 Temperature Sensitivity

To further explore the differences in temperature sensitivity of irrigated and non-irrigated soils, an average respiration curve was calculated using the average $\Delta H^{\ddagger}$, $\Delta S^{\ddagger}$ and $\Delta C_p^{\ddagger}$ across all sites and moisture contents (Figure 4.7, Box a). Absolute temperature sensitivity (first derivative of respiration rate) was calculated from this as the slope of the average MMRT curve (Figure 4.7. Box c). Box c shows the first derivative of the absolute respiration rate which shows the difference between the T_{inf} for the two treatments. Boxes b and d show the same information as boxes a and c but the temperature axis is constrained at 0-40°C to more clearly show the magnitude of the difference in temperature sensitivity between the irrigated and non-irrigated soils at temperatures realistic in the field.

Between 0-40°C, the non-irrigated soil had higher respiration rates than irrigated soil, (Figure 4.7, Box b) and were also more temperature sensitive, but as temperatures increased the irrigated respiration rates increased more quickly than the non-irrigated respiration rates.

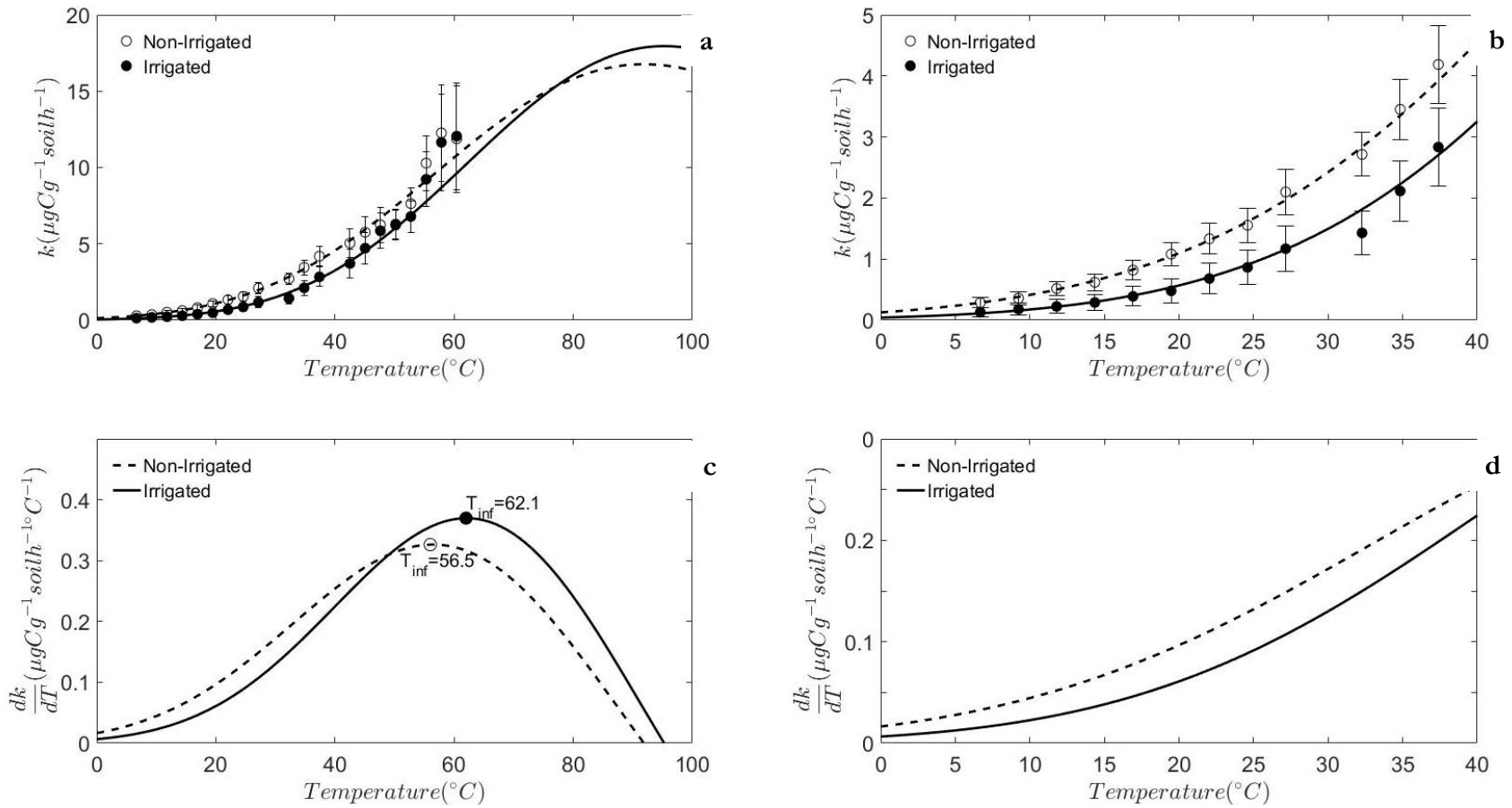


Figure 4.7 (a) MMRT curve calculated using average $\Delta H^{\ddagger}$, $\Delta S^{\ddagger}$ and $\Delta C_p^{\ddagger}$ values across 13 Canterbury sites for irrigated and non-irrigated treatments (b) Same curve as box a but constrained from 0-40°C to show difference in sensitivity between treatments and error bars added. The error bars show differences in average respiration rate over five moisture contents. (c) First derivative of average respiration rate across 13 Canterbury sites for irrigated and non-irrigated treatments Note: temperature range 0-100°C to show modelled T_{opt} and average T_{inf} values for each treatment added. (d) First derivative of average respiration rate across 13 Canterbury sites for irrigated and non-irrigated treatments constrained from 0-40°C.

4.5 Discussion

4.5.1 Temperature Response

It is generally accepted that microbes have an optimum temperature and moisture content at which they function to maximum efficiency. Consequently, microbes are compromised when soils are very wet or very dry (Davidson *et al.*, 2000; Sierra *et al.*, 2015). On average, irrigated soils had a higher T_{opt} and T_{inf} than non-irrigated soils by an average of 8.8°C and 7.6°C respectively. This indicated that the microbial communities in the irrigated soil have responded to extended periods of higher moisture content by shifting their T_{opt} and T_{inf} upwards. An upwards shift of their temperature response allows them to be active at higher temperatures than the microbes in the non-irrigated soils (Figure 4.7., Box a). There was more moisture available in the irrigated soil at seasonally dry periods. Consequently, at the irrigated sites the microbes did not face the same moisture limitation as the microbes in the non-irrigated soils. Microbes in the irrigated soil, however, were constrained in comparison to the microbes in the non-irrigated soil with regards to the availability of C substrate. In other research where rainfall was manipulated using rainout shelters, temperature sensitivity decreased with decreasing moisture content, which is consistent with the lower T_{inf} in the non-irrigated soils (Harper *et al.*, 2005; Nakano *et al.*, 2008).

The absolute respiration rates at 10°C and 20°C (Table 4.2) were higher in the non-irrigated soils by an average of 43% at R10 and 48% at R20. The lower respiration rate in irrigated soils is inconsistent with previously published data, where it was generally observed that irrigation increased soil microbial respiration (Sainju *et al.*, 2008; Condron *et al.*, 2014; Smith & Brye, 2014; Trost *et al.*, 2014; Gong *et al.*, 2015). Rain influences soil moisture content in a similar way to irrigation and in studies where rainfall was manipulated, respiration rates decreased with decreasing moisture. Harper *et al.* (2005) found that respiration declined with decreasing precipitation while Nakano, *et al.* (2008) found that decreasing precipitation and soil water content resulted in decreasing respiration rates. Talmon, *et al.* (2010) found that reducing rainfall amounts and increasing the dry periods between rainfall events decreased respiration rates by 20%.

The lower absolute respiration rates in irrigated soils found in this study support the loss of soil C under irrigation found by Mudge, *et al.* (2017). The data suggested that

under irrigation the most readily available, labile pool of soil C is lost first, which caused microbial communities to decompose the less readily available, recalcitrant soil C which resulted in lower absolute respiration rates in irrigated soils. It should be noted, however, the non-irrigated soils had been moisture limited for a long period and so when moisture adjusted for this analysis, may have been released of the moisture limitation which meant that respiration increased rapidly. This is commonly observed and is known as the 'Birch Effect' (Jarvis *et al.*, 2007; Unger *et al.*, 2010). Rapid increases of respiration are likely to happen more frequently due to increasing climatic variability under climate change and increased area of irrigated land. When soil is first irrigated, the moisture limitation will be lifted and so a rapid increase of respiration is likely to occur. Non-irrigated soils had a higher respiration rate than irrigated soils even at low moisture contents which indicates this rapid increase in respiration is not just a response to the sudden addition of water.

4.5.2 Carbon Substrate Availability

C substrate availability is one of the controlling factors of soil microbial activity. As described in section 4.2, soil C is categorized into three pools; readily available labile C that turns over quickly, an intermediate pool of C that turns over more slowly, and a recalcitrant pool of C that is not readily available to soil microbes (Davidson & Janssens, 2006). Mudge, *et al.* (2017) found more C in non-irrigated soils compared with irrigated soils, and therefore the microbes in the non-irrigated soils were likely to be less C substrate limited (when both sets of soils are standardised at same moisture content). Greater substrate availability in the non-irrigated soils is consistent with the higher respiration rates observed in this study. Mudge, *et al.* (2017) found no relationship between length of irrigation and the difference in soil C content between irrigated and non-irrigated soils. This may suggest that the C contents of irrigated soils decreased quickly after irrigation began. This suggestion is supported by the fact that there was no correlation between length of time under irrigation and temperature sensitivity was found in this study (Table 4.3). Therefore, it is likely that the more labile soil C was decomposed soon after initial irrigation, leaving only the intermediate and recalcitrant pools of C for soil microbes to decompose, which would cause the difference in absolute respiration rates found between the irrigated and non-irrigated soils.

The difference in temperature sensitivity found between the irrigated and non-irrigated soil can also be related back to the type of C being decomposed. Forms of C that have

large molecular weight, aromatic structure or are insoluble need to be broken down into simpler forms of C before microbes can use them as energy (Conant, *et al.,* 2011). The activation energy of these complex C compounds is considerably higher than those of more labile C and so the decomposition of recalcitrant soil C has a higher intrinsic temperature sensitivity than the decomposition of more labile soil C (Davidson & Janssens, 2006). Given much of the labile soil C is thought to be decomposed soon after irrigation; leaving more recalcitrant C pools, this is one possible explanation for the irrigated soil having a higher T_{inf} than the non-irrigated soil.

4.5.3 Shift in Soil Microbial Community Composition

Another possible explanation for the differences in temperature sensitivity found between the irrigated and non-irrigated soils is that there has been a shift in soil microbial community composition due to increased soil moisture when irrigation commences. In some cases, study sites have been irrigated continuously for 20 years and the prolonged seasonal difference in moisture content between irrigated and non-irrigated soils may enable microbes to adapt and for microbial community composition to change substantially.

The methods used in this study, whereby soils were moisture adjusted 24 hours prior to incubation was chosen as it was thought there would be insufficient time for microbial communities to adapt and therefore more likely representative of field communities.

Soil moisture content is a key factor that influences microbial community structure (Manzoni *et al.*, 2012; Ma *et al.*, 2015). So far, the term 'microbe' has been used loosely to describe any micro-organism living in the soil. There are, of course, innumerable species of micro-organisms living in the soil which can be grouped together into three general categories; soil fauna, bacteria and fungi. As soil moisture fluctuates, the type of micro-organisms that can be active at any one time, changes. Fungi, for example, are more resilient to dry conditions due to its filamentous nature and long hyphae that can access water that may be occluded inside soil aggregates that other microbes cannot reach (Ma *et al.*, 2015). Gram-negative bacteria are also more prevalent in dry soils while greater populations of gram-positive bacteria were found in wet soils (Ma *et al.*, 2015).

Other studies investigating the effect of moisture on microbial community structure have included the use of rainfall manipulation (Canarini *et al.*, 2016; Zhao *et al.*, 2016). Zeglin *et al.* (2013) used rain-out shelters to adjust rainfall frequency to determine the effect of reduced rainfall on microbial communities. There were two rainfall treatments used; 'altered' where there were longer periods between rainfall events and 'ambient' where the normal rainfall was kept. It was found that the microbial biomass and fungi: bacteria ratios were higher in the 'altered' treatment where soils had a lower moisture content; suggesting a shift in microbial population in drier soils. Their findings indicated enhanced microbial activity caused a greater reduction in soil C. They further suggested that C sequestration potential could be higher in soils with extended periods between wetting (altered soils), which could explain the difference in R10 and R20 between the irrigated and non-irrigated soil found in the Canterbury soils and help to explain the C loss found by Mudge, *et al.* (2017).

The increase in T_{opt} and T_{inf} found in the irrigated soil could also possibly be due to adaptation of the microbial community to increased moisture at warm times of the year. Irrigation occurs in spring/summer during typically high temperatures and low rainfall. Without irrigation, the microbes become water-limited which decreases the microbes' ability to decompose C (Davidson & Janssens, 2006). It is possible that the microbes in the irrigated soil have adapted to having no moisture limitation in the hottest part of the year which has enabled them to have a higher T_{opt} and T_{inf} than the non-irrigated soils. There has been very little research done on this topic so we are unable to state conclusively that this is a possible reason.

4.6 Implications and Conclusions

Irrigation had a significant effect on soil microbial activity. Soil microbial respiration had a higher T_{opt} and T_{inf} by 7.9°C and 7.6°C respectively under irrigation. This shift in temperature response was thought to be due to a change in microbial community composition or due to microbes adapting to an increase in moisture during the hottest part of the year. Absolute respiration rates were nearly 50% higher in the non-irrigated soils compared with the irrigated soil which was possibly due to a decrease in readily available soil C under irrigation. The differences in temperature response and absolute respiration rates are in agreement with the loss of soil C found by Mudge, *et al.* (2017) in soils under irrigation.

These results have important implications with regards to increased temperature and rainfall variability induced by climate change. Drought frequencies are predicted to

increase, especially in the Eastern parts of New Zealand, where Canterbury is located (Ministry for the Environment, 2016). As a result, irrigation will become an ever more crucial agricultural management practice to maintain or increase production. An expansion of irrigation coupled with increasing temperatures will mean that microbes, especially in irrigated soil, will be able to be active at higher temperatures for longer periods. This in turn could potentially deplete the soil of C stocks and increase CO_2 production. Further research is required to determine whether seasonal variation in temperature sensitivity of microbial respiration exists across the different soil types investigated. Another topic for further research would be DNA analysis of the soil microbial communities to determine any differences in community structure between treatments.

Chapter 5.

Seasonal Variation of Temperature and Moisture Sensitivity of Soil Microbial Respiration in Adjacent Irrigated and Non-Irrigated Soil

5.1 Abstract

Irrigation has a significant effect on the soil C cycle. Recent studies have shown that irrigation causes a reduction in soil C and one mechanism for this loss is an increase in soil microbial respiration caused by the increase in soil moisture from irrigation.

Soil microbial respiration fluctuates seasonally. In summer when temperatures are warmer, soil microbial respiration rates increase despite a reduction in rainfall. In winter, there is higher rainfall but cooler temperatures and so soil microbial respiration rates decrease. Irrigation changes this seasonal cycle by increasing moisture contents during summer when temperatures are warmest. In this study, soil samples were taken from two irrigated sites at a farm in Rangiriri in February, during late summer, and June, during winter to establish whether irrigation influences soil microbial respiration and whether this effect varies seasonally. Additional samplings were planned for spring and early summer but due to time constraints these were not undertaken. Samples from irrigated and adjacent non-irrigated areas were wet up to five different moisture contents and incubated on a temperature gradient block for five hours before gas samples were taken and analysed on an infra-red gas analyser. MMRT was then fitted to the respiration rates to determine T_{opt}, T_{inf}, $\Delta C_p^{\ddagger}$, R10 and R20.

There was no significant difference found in T_{opt}, T_{inf}, R10 or R20 which indicated that there was no irrigation effect evident at the farm in Rangiriri. This study was considerably limited by time and so the complete four season analysis was not completed. It was possible that the irrigation effect was not seen in this study due to the soil having an organic component which meant the irrigation effect was minimised due to increased original soil C content. Another possibility was that the Waikato region has a high annual rainfall and so the non-irrigated soils do not face a major moisture limitation.

5.2 Introduction

As discussed in Chapter 4, Section 4.2, it is crucial to understand how irrigation affects the cycling of soil carbon (C) and soil microbial activity. Along with the broad analysis undertaken on the Canterbury soils, a preliminary effort at a seasonal analysis was made using soils from a local farm at Rangiriri in the Waikato region of the North Island, New Zealand (Figure 5.1). We were unable to collect as many samples as we had first planned due to time constraints. This chapter will present the results from seasonal sampling undertaken in February and June 2016.

Previous studies have shown that climatic variability associated with the change in seasons influences soil microbial respiration (Conant *et al.*, 2011; Bradford, 2013). Soil microbial activity is governed by temperature, moisture and C substrate availability (Davidson & Janssens, 2006) (Chapter 2, Section 2.4; Chapter 4, Section 4.2.); three factors that fluctuate seasonally. Generally, most regions in New Zealand experience relatively high temperatures and low rainfall in the summer months and low temperatures and higher rainfall over the winter months. Production (plant growth) peaks in spring and so is likely to increase soil C stocks. Periods of high rainfall also mean that adjacent irrigated and non-irrigated soils are likely to be at similar soil moisture contents for at least part of the year. As a consequence of these seasonal effects, some studies have shown that soil microbial respiration rates tend to be higher in summer months and lower in winter months (Suseela *et al.*, 2012). However, others have shown seasonal changes in temperature sensitivity cause changes in labile C fractions (Davidson *et al.*, 2000; Kirschbaum, 2013). For example, as temperature increases, decomposition is accelerated resulting in a reduction in readily decomposable, labile C and subsequently a reduction in respiration rate as other less readily available C sources must be utilised (Kirschbaum, 2013). Another study done by Robinson, *et al.* (2017) suggested that temperature sensitivity of soil respiration was partly dependant on season of collection. They also suggested soil microbes may alter their temperature response with season, potentially tuning their metabolism in response to changes in temperature (Robinson, *et al.,* 2017).

Irrigation changes the inherent effect of seasonality by increasing soil moisture content and releasing moisture limitations during the hottest part of the year. This creates a large difference in moisture content between irrigated soils and non-irrigated soils, and therefore a difference in microbial activity between the two treatments (Chapter 4, Section 4.5.1.).

The aim of this analysis was to determine whether there was a difference in temperature and moisture sensitivity between irrigated and non-irrigated soils at a dairy farm at Rangiriri in the Waikato region of the North Island, New Zealand, and whether this difference varied seasonally. The methods used in this research were an extension of methods developed by Robinson, *et al.* (2017) and four seasonal samplings were planned. It was hypothesised that there would be a seasonal effect on the temperature sensitivity of soil microbial respiration.

5.3 Methods

5.3.1 Site Description

Samples were taken from a dairy farm at Rangiriri in the Waikato region of the North Island, New Zealand. There were three large pivot irrigators on the farm and samples were taken from under two of these irrigators (Site 1 and Site 2) as the third was effluent irrigated (Figure 5.1). The irrigators had been in place for 2-3 years. Soils were collected during summer, in February, and repeated during winter in June. Recent soils were found throughout the farm (Landcare Research, 2010). The Waikato region has a mean annual rainfall of 1100 mm and a mean annual temperature of 14°C (NIWA, 2012). A bucket sampler was used to take soil (0-0.1 m) at random from within an irrigated area and an adjacent non-irrigated area. Following collection, the samples were sieved and stored at 4°C until required.

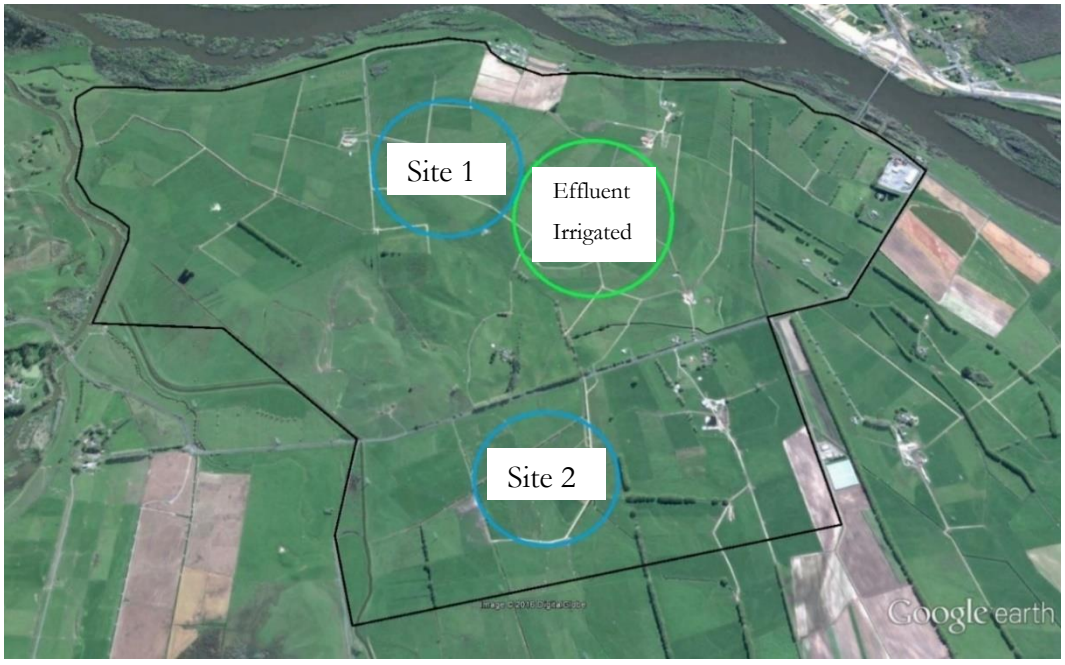


Figure 5.1. Outline of farm at Rangiriri showing three pivot irrigated areas; Site 1, Site 2 and the third, effluent irrigated area.

5.3.2 General Methodology

Soils were moisture adjusted and pre-incubated for 24 hours before analysis. Soil samples were incubated across a temperature gradient for 5 hours before gas samples were taken and injected into and infra-red gas analyser (IRGA) for CO_2 analysis. The methods used to measure microbial respiration were developed by Robinson, *et al.* (2017) and are summarised below.

Prior to respiration analysis the soils were moisture adjusted and pre-incubated for 24 hours at 20°C (Chapter 3, Section 5). The moisture contents were calculated as a percentage of the maximum water holding capacity (%MWHC) and there were five moisture contents used for this analysis: 80%MWHC, 65%MWHC. 50%MWHC, 35%MWHC and 20%MWHC. This ensured a wide range of moistures that the soil was likely to experience throughout the year as well as a wide range of temperatures.

The temperature gradient block for incubating soil (Figure 5.2) was turned on the night before analysis to ensure a stable temperature gradient between 5°C at the cold end and 60°C at the hot end. Thermistors were spaced evenly along the block to measure and record 7 temperatures which allowed for calculation of a discrete temperature at each tube hole.

A laptop was connected to the block to record thermistor temperatures and temperature gradient across the block.

Following pre-incubation soil samples were weighed into labelled Hungate tubes at 3 g (± 0.05 g) per tube. There were 20 tubes of irrigated soil and 20 tubes of non-irrigated soil per moisture content. The temperature gradient block had 132 holes in total with seven of the holes containing thermistors which meant that up to three moisture contents could be incubated on the block at once.

Once the soil was weighed out, the tubes and soil were transported to the temperature gradient block. The tubes were capped using a rubber stopper and an aluminium cap that was crimped around the top of the tube in order to prevent gas leakage. The tubes were capped individually immediately prior to being placed in the block. Three empty tubes were also capped and placed in the block as blanks.

Irrigated tubes from one moisture content were placed in the top row of the block first, starting at the cold end of the block and the time noted when the first tube was placed. A space was left between each of the irrigated tubes and the non-irrigated tubes from the same moisture content were placed in the gaps which meant the tubes were

placed with treatments alternating (Figure 5.2). Site 1 and 2 were both analysed at the same moisture content at the same time with site 1 in the top row and site 2 in the middle row. Once all the tubes had been placed the Perspex lid was closed tightly to prevent fluctuations in temperature and the tubes were left to incubate for five hours from when the first tube was placed.

After five hours of incubation the headspace of each tube was sampled for CO_2 using labelled 1 mL insulin syringes. Gas samples were taken in the order in which the tubes were originally placed in the block. The syringe was then inserted into a large rubber bung for transport to the IRGA. Robinson *et al*, (2017) determined that this was a sufficient way to transport the syringes and no leakage occurred. This was repeated for each tube with individual syringes.

A standard curve was produced for the IRGA using a 1% CO_2 standard before and after the gas samples were injected. The insulin syringes were inserted into the IRGA in the same order the tubes were sampled from the block.

The CO_2 concentrations from each tube were read by the computer as peaks heights in mV.

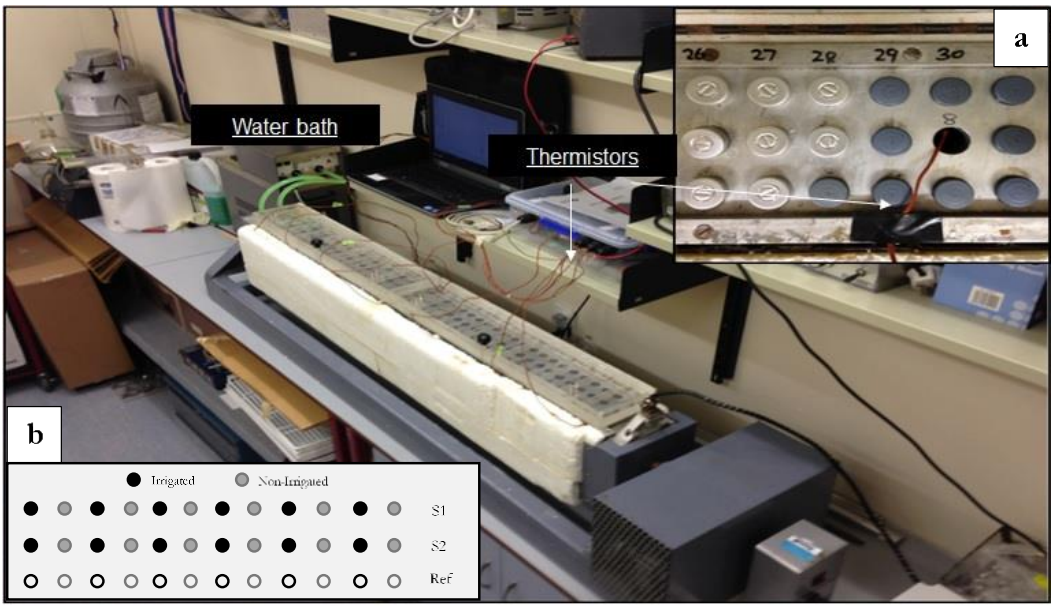


Figure 5.2 Temperature gradient block. Inset a: Hungate tubes and thermistors. Inset b: Tube layout showing Site 1 in the top row and Site 2 in the middle row. Irrigated and non-irrigated samples at the same moisture content from both sites were incubated at the same time. The 'Ref' row contained blanks and thermistors. Image modified from Robinson, (2016)

5.3.3 Data Analysis

Respiration rates (Rs) were calculated using the peak heights produced in MATLAB. Rs was determined using the following calculation:

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

Where Rs is the respiration rate in μL CO_2 g $soil^{-1}$ hr^{-1}, H_s is the peak height of the sample (mV), V_i is the injection volume (mL), H_{st} is the height of the standard (mV), H_b is the peak height of the blank (mV), S is the CO_2 concentration of the standard, either 1% or 2% CO_2 per mL of gas, V is the headspace volume of the Hungate tube (mL), ODW is the oven dry weight of the soil being analysed (g) and t is the length of time the soil was incubated for (hr). This calculation was completed individually for each sample and resulted in respiration curves for the temperature gradient. Data analysis was undertaken on respiration data to 52°C (Chapter 3, Section 3).

Rs data was fitted using MMRT and the temperature gradient data collected from the thermistors on the block. The temperature optima (T_{opt}) and temperature inflection point (T_{inf}) were calculated using the calculations below:

$$T_{opt} = \frac{\left(\Delta H^{\ddagger} - \left(\Delta C_p^{\ddagger} \times 280\right)\right)}{\left(\left(-\Delta C_p^{\ddagger}\right) - 8314\right)} \qquad T_{inf} = \frac{T_{opt}}{1 + \left(2.883 \div \left(\sqrt{-\Delta C_p^{\ddagger}}\right)\right)}$$

Where $\Delta H^{\ddagger}$ is the change in enthalpy and $\Delta C_p^{\ddagger}$ is the change in heat capacity.

Bootstrapping was also carried out during the fitting of MMRT curves. The curve was fitted 1000 times and the best fit was used to calculate T_{opt} and T_{inf}. If there was no fit, then the bootstrapping was increased to 5000. If there was still no fit, the data was noted as a non- fit. Following MMRT curve fitting, 3-D temperature-moisture webs were drawn. To create these webs, MMRT was fitted to the temperature response at each moisture content and then a surface created by a loess interpolation was fitted between curves at each moisture content R10 and R20 were calculated using the respiration rate at the closest temperature to 10°C and 20°C as there was some very slight temperature fluctuation on the block. Statistical differences T_{opt}, T_{inf}, $\Delta C_p^{\ddagger}$, R10 and R20 between irrigated and non-irrigated treatments, moisture content, soil type and length of irrigation were tested using standard two-way analysis of variance (ANOVA) methods

5.4 Results

Table 5.1 shows the T_{opt}, T_{inf}, $\Delta C_p^{\ddagger}$, R10 and R20 values for each site for both February and June seasonal samplings. In February, the average T_{opt} over all moisture contents was 81°C for the irrigated soils and 78°C for the non-irrigated soils for Site 1 while Site 2 had an average T_{opt} of 76°C and 83°C for the irrigated and non-irrigated soils respectively (Table 5.1). In June, the average T_{opt} over all moisture contents was 72°C for the irrigated soil and 75°C for the non-irrigated soils at Site 1. At Site 2, the average T_{opt} was 77°C for the irrigated soils and 72°C for the non-irrigated soils (Table 5.1). There was very little difference in the average R10 and R20 values between treatments and seasons.

Table 5.1 T_{opt} (°C), T_{inf} (°C), $\Delta C_p^{\ddagger}$, R10 (µg C g^{-1} hr^{-1}) and R20 (µg C g^{-1} hr^{-1}) values for the February and June samplings at Rangiriri in the Waikato region of the North Island, New Zealand

	Site 1					Site 2				
February	**T_{opt}**	**T_{inf}**	**$\Delta C_p^{\ddagger}$**	**R10**	**R20**	**T_{opt}**	**T_{inf}**	**$\Delta C_p^{\ddagger}$**	**R10**	**R20**
80I	77.297	51.139	-1504.571	0.915	2.061	85.754	53.753	-1036.500	0.604	1.710
65I	86.037	58.216	-1406.287	0.289	1.185	78.782	51.407	-1384.392	0.345	0.944
50I	70.300	46.088	-1674.770	0.340	0.554	50.021	32.241	-2694.403	0.344	1.196
35I	85.505	57.845	-1397.821	0.279	1.074	79.840	52.702	-1455.671	0.313	0.852
20I	85.377	58.563	-1490.988	0.180	0.670	86.511	58.811	-1401.184	0.139	0.525
80NI	78.757	50.132	-1255.437	0.425	1.588	76.293	47.524	-1232.377	0.700	2.166
65I	89.844	60.612	-1281.954	0.362	1.243	81.763	53.097	-1281.157	0.365	1.309
50NI	71.121	47.777	-1808.287	0.374	1.110	84.180	54.712	-1219.688	0.417	1.238
35NI	88.876	59.129	-1256.757	0.236	0.918	86.032	57.233	-1294.461	0.264	1.075
20NI	59.062	37.695	-2009.712	0.175	0.688	87.964	59.587	-1396.647	0.173	0.632
June										
80I	-	-	-	-	-	-	-	-	-	-
65I	74.029	50.544	-1791.202	0.393	0.603	74.029	50.544	-2161.441	0.150	0.517
50I	67.171	44.657	-1976.881	0.931	1.180	78.752	47.499	-1058.907	0.557	1.029
35I	65.796	44.450	-1995.702	0.869	1.158	71.950	44.633	-1308.674	0.362	0.967
20I	80.334	56.951	-1899.925	0.082	0.271	84.660	57.913	-1460.130	0.083	0.255
80NI	-	-	-	-	-	-	-	-	-	-
65I	70.752	47.437	-1823.784	0.081	0.621	72.485	43.601	-1190.718	0.245	0.675
50NI	72.178	42.025	-1103.277	0.741	1.830	70.908	40.436	-1043.773	0.742	1.785
35NI	72.957	44.606	-1246.421	0.454	1.347	62.896	40.235	-1773.817	0.146	1.103
20NI	84.798	59.409	-1647.141	0.054	0.143	84.150	57.078	-1490.077	0.013	0.059

There was no significant difference between the T_{opt}, T_{inf}, R10 and R20 values for the irrigated and non-irrigated treatments. There was a significant difference in the $\Delta C_p^{\ddagger}$ between treatments (Table 5.2). and a relationship between soil moisture content and R10 and R20.

Table 5.2 P-values from ANOVA of average T_{opt}, T_{inf}, $\Delta C_p^{\ddagger}$, R10 and R20 values from 2 paired irrigated and non-irrigated sites at Rangiriri in the Waikato region of the North Island, New Zealand.

Factor	T_{opt}	T_{inf}	ΔC_p	R10	R20
Moisture	0.553	0.037	0.184	0.001	0.001
Irrigation	0.837	0.351	0.001	0.484	0.280
Moisture*Irrigation	0.232	0.632	0.537	0.393	0.464
Season (Feb/June)	0.173	0.124	0.797	0.302	0.151

5.5 Discussion

Across the two seasons sampled (February and June) there was no significant difference found in the temperature sensitivity (T_{opt} and T_{inf}) or absolute respiration rates (R10 and R20) between the irrigated soil and the non-irrigated soil at Rangiriri (Table 5.2). This contrasted with the findings from the Canterbury study, where there were significant differences found in all the calculated factors except for the $\Delta C_p^{\ddagger}$ (Table 4.3).

One limitation of this study was that only part of the seasonal analysis was undertaken and so we are unable to determine conclusively whether a statistical difference in temperature sensitivity exists between the irrigated and non-irrigated treatments across all four seasons. Another limitation was that there was some confusion as to effluent irrigation on the farm at Rangiriri. If the sampled soils were effluent irrigated it would influence soil C content and therefore soil microbial respiration.

There are several possible explanations for the lack of difference in temperature sensitivity and absolute respiration rate between irrigated and non-irrigated treatments found at the Rangiriri sites. It is possible that there is a geographical effect component whereby the Canterbury and Rangiriri sites respond differently. The Waikato region experiences around 1100 mm of rain a year compared with around 650 mm of rain per year in the Canterbury region of the South Island (NIWA, 2016). Soils in the

Canterbury region, therefore, become considerably drier than the soils at Rangiriri due to the lower rainfall and so face a greater moisture limitation.

Another possible reason for the difference in microbial respiration rates and temperature sensitivity between Canterbury and Rangiriri studies is that it is likely there was an organic component to the soil at the Rangiriri farm as indicated by Landcare Research (2010) which could mean there was additional soil C. As there is more C in the soil to start with (relative to the Canterbury sites) the irrigation effect could take longer to become apparent as the microbes are able to access more labile C and will not face the same C substrate limitation as the irrigated Canterbury soils.

The two sites sampled at Rangiriri had only been irrigated for 2-3 years and so in the context of a wetter climate, irrigation may not have been in place long enough to cause any effect, especially if the effect is reduced by additional C availability.

5.6 Conclusions

Across the two seasons (February and June) that were analysed, irrigation had no effect on the temperature sensitivity of soil microbial respiration at the Rangiriri sites. Only two of four seasonal samplings were completed so no irrefutable conclusions about seasonality could be drawn. It is possible that the soil at Rangiriri does not face the same C substrate and moisture limitations seen in the Canterbury soils, hence no significant irrigation treatment effect was seen at Rangiriri which is inconsistent with the Canterbury study.

To observe any seasonal patterns, the analysis of the remaining seasons would need to be completed and soil C analysis to determine the soil C content. It would also be beneficial to undertake a seasonal analysis on the Canterbury study A seasonal analysis on these sites would determine whether the difference in temperature sensitivity is still present during winter months when both treatments are at similar moisture contents. Seasonal sampling in areas with greater climatic variability could reveal if mean annual temperature, mean annual rainfall, land use or management practices are the main drivers in the temperature sensitivity of soil microbial respiration and C cycling.

Chapter 6.

Conclusions and Further Research

6.1 Background

Recent research has shown a loss of soil C under irrigation but exactly why this occurs was not well understood (Schipper *et al.*, 2013; Condron *et al.*, 2014; Mudge *et al.*, 2017). In 34 paired irrigated and non-irrigated sites throughout New Zealand, Mudge, *et al.* (2017) found that there was a loss of 7 t C ha^{-1} under irrigation. One possible mechanism for this loss was an increase in microbial activity under irrigation. The main aim of this research was to determine whether there was a difference in temperature and moisture sensitivity of microbial activity between irrigated and non-irrigated soil. Soil samples from 13 paired irrigated and non- irrigated sites in Canterbury and two sites from Rangiriri in the Waikato were incubated for five hours on a temperature gradient block at five different moisture contents. Gas samples from the incubated soils were then analysed for CO_2 in an IRGA and the temperature sensitivity determined using MMRT. MMRT was fitted to the temperature response at each moisture content and the T_{opt} and T_{inf} of the soil along with the $\Delta C_p^{\ddagger}$, R10 and R20 were calculated for each site at each moisture content.

6.2 Canterbury

Irrigation had a significant effect on soil microbial respiration. Analysis of soil from the Canterbury region of the South Island, New Zealand, showed that microbes in soil under irrigation had a higher T_{opt} and T_{inf} by 8.8°C and 7.6°C respectively. The R10 rates were 43% higher and the R20 rates 49% higher in the non-irrigated soil compared with the irrigated soil. Under irrigation the moisture limitation is removed during the hottest and driest months of the year and as a result, microbial communities are active for longer periods. As discussed in chapter 4, these differences in temperature sensitivity and absolute respiration rate were thought to be due to the decrease in the labile, readily available soil C under irrigation or a change in microbial community structure due to the moisture content difference between treatments.

6.3 Rangiriri

There was no significant difference in T_{opt}, T_{inf}, R10 or R20 between treatments or seasons sampled in the Rangiriri soil. This was thought to be due to geographical differences in rainfall between the wetter Waikato and drier Canterbury regions or due to an organic component in the Rangiriri soil influencing soil C availability. There was also some confusion as to effluent irrigation on the farm which would also alter soil C concentrations.

6.4 Management Implications

Irrigation is a crucial management practice used to increase production, especially in drought prone areas such as the Canterbury region of the South Island, New Zealand. Annual rainfall in Canterbury is only around 600 mm yet it is a high producing region of New Zealand. With a changing global climate, areas such as Canterbury are likely to be subjected to more frequent and long lasting droughts. This will mean that the area of land that needs to be irrigated will increase substantially, possibly resulting in the loss of soil C. It is likely that the C lost under irrigation is the result of increased microbial activity during hotter months, increasing the decomposition of the most readily available, labile form of soil C. Consequently, increasing the area of land under irrigation will likely lead to a change in C cycling and result in more CO_2 released into the atmosphere.

6.5 Limitations

Time was one of the main limitations of this study. The Rangiriri seasonal analysis was incomplete making it impossible to determine whether there was a seasonal effect of irrigation on the temperature sensitivity of soil microbial respiration. This is one area where further research would be beneficial.

Another limitation to this research was receiving mixed information from farmers at the Rangiriri farm. The farm at Rangiriri had three pivot irrigators and there was some confusion about where the effluent was irrigated. Setting up clear communication lines and working with just the farm owner or manager would have resolved this problem.

6.6 Further Research

This study has produced some questions that would benefit from further research. It would be beneficial to continue the research into the effect of irrigation on C stocks throughout New Zealand to determine if there is any geographical influence (annual rainfall or mean annual temperature) on the sensitivity of soil microbial respiration under irrigation. Mudge, *et al.* (2017) analysed 34 sites throughout New Zealand for soil C concentrations, 13 of which have been analysed here and it would be worthwhile continuing this work on the remaining sites. A global research effort to better understand the interaction between soil type, land use and irrigation treatment effect on C stocks would be beneficial, especially in drought prone regions where climate change will exacerbate the need for irrigation.

Seasonal changes in microbial activity could also be explored further. It would be interesting to find out if the differences in temperature sensitivity and absolute respiration rates found in the Canterbury soils were sustained through winter when there are cooler temperatures and the two treatments are at similar soil moisture contents because of increased rainfall.

The increase in respiration rate found in the non-irrigated soil was possibly due to the soil being dry for such a long period and then having water added to adjust the %MWHC. This would cause a sudden increase in respiration known as the 'Birch Effect' which is possibly what was measured here. To rule out the 'Birch Effect' being the sole cause of the difference in respiration between treatments, both treatments could be analysed for varying periods of time post-wetting or analysed in their field conditions to see if the difference is still prevalent in the field.

It would also be beneficial to undertake C fractionation analysis on the bulk soil from each site and treatment to determine whether the microbes in the irrigated soil are substrate limited and having to decompose more recalcitrant C than the microbes in the non-irrigated soil.

DNA analysis could be undertaken to accurately determine whether there is a change in microbial community composition between irrigated and non-irrigated soils. DNA analysis would give information on the species and community of microbes living in each treatment. If different species were found living in each treatment it could help to explain the differences in temperature sensitivity and absolute respiration rate found in the Canterbury soils.

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Appendices

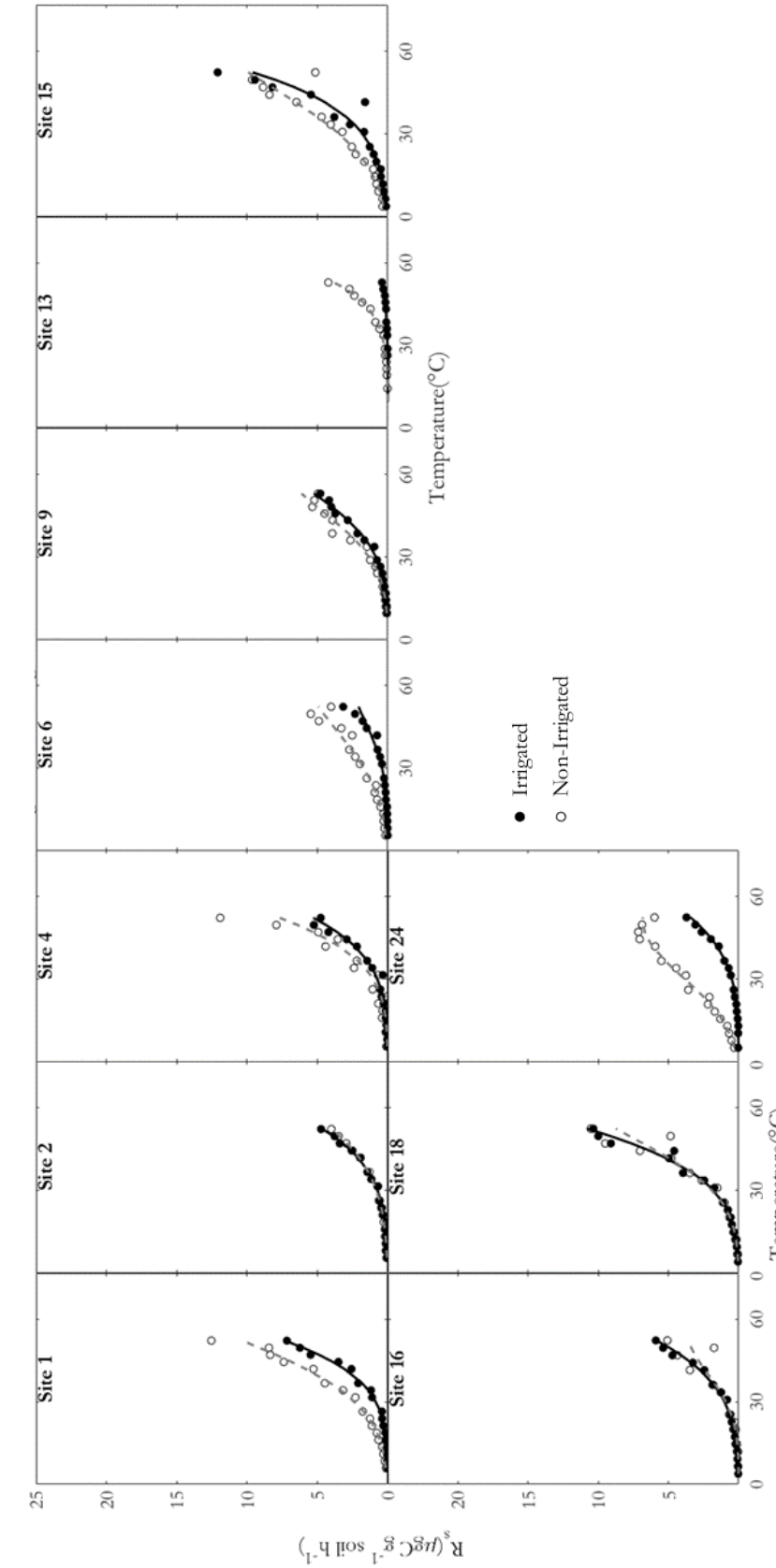

Figure A.1. Respiration rates in response to temperature at 20 %MWHC for the 13 irrigated and non-irrigated Canterbury sites fitted with MMRT model Sites are labelled in accordance with the original Canterbury sites from Mudge, *et al.* (2017). Due to lack of soil only 10 sites were completed at 20% MWHC

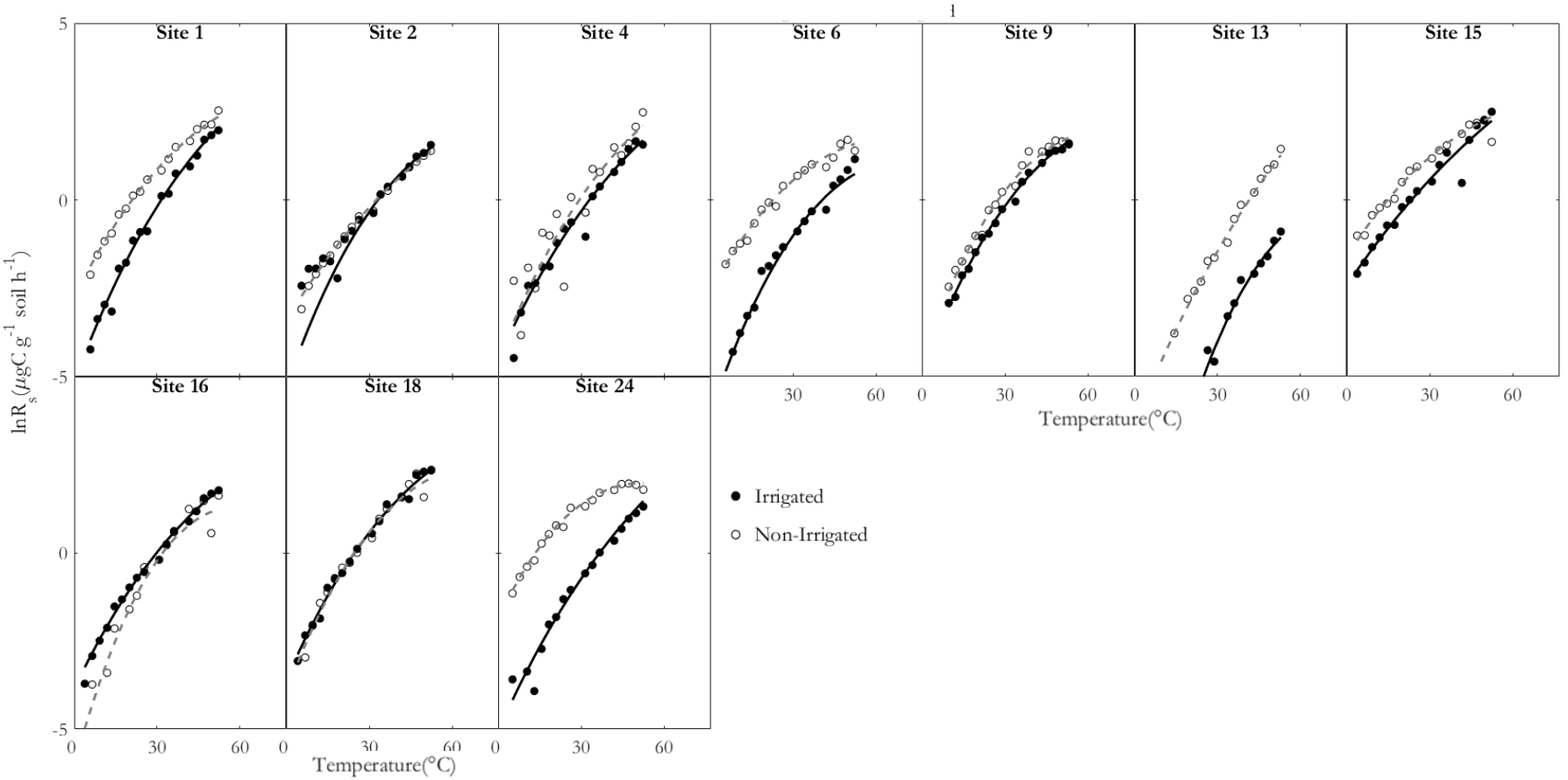

Figure A 2 Natural log of respiration rates in response to temperature at 20 %MWHC for the 13 irrigated and non-irrigated Canterbury sites fitted with the MMRT model. The sites are labelled in accordance with the original Canterbury sites from Mudge, et al. (2017). Due to lack of soil only 10 sites were completed at 20% MWHC

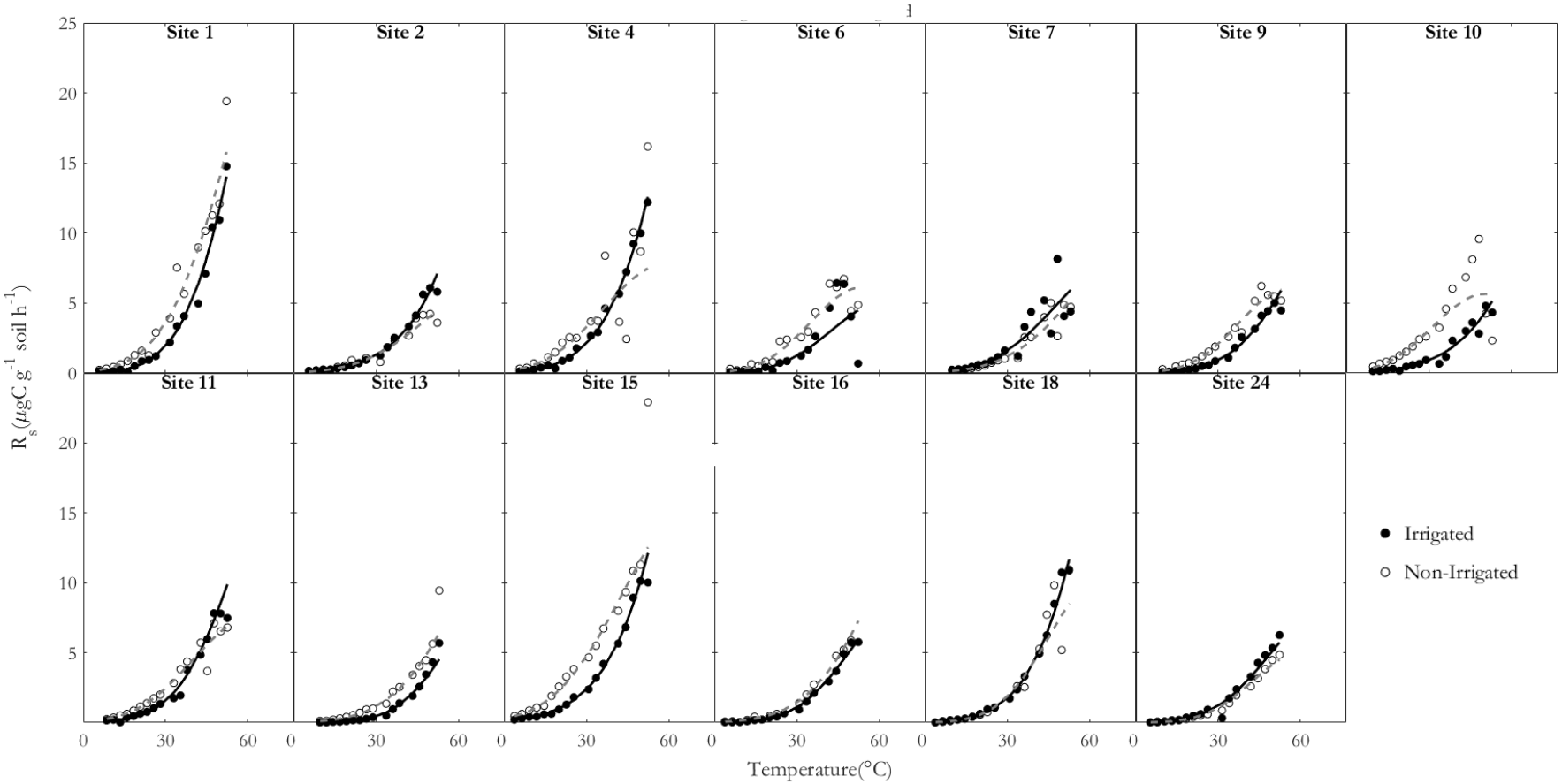

Figure A.3 Respiration rates in response to temperature at 35 %MWHC for the 13 irrigated and non-irrigated Canterbury sites fitted with the MMRT model. Sites are labelled in accordance with the original Canterbury sites from Mudge, *et al.* (2017)

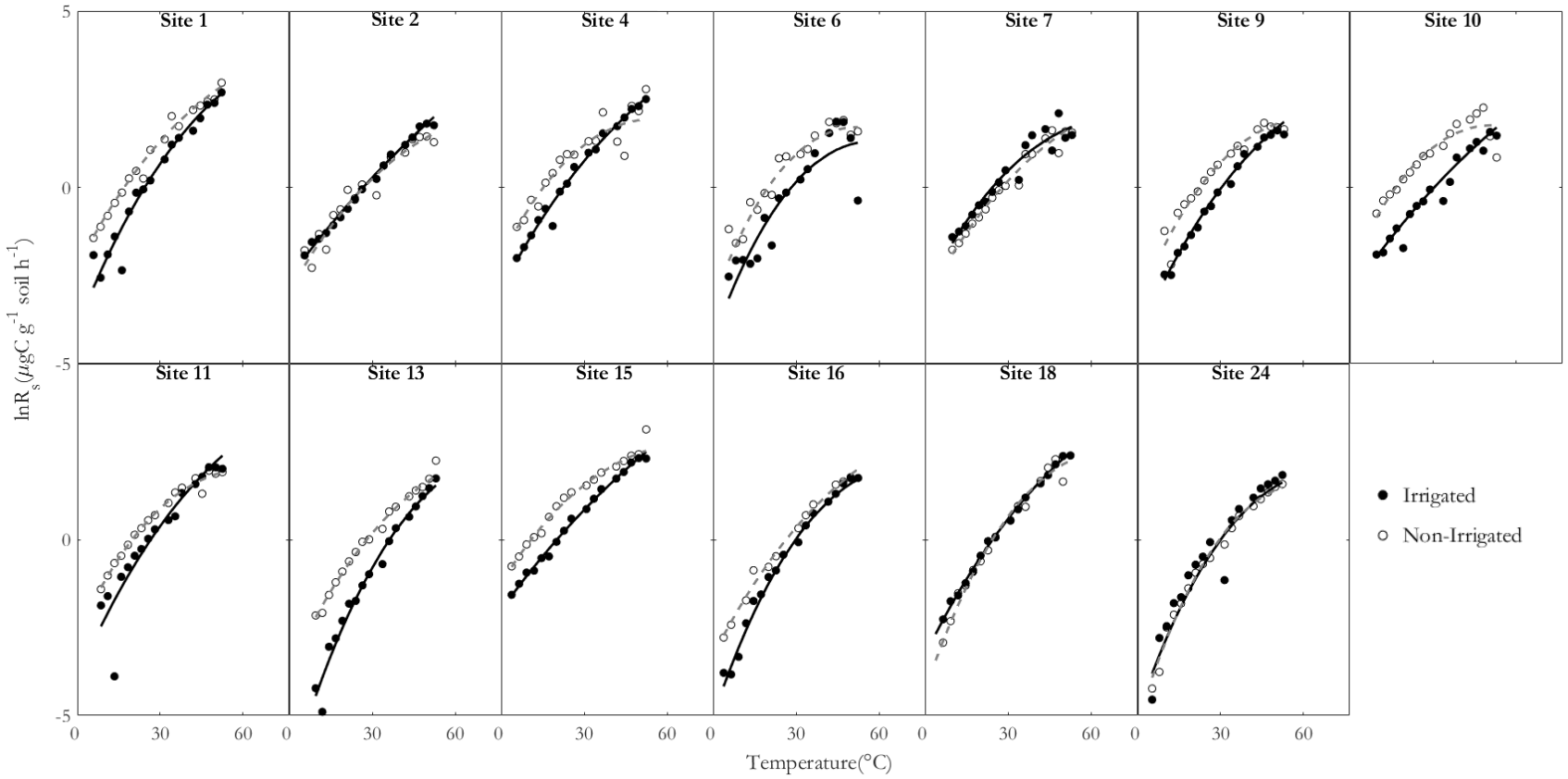

Figure A.4 Natural log of respiration rates in response to temperature at 35 %MWHC for the 13 irrigated and non-irrigated Canterbury sites fitted with the MMRT model. The sites are labelled in accordance with the original Canterbury sites from Mudge, et al. (20170)

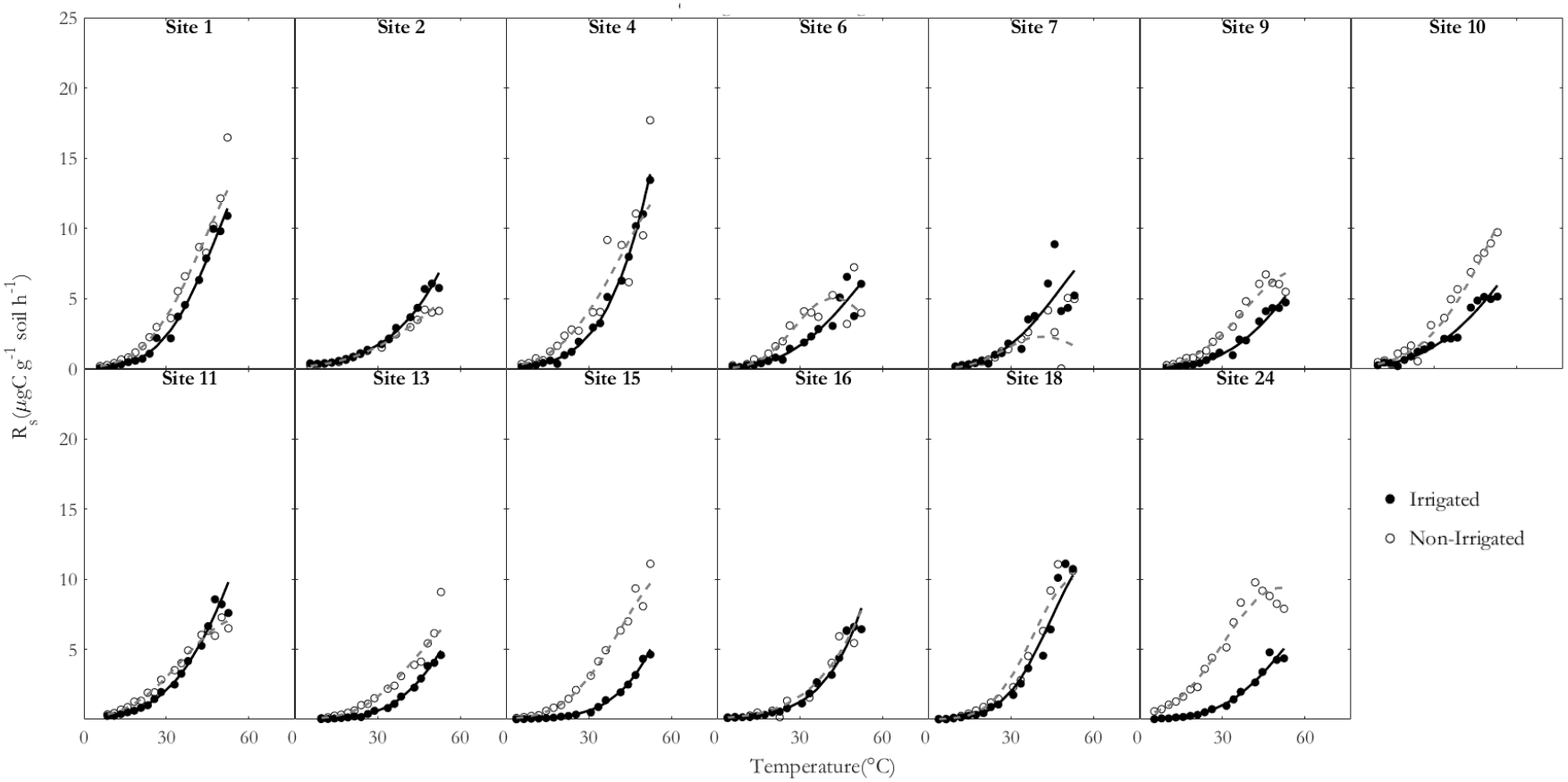

Figure A.5 Respiration rates in response to temperature at 50 %MWHC for the 13 irrigated and non-irrigated Canterbury sites fitted with the MMRT model. Sites are labelled in accordance with the original Canterbury sites from Mudge, et al. (2017)

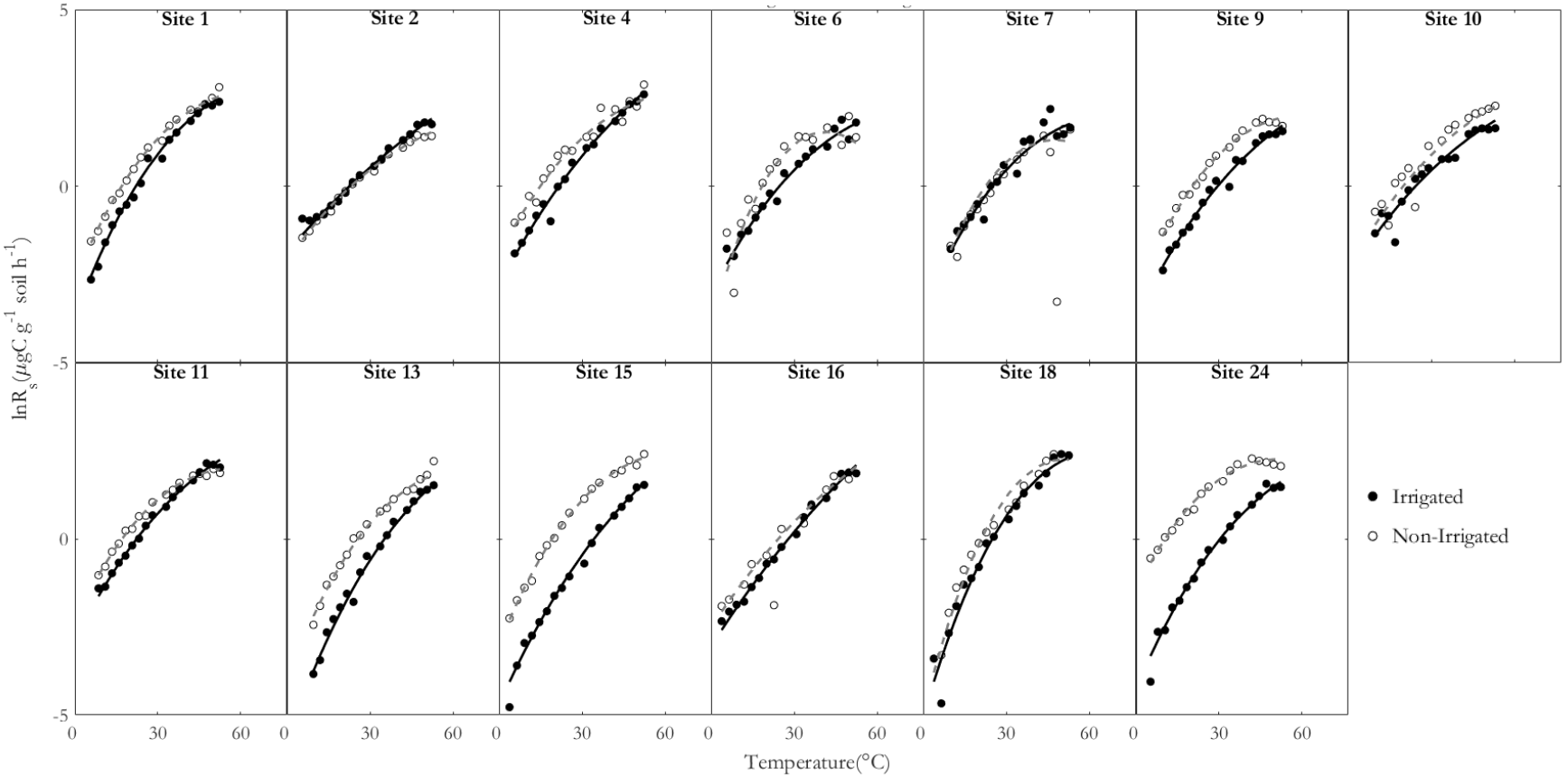

Figure A.6 Natural log of respiration rates in response to temperature at 50 %MWHC for the 13 irrigated and non-irrigated Canterbury sites fitted with the MMRT model. The sites are labelled in accordance with the original Canterbury sites from Mudge, et al. (2017)

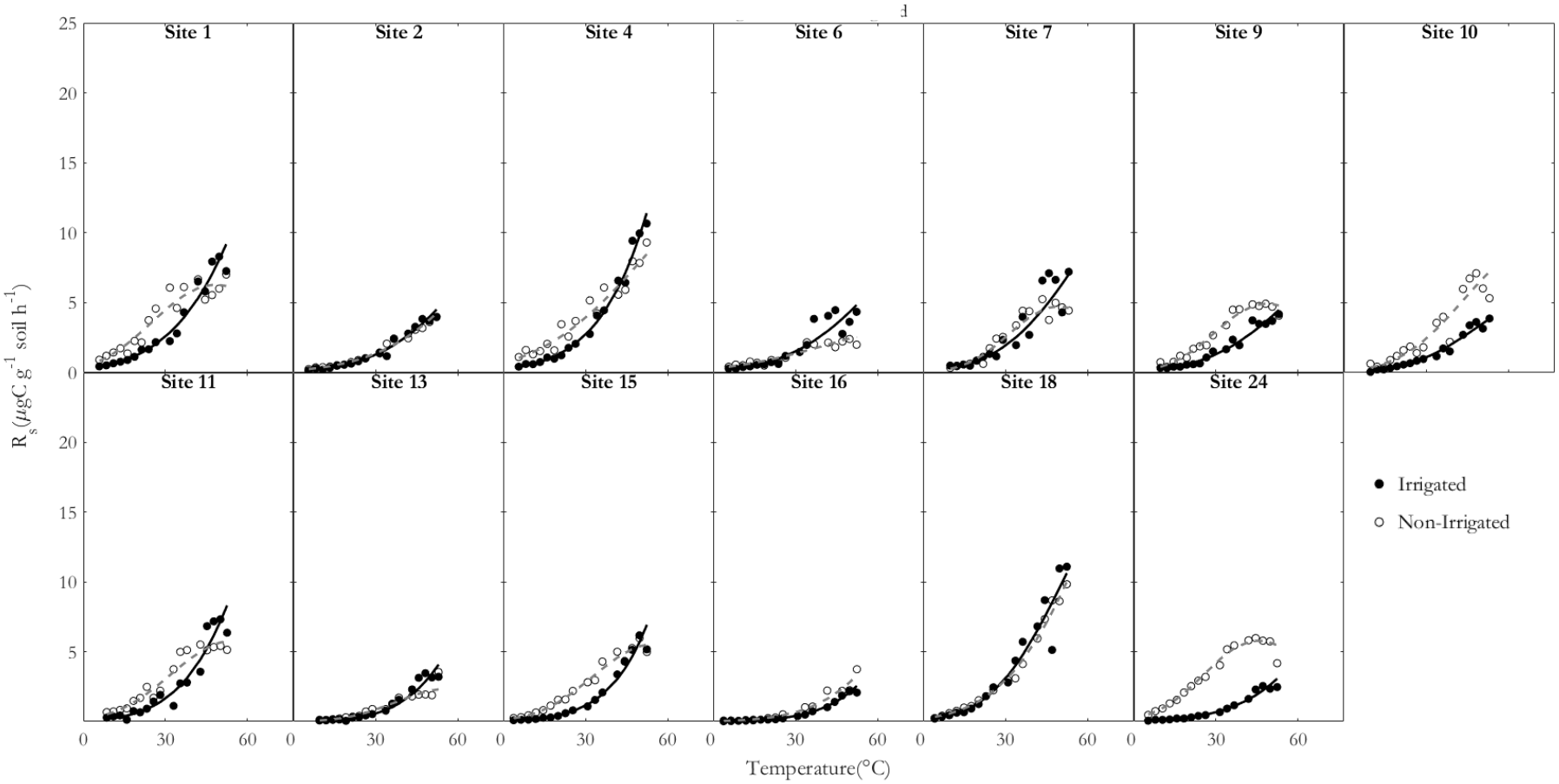

Figure A.7 Respiration rates in response to temperature at 80 %MWHC for the 13 irrigated and non-irrigated Canterbury sites fitted with the MMRT model. Sites are labelled in accordance with the original Canterbury sites from Mudge, et al. (2017)

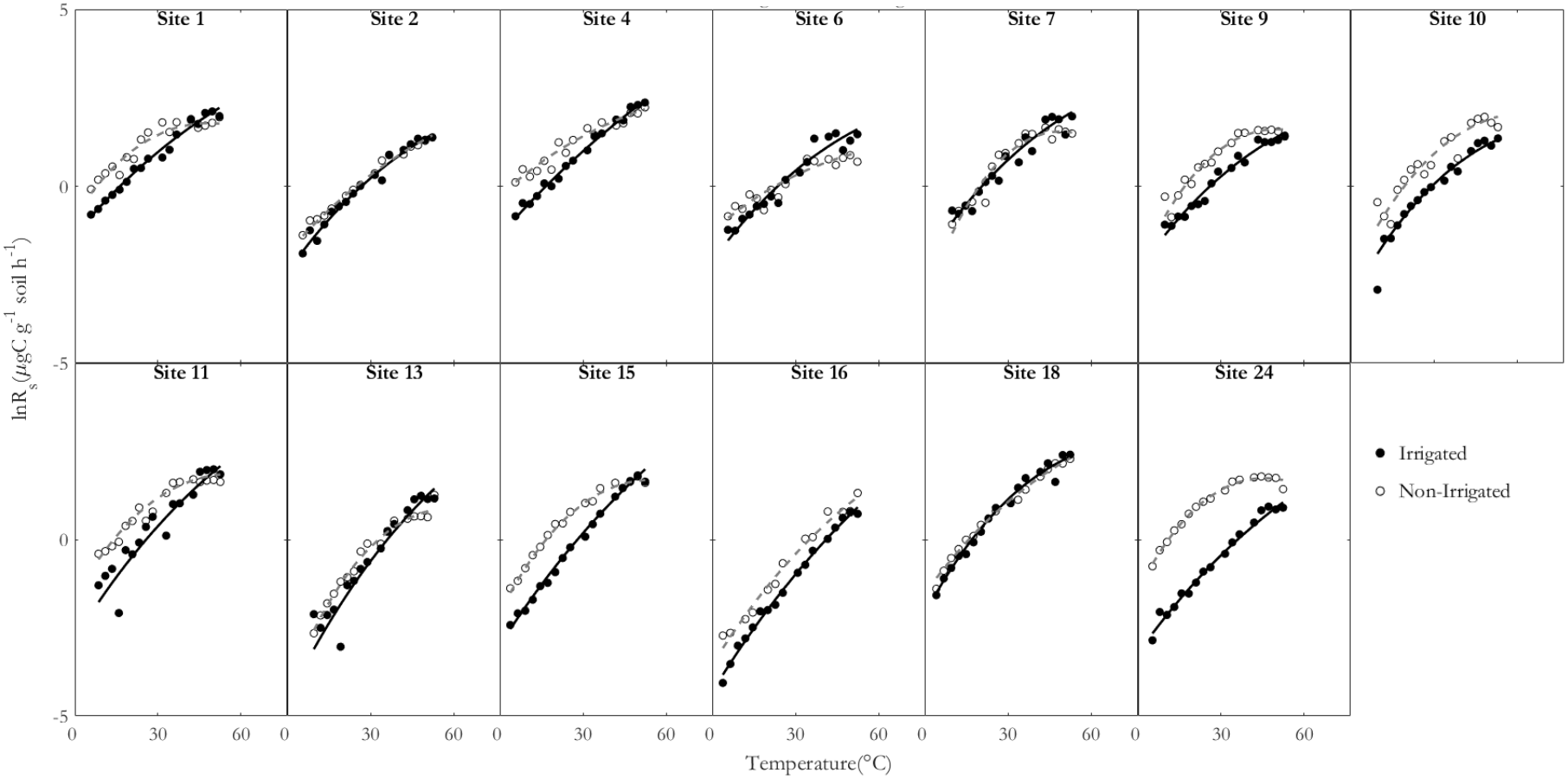

Figure A.8 Natural log of respiration rates in response to temperature at 80 %MWHC for the 13 irrigated and non-irrigated Canterbury sites fitted with the MMRT model. The sites are labelled in accordance with the original Canterbury sites from Mudge, et al. (2017)

Table A.1 Raw respiration data (µg C g^{-1} hr^{-1}) and calculated temperature (°C) for Canterbury site 1.

Temp	80 %MWHC		65 %MWHC		Temp	50 %MWHC		35 %MWHC		20 %MWHC	
	I	NI	I	NI		I	NI	I	NI	I	NI
6.823	0.150	0.251	0.248	0.251	5.542	0.399	0.232	0.145	0.168	0.088	0.045
9.363	0.288	0.380	0.282	0.351	8.136	0.378	0.280	0.213	0.102	0.143	0.088
11.903	0.213	0.394	0.301	0.447	10.730	0.418	0.373	0.232	0.266	0.143	0.122
14.443	0.340	0.437	0.481	0.520	13.324	0.448	0.463	0.274	0.171	0.191	0.167
16.984	0.487	0.532	0.220	0.694	15.919	0.578	0.488	0.342	0.456	0.176	0.207
19.524	0.562	0.577	0.669	0.875	18.513	0.649	0.719	0.428	0.535	0.109	0.281
22.064	0.642	0.763	1.082	1.067	21.107	0.827	0.869	0.544	0.932	0.329	0.356
24.604	0.815	0.905	0.919	1.190	23.701	1.120	1.059	0.703	0.731	0.416	0.465
27.144	1.038	1.010	1.442	1.613	26.295	1.364	1.284	0.947	1.085	0.571	0.627
32.225	1.375	1.441	1.767	1.948	31.484	1.766	1.520	1.270	0.797	0.689	0.733
34.765	1.181	2.073	2.339	2.529	34.078	2.168	2.106	1.863	1.841	1.173	1.158
37.305	2.410	2.439	3.017	2.893	36.672	2.925	2.465	2.531	2.343	1.450	1.295
42.386	2.791	2.461	4.648	3.569	41.861	3.681	2.979	3.328	2.682	1.970	1.922
44.926	3.278	3.070	5.302	3.743	44.455	4.346	3.499	4.121	3.914	2.570	2.514
47.466	3.839	3.195	5.996	4.455	47.049	5.696	4.220	5.622	4.158	3.414	2.944
50.006	3.724	3.623	6.430	4.049	49.643	6.077	4.015	6.090	4.231	3.774	3.490
52.546	3.991	3.970	6.267	4.148	52.237	5.761	4.127	5.807	3.599	4.743	4.009
55.087	2.523	3.034	5.916	4.062	54.832	5.181	3.412	2.847	3.264	4.979	3.277
57.627	4.383	3.033	6.667	3.163	57.426	4.444	3.395	4.906	3.384	5.109	3.450
60.167	3.992	2.791	6.590	2.694	60.020	4.525	2.738	4.331	1.000	4.890	2.919

Table A.2 Raw respiration data (µg C g^{-1} hr^{-1}) and calculated temperature (°C) for Canterbury site 2

Temp	80 %MWHC		65 %MWHC		Temp	50 %MWHC		35 %MWHC		20 %MWHC	
	I	NI	I	NI		I	NI	I	NI	I	NI
6.823	0.150	0.251	0.248	0.251	5.542	0.399	0.232	0.145	0.168	0.088	0.045
9.363	0.288	0.380	0.282	0.351	8.136	0.378	0.280	0.213	0.102	0.143	0.088
11.903	0.213	0.394	0.301	0.447	10.730	0.418	0.373	0.232	0.266	0.143	0.122
14.443	0.340	0.437	0.481	0.520	13.324	0.448	0.463	0.274	0.171	0.191	0.167
16.984	0.487	0.532	0.220	0.694	15.919	0.578	0.488	0.342	0.456	0.176	0.207
19.524	0.562	0.577	0.669	0.875	18.513	0.649	0.719	0.428	0.535	0.109	0.281
22.064	0.642	0.763	1.082	1.067	21.107	0.827	0.869	0.544	0.932	0.329	0.356
24.604	0.815	0.905	0.919	1.190	23.701	1.120	1.059	0.703	0.731	0.416	0.465
27.144	1.038	1.010	1.442	1.613	26.295	1.364	1.284	0.947	1.085	0.571	0.627
32.225	1.375	1.441	1.767	1.948	31.484	1.766	1.520	1.270	0.797	0.689	0.733
34.765	1.181	2.073	2.339	2.529	34.078	2.168	2.106	1.863	1.841	1.173	1.158
37.305	2.410	2.439	3.017	2.893	36.672	2.925	2.465	2.531	2.343	1.450	1.295
42.386	2.791	2.461	4.648	3.569	41.861	3.681	2.979	3.328	2.682	1.970	1.922
44.926	3.278	3.070	5.302	3.743	44.455	4.346	3.499	4.121	3.914	2.570	2.514
47.466	3.839	3.195	5.996	4.455	47.049	5.696	4.220	5.622	4.158	3.414	2.944
50.006	3.724	3.623	6.430	4.049	49.643	6.077	4.015	6.090	4.231	3.774	3.490
52.546	3.991	3.970	6.267	4.148	52.237	5.761	4.127	5.807	3.599	4.743	4.009
55.087	2.523	3.034	5.916	4.062	54.832	5.181	3.412	2.847	3.264	4.979	3.277
57.627	4.383	3.033	6.667	3.163	57.426	4.444	3.395	4.906	3.384	5.109	3.450
60.167	3.992	2.791	6.590	2.694	60.020	4.525	2.738	4.331	1.000	4.890	2.919

Table A.3 Raw respiration data (µg C g^{-1} hr^{-1}) and calculated temperature (°C) for Canterbury site 4

Temp	80 %MWHC		65 %MWHC		Temp	50 %MWHC		35 %MWHC		20 %MWHC	
	I	NI	I	NI		I	NI	I	NI	I	NI
5.525	0.428	1.118	0.263	1.401	5.518	0.149	0.355	0.135	0.326	0.011	0.101
8.123	0.621	1.618	0.476	1.230	8.114	0.201	0.427	0.183	0.395	0.041	0.022
10.722	0.605	1.312	0.498	1.630	10.711	0.285	0.756	0.256	0.701	-0.088	0.147
13.320	0.758	1.540	0.584	1.992	13.307	0.434	0.631	0.395	0.582	0.095	0.082
15.918	1.085	2.054	0.737	2.791	15.903	0.601	1.243	0.546	1.140	0.149	0.395
18.516	1.000	1.596	0.618	1.956	18.500	0.370	1.645	0.334	1.496	0.152	0.365
21.114	1.239	3.459	1.480	4.287	21.096	0.985	2.372	0.886	2.183	0.296	0.671
23.713	1.776	2.580	1.594	4.910	23.693	1.218	2.805	1.108	2.560	0.442	0.086
26.311	2.064	3.698	2.022	5.634	26.289	1.944	2.723	1.781	2.524	0.531	1.079
31.507	2.763	5.166	2.750	7.348	31.482	2.949	4.030	2.665	3.694	0.353	0.699
34.105	4.090	4.234	3.910	8.400	34.078	3.256	4.056	2.926	3.722	1.106	2.400
36.704	4.455	6.086	5.078	9.633	36.675	5.124	9.178	4.624	8.387	1.460	2.201
41.900	6.581	5.576	6.784	12.311	41.867	6.280	8.817	5.659	3.653	2.199	4.420
44.498	6.438	5.924	8.080	11.957	44.464	7.981	6.171	7.226	2.440	2.918	3.549
47.096	9.429	7.965	10.754	13.017	47.060	10.165	11.055	9.237	10.055	4.216	4.939
49.695	9.954	7.842	12.768	12.270	49.657	11.016	9.507	9.997	8.676	5.254	7.915
52.293	10.664	9.303	16.929	20.407	52.253	13.443	17.700	12.204	16.174	4.767	11.907
54.891	25.868	13.216	13.339	34.962	54.849	25.913	40.594	23.718	14.377	7.943	15.416
57.489	33.919	23.196	44.950	43.442	57.446	42.188	45.515	14.637	41.979	8.475	29.430
60.087	37.346	27.943	20.186	50.936	60.042	44.692	53.372	41.003	49.437	10.261	32.061

Table A.4 Raw respiration data (µg C g^{-1} hr^{-1}) and calculated temperature (°C) for Canterbury site 6

Temp	80 %MWHC		65 %MWHC		Temp	50 %MWHC		35 %MWHC		20 %MWHC	
	I	NI	I	NI		I	NI	I	NI	I	NI
5.794	0.292	0.428	0.190	0.372	5.654	0.170	0.268	0.326	0.080	-0.001	0.162
8.383	0.285	0.571	0.268	0.561	8.245	0.138	0.049	0.395	0.126	0.014	0.236
10.973	0.399	0.527	0.171	0.920	10.835	0.254	0.350	0.701	0.128	0.023	0.290
13.562	0.449	0.793	0.265	0.661	13.426	0.282	0.686	0.582	0.115	0.038	0.315
16.151	0.567	0.711	0.307	0.775	16.016	0.411	0.526	1.140	0.133	0.048	0.515
18.740	0.608	0.510	0.543	1.506	18.606	0.567	1.094	1.496	0.421	-	0.757
21.329	0.749	0.904	1.138	1.201	21.197	0.813	1.612	2.183	0.193	0.134	0.926
23.919	0.623	0.732	1.238	1.277	23.787	0.652	1.965	2.560	0.734	0.154	0.835
26.508	1.197	1.063	1.541	2.236	26.378	1.444	3.092	2.524	0.862	0.208	1.483
31.686	1.482	1.473	1.646	3.172	31.558	1.883	4.103	3.694	1.248	0.263	1.977
34.275	1.982	2.169	2.259	4.081	34.149	2.316	4.014	3.722	1.677	0.411	2.316
36.865	3.845	2.035	3.177	3.598	36.739	2.843	3.708	8.387	2.625	0.547	2.730
42.043	4.062	2.150	2.740	3.691	41.920	3.061	5.257	3.653	4.659	0.724	2.529
44.632	4.467	1.822	5.200	3.942	44.510	5.092	5.083	2.440	6.428	0.757	3.304
47.221	2.774	2.230	6.820	2.565	47.101	6.555	3.203	10.055	6.363	1.498	4.894
49.811	3.635	2.407	7.085	3.748	49.691	3.763	7.240	8.676	4.045	1.788	5.472
52.400	4.345	1.999	3.387	3.333	52.282	6.056	3.993	16.174	0.688	2.327	4.026
54.989	4.043	2.162	3.063	2.597	54.872	5.029	2.746	14.377	4.334	3.170	1.798
57.578	4.841	1.892	4.759	1.742	57.462	4.840	0.803	41.979	4.369	3.746	1.949
60.167	3.301	2.567	5.178	3.204	60.053	4.268	3.088	49.437	-	2.371	0.659

Table A.5 Raw respiration data (µg C g^{-1} hr^{-1}) and calculated temperature (°C) for Canterbury site 7

Temp	80 %MWHC		65 %MWHC		Temp	50 %MWHC		35 %MWHC		20 %MWHC	
	I	NI	I	NI		I	NI	I	NI	I	NI
9.890	0.503	-	0.062	0.435	9.888	0.169	0.185	0.244	0.171	-	-
12.290	0.459	0.342	0.167	0.346	12.287	0.280	0.135	0.285	0.206	-	-
14.690	0.576	0.497	0.397	0.614	14.686	0.338	0.317	0.335	0.269	-	-
17.090	0.495	0.581	0.891	0.383	17.086	0.417	0.446	0.460	0.358	-	-
19.489	0.853	0.644	1.163	0.954	19.485	0.603	0.520	0.602	0.427	-	-
21.889	1.126	0.869	1.662	1.106	21.884	0.388	0.675	0.664	0.536	-	-
24.289	1.343	0.628	1.999	0.982	24.283	1.014	0.823	0.880	0.745	-	-
26.689	1.170	1.747	2.446	1.702	26.682	1.129	1.260	1.154	0.923	-	-
29.089	2.346	2.433	2.989	1.945	29.082	1.814	1.398	1.620	1.042	-	-
33.888	1.968	2.555	3.955	2.787	33.880	1.424	2.129	1.235	1.059	-	-
36.288	3.989	3.375	4.162	3.662	36.279	3.530	2.617	3.304	2.563	-	-
38.688	2.700	4.419	6.454	4.615	38.678	3.770	3.670	4.373	2.564	-	-
43.487	6.588	4.389	8.245	3.630	43.477	6.079	4.167	5.198	3.994	-	-
45.887	7.108	5.252	9.352	5.704	45.876	8.874	2.619	2.844	5.018	-	-
48.287	6.635	3.767	9.308	5.230	48.275	4.120	0.038	8.156	2.636	-	-
50.687	4.312	4.992	10.001	5.031	50.674	4.348	5.052	4.072	4.875	-	-
53.087	7.208	4.665	10.426	6.050	53.074	5.227	4.975	4.401	4.729	-	-
55.486	5.341	4.439	13.939	3.253	55.473	5.606	4.187	2.210	0.265	-	-
57.886	7.392	2.812	4.119	3.139	57.872	5.135	2.666	3.387	3.267	-	-
60.286	5.407	0.350	-	2.863	60.271	3.373	3.510	3.184	4.090	-	-

Table A.6 Raw respiration data (µg C g^{-1} hr^{-1}) and calculated temperature (°C) for Canterbury site 9

Temp	80 %MWHC		65 %MWHC		Temp	50 %MWHC		35 %MWHC		20 %MWHC	
	I	NI	I	NI		I	NI	I	NI	I	NI
9.944	0.338	0.746	0.329	0.189	9.675	0.092	0.273	0.084	0.291	0.054	0.085
12.485	0.326	0.418	0.232	0.778	12.084	0.163	0.348	0.083	0.112	0.064	0.138
15.026	0.425	0.773	0.130	0.899	14.493	0.191	0.536	0.156	0.485	0.118	0.176
17.566	0.421	1.205	0.565	1.090	16.901	0.267	0.779	0.188	0.618	0.142	0.248
20.107	0.572	1.065	0.621	1.558	19.310	0.315	0.794	0.259	0.732	0.227	0.361
22.647	0.601	1.708	0.985	1.601	21.719	0.424	1.031	0.320	0.890	0.343	0.372
25.188	0.659	1.881	1.135	2.287	24.128	0.627	1.299	0.504	1.213	0.385	0.747
27.729	1.084	1.956	1.252	0.545	26.537	0.900	1.929	0.587	1.544	0.518	0.874
30.269	1.515	2.671	1.366	2.672	28.945	1.164	2.370	0.867	1.896	0.769	1.251
35.350	1.674	3.395	1.834	3.578	33.763	0.980	3.012	1.097	2.599	0.952	1.481
37.891	2.364	4.500	2.450	5.563	36.172	2.096	3.892	1.820	3.234	1.667	2.651
40.432	1.959	4.531	3.174	4.469	38.581	2.033	4.820	2.573	2.912	2.160	3.939
45.513	3.739	4.880	3.853	7.062	43.398	3.390	6.052	3.156	5.160	2.847	3.920
48.053	3.488	4.764	3.679	6.680	45.807	4.110	6.727	4.108	6.218	3.737	4.485
50.594	3.485	4.929	4.629	6.532	48.216	4.331	6.131	4.437	5.591	4.017	5.360
53.135	3.699	4.712	2.542	5.979	50.625	4.343	6.041	5.016	5.486	4.166	5.225
55.675	4.187	4.070	4.500	5.436	53.033	4.732	5.492	4.477	5.177	4.797	4.962
58.216	3.001	2.950	3.854	3.466	55.442	5.368	4.119	4.958	4.213	4.299	4.377
60.756	5.049	3.841	6.566	5.014	57.851	6.009	4.044	5.023	4.152	4.416	4.041
63.297	4.232	4.145	4.323	4.254	60.260	3.533	3.622	1.383	3.672	1.006	2.603

Table A.7 Raw respiration data (µg C g^{-1} hr^{-1}) and calculated temperature (°C) for Canterbury site 10

Temp	80 %MWHC		65 %MWHC		Temp	50 %MWHC		35 %MWHC		20 %MWHC	
	I	NI	I	NI		I	NI	I	NI	I	NI
9.693	-0.054	0.638	0.160	0.434	9.679	0.263	0.484	0.149	0.476	-	-
12.103	0.226	0.428	0.328	0.670	12.089	0.464	0.603	0.158	0.687	-	-
14.513	0.230	0.343	0.249	0.707	14.500	0.431	0.330	0.234	0.818	-	-
16.924	0.331	0.905	0.476	1.205	16.910	-0.042	1.092	0.312	0.936	-	-
19.334	0.456	1.197	0.642	1.346	19.321	0.204	1.304	0.179	1.258	-	-
21.745	0.573	1.607	0.612	1.516	21.732	0.646	1.664	0.469	1.526	-	-
24.155	0.675	1.867	0.656	2.032	24.142	0.890	0.552	0.591	1.905	-	-
26.565	0.847	1.403	0.895	2.472	26.553	1.231	1.651	0.671	2.425	-	-
28.976	0.975	1.815	1.221	2.880	28.963	1.397	3.120	0.941	2.613	-	-
33.797	1.171	3.565	0.952	3.791	33.785	1.669	3.631	0.679	3.241	-	-
36.207	1.734	3.993	1.139	4.757	36.195	2.148	4.962	1.166	4.594	-	-
38.617	1.522	2.197	2.427	5.247	38.606	2.160	5.672	2.331	6.028	-	-
43.438	2.705	5.981	3.854	6.162	43.427	2.231	6.890	3.010	6.842	-	-
45.849	3.383	6.730	3.673	7.544	45.838	4.366	7.840	3.621	8.125	-	-
48.259	3.626	7.104	3.524	7.985	48.248	4.872	8.267	2.828	9.581	-	-
50.669	3.153	6.021	3.116	7.858	50.659	5.128	8.939	4.811	4.243	-	-
53.080	3.872	5.324	3.515	10.479	53.069	4.975	9.724	4.335	2.331	-	-
55.490	4.199	8.366	5.561	12.435	55.480	5.149	5.057	5.505	8.641	-	-
57.901	5.314	16.243	7.575	13.346	57.891	9.590	11.010	4.414	8.752	-	-
60.311	7.006	10.026	7.750	24.116	60.301	10.045	10.781	5.445	8.423	-	-

Table A.8 Raw respiration data (µg C g^{-1} hr^{-1}) and calculated temperature (°C) for Canterbury site 11

Temp	80 %MWHC		65 %MWHC		Temp	50 %MWHC		35 %MWHC		20 %MWHC	
	I	NI	I	NI		I	NI	I	NI	I	NI
9.797	0.274	0.668	0.095	0.402	8.559	0.246	0.356	0.154	0.244	-	-
12.338	0.358	0.720	0.338	0.239	11.006	0.258	0.456	0.201	0.360	-	-
14.879	0.436	0.828	0.444	0.773	13.454	0.376	0.695	0.020	0.512	-	-
17.420	0.125	0.938	0.587	0.413	15.902	0.508	0.873	0.347	0.630	-	-
19.961	0.741	1.476	0.741	1.179	18.349	0.620	1.261	0.456	0.861	-	-
22.502	0.658	1.692	1.011	1.375	20.797	0.830	1.328	0.631	1.145	-	-
25.043	0.919	2.480	1.419	1.843	23.244	1.009	1.902	0.764	1.383	-	-
27.584	1.434	1.701	1.545	1.400	25.692	1.454	1.931	1.023	1.732	-	-
30.125	1.904	2.210	2.235	2.540	28.140	1.953	2.831	1.336	2.001	-	-
35.207	1.118	3.746	1.613	1.722	33.035	2.486	3.510	1.742	2.829	-	-
37.748	2.734	4.990	2.490	4.503	35.482	3.262	4.012	1.937	3.814	-	-
40.289	2.792	5.120	4.720	5.333	37.930	4.161	4.924	3.760	4.368	-	-
45.371	3.565	5.516	6.020	3.832	42.825	5.259	6.037	4.844	5.713	-	-
47.912	6.831	5.108	7.540	7.227	45.273	6.653	6.325	5.976	3.684	-	-
50.453	7.177	5.333	8.519	7.065	47.720	8.567	5.968	7.826	7.111	-	-
52.994	7.325	5.418	5.407	7.524	50.168	8.216	7.281	7.806	6.530	-	-
55.535	6.363	5.138	9.147	6.812	52.616	7.592	6.496	7.479	6.802	-	-
58.076	7.624	4.932	5.989	18.479	55.063	13.587	13.558	8.544	11.226	-	-
60.617	16.442	4.751	6.991	22.303	57.511	12.617	15.697	6.793	11.830	-	-
63.158	18.550	5.948	11.644	-	59.958	-	14.262	11.261	9.478	-	-

Table A.9 Raw respiration data (µg C g^{-1} hr^{-1}) and calculated temperature (°C) for Canterbury site 13

Temp	80 %MWHC		65 %MWHC		Temp	50 %MWHC		35 %MWHC		20 %MWHC	
	I	NI	I	NI		I	NI	I	NI	I	NI
9.884	0.121	0.070	0.027	0.103	9.537	-0.022	0.087	-0.015	0.116	-0.047	-0.038
12.278	0.082	0.117	0.029	0.156	11.949	0.032	0.149	0.007	0.125	-0.036	-0.025
14.671	0.118	0.164	0.044	0.165	14.361	0.071	0.271	0.048	0.207	-0.037	0.023
17.065	0.137	0.215	0.079	0.305	16.773	0.103	0.343	0.060	0.297	-0.020	-0.006
19.459	0.048	0.303	0.109	0.334	19.185	0.143	0.473	0.099	0.402	-0.011	0.061
21.852	0.274	0.344	0.138	0.448	21.597	0.211	0.638	0.161	0.537	-0.021	0.076
24.246	0.311	0.407	0.161	0.521	24.009	0.167	1.015	0.175	0.701	-0.002	0.099
26.639	0.436	0.714	0.208	0.557	26.421	0.387	1.110	0.272	0.938	0.014	0.177
29.033	0.529	0.893	0.297	0.699	28.833	0.614	1.508	0.373	1.005	0.010	0.195
33.820	0.777	0.887	0.419	0.815	33.657	0.809	2.185	0.498	1.353	0.037	0.299
36.214	1.248	1.275	0.651	1.147	36.069	1.109	2.403	0.955	2.218	0.054	0.583
38.607	1.554	1.709	0.909	1.618	38.481	1.627	3.091	1.384	2.538	0.104	0.868
43.395	2.301	1.803	1.219	1.674	43.305	2.267	3.892	1.909	3.416	0.124	1.233
45.788	3.131	1.928	1.586	1.143	45.717	2.917	4.117	2.567	4.038	0.166	1.822
48.182	3.462	1.940	1.813	2.799	48.129	3.811	5.428	3.442	4.462	0.203	2.379
50.575	3.159	1.884	2.025	3.387	50.541	4.035	6.146	4.316	5.634	0.315	2.726
52.969	3.205	3.545	2.888	5.447	52.953	4.587	9.086	5.688	9.443	0.408	4.228
55.363	9.071	8.336	5.981	11.556	55.365	11.765	15.636	11.260	13.728	0.562	6.311
57.756	12.298	9.398	6.952	5.023	57.777	17.257	22.271	16.169	9.404	0.702	8.567
60.150	15.243	5.505	8.038	5.949	60.189	21.243	24.368	19.067	10.017	0.928	10.414

Table A.10 Raw respiration data (µg C g^{-1} hr^{-1}) and calculated temperature (°C) for Canterbury site 15

Temp	80 %MWHC		65 %MWHC		Temp	50 %MWHC		35 %MWHC		20 %MWHC	
	I	NI	I	NI		I	NI	I	NI	I	NI
4.952	0.089	0.251	0.042	0.210	3.776	0.008	0.105	0.208	0.467	0.124	0.364
7.597	0.124	0.310	0.084	0.332	6.475	0.027	0.175	0.284	0.618	0.170	0.367
10.242	0.133	0.445	0.111	0.426	9.174	0.052	0.250	0.392	0.870	0.263	0.649
12.887	0.182	0.643	0.182	0.724	11.873	0.064	0.303	0.413	1.069	0.346	0.801
15.533	0.267	0.814	0.240	0.914	14.572	0.094	0.614	0.590	1.196	0.485	0.904
18.178	0.293	1.137	0.291	1.307	17.271	0.128	0.832	0.619	1.903	0.493	1.031
20.823	0.397	1.554	0.431	1.760	19.970	0.199	1.024	0.937	2.578	0.815	1.656
23.468	0.593	1.581	0.620	2.238	22.669	0.248	1.466	1.285	3.284	1.005	2.274
26.113	0.802	2.189	0.901	2.868	25.368	0.344	2.096	1.813	3.829	1.281	2.554
31.404	1.084	2.814	1.257	3.580	30.766	0.496	3.121	2.379	4.663	1.678	3.227
34.049	1.544	2.958	2.075	5.247	33.465	0.887	4.141	3.191	5.497	2.689	4.051
36.694	2.081	4.294	2.868	6.426	36.164	1.369	4.935	4.196	6.729	3.802	4.704
41.985	3.375	4.999	4.077	6.795	41.562	1.934	6.345	5.657	7.990	1.615	6.485
44.630	4.291	4.334	5.363	7.893	44.261	2.484	6.991	6.824	9.344	5.459	8.409
47.275	5.139	5.260	8.199	8.296	46.960	3.163	9.345	8.938	10.857	8.199	8.861
49.920	6.173	5.968	9.154	7.139	49.659	4.322	8.072	10.131	11.299	9.439	9.640
52.565	5.170	4.982	8.702	7.012	52.358	4.628	11.102	10.023	22.919	12.088	5.149
55.211	7.392	4.479	13.386	10.357	55.057	4.460	20.807	10.894	29.269	12.556	5.090
57.856	10.633	4.680	25.583	6.326	57.756	9.641	14.136	13.270	22.265	11.706	5.098
60.501	15.975	3.650	3.072	1.837	60.455	2.003	11.554	14.989	13.146	10.296	3.888

Table A.11 Raw respiration data (μg C g^{-1} hr^{-1}) and calculated temperature (°C) for Canterbury site 16

Temp	80 %MWHC		65 %MWHC		Temp	50 %MWHC		35 %MWHC		20 %MWHC	
	I	NI	I	NI		I	NI	I	NI	I	NI
4.021	0.017	0.066	0.066	0.084	3.818	0.097	0.149	0.023	0.062	0.024	0.003
6.707	0.029	0.071	0.158	0.205	6.516	0.127	0.179	0.022	0.089	0.054	0.024
9.394	0.049	-	0.164	-	9.214	0.154	-	0.036	-	0.083	-
12.080	0.061	0.105	0.227	0.302	11.912	0.168	0.273	0.093	0.178	0.119	0.033
14.767	0.083	0.126	0.304	0.435	14.610	0.254	0.488	0.175	0.418	0.219	0.117
17.453	0.131	-	0.366	-	17.308	0.329	-	0.210	-	0.267	-
20.139	0.136	0.242	0.544	0.785	20.006	0.493	0.623	0.345	0.460	0.375	0.201
22.826	0.157	0.285	0.792	1.247	22.704	0.559	0.152	0.416	0.623	0.493	0.297
25.512	0.221	0.512	1.003	1.603	25.402	0.795	1.325	0.650	1.383	0.581	0.668
30.885	0.390	-	1.208	-	30.798	1.139	-	0.926	-	0.822	-
33.571	0.489	1.025	1.903	2.090	33.496	1.854	1.553	1.497	1.998	1.277	1.247
36.258	0.730	1.072	2.641	3.182	36.194	2.655	2.582	2.111	2.715	1.860	1.803
41.631	1.020	2.216	3.687	3.811	41.590	3.180	4.036	2.938	4.767	2.439	3.471
44.317	1.403	-	4.070	-	44.288	4.383	-	3.677	-	3.258	-
47.003	1.854	2.194	5.383	5.431	46.986	6.352	5.930	4.929	5.206	4.716	4.364
49.690	2.163	2.243	5.824	6.312	49.684	6.603	6.316	5.730	5.882	5.374	1.748
52.376	2.073	3.745	6.130	5.628	52.382	6.430	5.445	5.756	5.342	5.898	5.071
55.063	2.311	-	6.168	-	55.080	6.473	-	5.703	-	5.615	-
57.749	3.197	4.139	7.906	9.158	57.778	6.829	7.678	5.099	6.083	5.878	4.627
60.435	4.090	4.271	7.185	9.666	60.476	5.307	8.636	2.421	6.118	5.232	4.461

Table A.12 Raw respiration data (µg C g^{-1} hr^{-1}) and calculated temperature (°C) for Canterbury site 18

Temp	80 %MWHC		65 %MWHC		Temp	50 %MWHC		35 %MWHC		20 %MWHC	
	I	NI	I	NI		I	NI	I	NI	I	NI
3.844	0.207	0.248	0.117	0.249	4.188	-0.033	-0.005	0.006	0.005	0.046	-0.047
6.549	0.332	0.414	0.229	0.321	6.866	-0.009	0.037	0.104	0.053	0.096	0.051
9.253	0.447	0.593	0.368	0.513	9.543	0.069	0.123	0.173	0.099	0.130	0.127
11.958	0.629	0.764	0.352	0.656	12.220	0.148	0.252	0.205	0.217	0.155	0.241
14.663	0.662	0.994	0.592	0.911	14.897	0.273	0.417	0.290	0.271	0.371	0.326
17.368	0.924	1.109	0.492	1.111	17.574	0.326	0.637	0.400	0.420	0.488	0.474
20.073	1.247	1.519	0.985	1.283	20.252	0.450	0.886	0.633	0.541	0.565	0.657
22.777	1.817	1.814	1.229	1.680	22.929	0.882	1.205	0.957	0.737	0.780	0.753
25.482	2.453	2.235	1.539	2.046	25.606	1.062	1.474	1.060	1.080	1.117	1.002
30.892	2.800	2.968	2.537	2.817	30.960	1.744	2.305	1.712	1.735	1.728	1.520
33.597	4.359	3.094	3.520	4.114	33.638	2.551	2.805	2.363	2.604	2.450	2.628
36.301	5.712	4.121	4.444	5.772	36.315	3.647	4.519	3.304	2.535	3.963	3.476
41.711	6.815	5.951	5.982	7.040	41.669	4.542	6.307	4.932	5.272	4.955	4.792
44.416	8.694	7.345	7.693	10.350	44.346	6.422	9.188	6.249	7.720	4.592	7.025
47.121	5.126	8.677	10.416	12.111	47.024	10.100	11.063	8.492	9.825	9.103	9.507
49.825	10.965	8.623	11.382	12.292	49.701	11.087	11.113	10.741	5.188	10.001	4.849
52.530	11.093	9.836	10.176	12.005	52.378	10.734	10.599	10.888	10.939	10.353	10.539
55.235	16.773	17.823	5.326	16.263	55.055	8.620	14.167	9.952	9.175	9.086	8.723
57.940	20.346	28.298	7.096	22.735	57.732	8.965	18.564	9.248	9.911	9.844	8.277
60.645	-	28.780	7.883	12.395	60.410	12.063	14.890	8.673	9.415	8.192	8.015

Table A.13 Raw respiration data (µg C g^{-1} hr^{-1}) and calculated temperature (°C) for Canterbury site 24

Temp	80 %MWHC		65 %MWHC		Temp	50 %MWHC		35 %MWHC		20 %MWHC	
	I	NI	I	NI		I	NI	I	NI	I	NI
4.631	0.058	0.470	0.050	0.463	5.207	0.017	0.577	-0.014	0.520	-0.028	0.317
7.263	0.129	0.741	0.036	0.759	7.831	0.072	0.733	0.023	0.730	-0.007	0.506
9.895	0.118	0.934	0.108	1.006	10.455	0.075	1.047	0.082	1.067	0.035	0.675
12.527	0.148	1.295	0.186	1.185	13.079	0.143	1.272	0.118	1.265	0.020	0.806
15.159	0.217	1.547	0.205	1.535	15.703	0.173	1.627	0.163	1.641	0.066	1.301
17.791	0.216	2.078	0.237	1.958	18.327	0.254	2.142	0.250	2.585	0.131	1.700
20.423	0.296	2.535	0.364	2.268	20.951	0.324	2.308	0.389	2.680	0.162	2.180
23.055	0.403	2.926	0.424	2.706	23.575	0.512	3.602	0.499	3.685	0.269	2.083
25.687	0.457	3.200	0.536	3.378	26.199	0.729	4.392	0.592	4.337	0.349	3.582
30.951	0.668	4.030	0.741	3.559	31.447	0.969	5.137	0.869	5.227	0.558	3.757
33.583	0.923	5.174	1.116	4.869	34.071	1.425	6.929	1.382	5.524	0.706	4.449
36.215	1.160	5.451	1.278	5.392	36.695	1.969	8.334	1.956	7.313	1.011	5.503
41.479	1.620	5.816	1.316	5.945	41.943	2.640	9.778	2.584	8.172	1.409	5.946
44.111	2.292	5.978	2.341	6.851	44.567	3.383	9.175	3.158	10.010	1.977	7.049
46.743	2.546	5.790	3.241	6.466	47.191	4.787	8.801	3.831	4.554	2.638	7.148
49.375	2.350	5.743	3.279	6.141	49.815	4.246	8.248	4.471	3.976	3.084	6.878
52.007	2.466	4.180	2.941	4.844	52.439	4.354	7.894	4.849	3.489	3.714	5.993
54.639	2.425	7.122	3.074	6.732	55.063	4.297	8.979	4.488	3.522	4.134	6.033
57.271	2.714	8.596	3.159	9.911	57.687	4.577	9.012	4.802	2.927	4.447	5.591
59.903	2.858	8.206	1.615	6.422	60.311	4.175	9.362	1.790	3.159	3.935	6.050

Table A.14 Raw respiration data (µg C g^{-1} hr^{-1}) and calculated temperature (°C) from the February sampling at Rangiriri site 1

Temp	80 %MWHC		65 %MWHC		Temp	50 %MWHC		35 %MWHC		20 %MWHC	
	I	NI	I	NI		I	NI	I	NI	I	NI
5.553	0.701	0.413	0.189	0.253	6.823	0.234	0.276	0.160	0.173	0.095	0.120
8.093	0.915	0.425	0.289	0.362	9.363	0.340	0.374	0.279	0.236	0.180	0.175
10.633	1.224	0.620	0.617	0.516	11.903	0.589	0.453	0.400	0.367	0.285	0.297
13.173	1.219	0.845	0.576	0.564	14.443	0.603	0.592	0.539	0.514	0.399	0.346
15.713	1.336	0.983	0.865	0.920	16.984	0.817	0.680	0.695	0.721	0.500	0.493
18.254	2.061	1.588	1.185	1.243	19.524	0.554	1.110	1.074	0.918	0.670	0.688
20.794	2.447	1.821	1.482	1.663	22.064	1.492	1.457	1.503	1.213	0.996	0.956
23.334	3.268	2.270	2.373	2.294	24.604	2.272	1.985	2.045	1.760	1.528	1.197
25.874	5.060	3.122	3.301	2.554	27.144	3.276	2.680	2.965	2.207	2.210	1.621
30.955	6.416	3.819	3.842	4.051	32.225	3.863	3.271	3.813	2.987	2.761	2.178
33.495	8.370	5.054	6.831	5.873	34.765	5.762	4.908	5.739	4.705	4.257	3.582
36.035	11.036	6.254	9.183	7.842	37.305	7.952	6.793	7.911	6.130	6.280	4.955
41.115	18.599	7.935	11.862	10.854	42.386	10.648	8.790	10.648	7.938	8.296	6.734
43.656	18.111	10.532	15.461	13.693	44.926	13.469	11.505	16.883	10.367	10.805	8.733
46.196	19.821	13.625	18.053	16.852	47.466	4.728	15.966	18.047	14.888	14.175	13.005
48.736	24.040	15.533	19.013	19.535	50.006	19.110	17.064	24.296	16.930	13.628	15.502
51.276	48.135	20.959	24.843	24.384	52.546	25.037	22.235	48.461	21.014	18.220	15.104
53.816	63.994	34.143	56.211	39.591	55.087	42.843	35.595	70.013	31.781	23.465	19.037
56.357	64.470	40.581	70.595	50.551	57.627	63.777	44.782	68.857	51.139	33.628	26.492
58.897	-	56.006	75.574	74.855	60.167	75.783	59.706	-	48.589	41.444	40.550

Table A.15 Raw respiration data (µg C g^{-1} hr^{-1}) and calculated temperature (°C) from the February sampling at Rangiriri site 2

Temp	80 %MWHC		65 %MWHC		Temp	50 %MWHC		35 %MWHC		20 %MWHC	
	I	NI	I	NI		I	NI	I	NI	I	NI
5.553	0.597	0.594	0.242	0.319	6.823	0.306	0.272	0.297	0.243	0.113	0.130
8.093	0.604	0.700	0.345	0.365	9.363	0.344	0.417	0.313	0.264	0.139	0.173
10.633	0.748	0.858	0.508	0.467	11.903	0.432	0.600	0.388	0.437	0.259	0.279
13.173	0.860	1.460	0.488	0.794	14.443	0.664	0.708	0.483	0.518	0.304	0.355
15.713	1.370	1.846	0.673	0.888	16.984	0.794	1.097	0.582	0.830	0.408	0.542
18.254	1.710	2.166	0.944	1.309	19.524	1.196	1.238	0.852	1.075	0.525	0.632
20.794	2.117	2.712	1.540	1.730	22.064	1.451	2.193	1.278	1.476	0.752	0.840
23.334	2.340	3.747	2.253	2.484	24.604	2.293	2.500	1.686	1.895	1.058	1.150
25.874	3.427	4.535	2.506	2.586	27.144	2.391	3.113	2.064	2.591	1.535	1.693
30.955	4.720	5.922	3.480	3.307	32.225	3.325	3.595	4.424	3.137	1.907	2.159
33.495	5.408	7.216	5.098	5.237	34.765	4.809	4.829	4.980	4.533	3.050	3.474
36.035	6.680	9.718	6.061	7.218	37.305	5.511	7.067	5.126	6.157	4.076	4.462
41.115	8.041	10.248	9.307	7.538	42.386	8.340	9.768	6.858	8.709	5.279	6.566
43.656	10.536	14.582	0.078	12.944	44.926	9.551	12.587	7.489	10.889	7.484	10.628
46.196	12.095	15.090	10.833	14.672	47.466	11.604	13.547	11.190	13.377	8.899	13.892
48.736	12.898	15.339	11.513	12.986	50.006	2.122	14.213	16.270	13.947	10.702	13.280
51.276	16.106	19.193	11.955	13.389	52.546	16.172	18.551	14.550	14.657	10.433	11.894
53.816	27.496	31.528	28.167	25.725	55.087	27.238	30.031	22.707	23.166	13.472	16.011
56.357	34.797	43.092	26.961	27.401	57.627	26.976	39.464	30.877	24.507	16.028	16.403
58.897	-	-	20.230	15.405	60.167	36.307	31.874	22.867	17.107	14.403	13.100

Table A.16 Raw respiration data (µg C g^{-1} hr^{-1}) and calculated temperature (°C) from the February sampling at June site 1

Temp	80 %MWHC		65 %MWHC		Temp	50 %MWHC		35 %MWHC		20 %MWHC	
	I	NI	I	NI		I	NI	I	NI	I	NI
6.823	-	-	0.124	0.124	5.553	0.203	0.752	0.145	0.386	0.050	0.076
9.363	-	-	0.393	0.081	8.093	0.931	0.741	0.869	0.454	0.082	0.083
11.903	-	-	0.498	0.258	10.633	1.296	0.913	0.758	0.482	0.096	0.129
14.443	-	-	0.201	0.321	13.173	0.535	1.188	0.470	0.714	0.129	0.142
16.984	-	-	0.995	0.501	15.713	2.390	1.543	0.595	1.014	0.212	0.180
19.524	-	-	0.603	0.621	18.254	1.180	1.830	1.158	1.347	0.271	0.255
22.064	-	-	1.566	0.855	20.794	5.698	2.327	3.073	1.393	0.280	0.367
24.604	-	-	2.884	0.791	23.334	3.920	2.155	3.574	2.139	0.521	0.568
27.144	-	-	3.220	1.677	25.874	7.351	4.578	5.640	2.767	0.705	0.730
32.225	-	-	2.721	2.312	30.955	5.289	5.007	4.274	3.501	0.970	1.084
34.765	-	-	6.558	1.905	33.495	12.040	4.866	5.916	3.113	1.691	1.841
37.305	-	-	5.466	4.538	36.035	8.150	4.214	8.995	5.602	2.101	2.368
42.386	-	-	5.350	5.471	41.115	10.982	7.498	9.479	4.616	3.254	3.330
44.926	-	-	13.633	4.411	43.656	20.941	5.177	17.054	6.908	4.219	4.633
47.466	-	-	16.028	6.394	46.196	23.787	10.896	18.109	7.879	5.468	5.556
50.006	-	-	18.729	8.761	48.736	28.860	12.119	19.119	7.878	6.490	6.840
52.546	-	-	25.720	12.281	51.276	39.732	14.455	21.090	9.942	7.775	6.741
55.087	-	-	30.755	24.029	53.816	40.430	6.263	33.080	6.119	11.288	12.536
57.627	-	-	73.226	32.666	56.357	88.370	30.698	15.751	11.033	5.551	6.967
60.167	-	-	73.124	37.066	58.897	109.837	56.761	-	5.616	8.087	7.400

Table A.17 Raw respiration data (µg C g^{-1} hr^{-1}) and calculated temperature (°C) from the June sampling at Rangiriri site 2

Temp	80 %MWHC		65 %MWHC		Temp	50 %MWHC		35 %MWHC		20 %MWHC	
	I	NI	I	NI		I	NI	I	NI	I	NI
6.823	-	-	0.100	0.132	5.553	0.487	0.651	0.350	0.417	0.023	0.011
9.363	-	-	0.150	-0.170	8.093	0.557	0.742	0.362	0.146	0.054	0.013
11.903	-	-	0.083	0.245	10.633	0.867	1.030	0.472	0.606	0.064	0.018
14.443	-	-	0.099	0.375	13.173	0.717	0.619	0.349	0.802	0.098	0.032
16.984	-	-	0.236	0.539	15.713	0.664	1.546	0.807	0.923	0.118	0.040
19.524	-	-	0.517	0.675	18.254	1.029	1.785	0.967	1.103	0.143	0.059
22.064	-	-	0.693	0.409	20.794	1.910	1.495	0.905	0.741	0.206	0.086
24.604	-	-	0.665	1.135	23.334	2.161	2.748	1.486	0.945	0.265	0.106
27.144	-	-	1.190	1.343	25.874	3.020	3.275	2.578	2.159	0.370	0.152
32.225	-	-	1.369	0.833	30.955	1.937	3.484	2.241	2.737	0.449	0.196
34.765	-	-	1.205	1.035	33.495	3.146	3.042	3.229	1.875	1.039	0.337
37.305	-	-	2.129	2.919	36.035	5.810	6.249	4.232	3.425	1.480	0.418
42.386	-	-	1.418	1.549	41.115	6.788	4.463	4.855	3.025	1.490	0.574
44.926	-	-	1.997	4.462	43.656	6.183	8.422	5.323	6.205	2.100	0.938
47.466	-	-	3.599	4.876	46.196	7.497	6.612	6.111	6.619	2.759	1.161
50.006	-	-	2.416	4.081	48.736	8.640	6.732	6.063	6.573	3.330	1.869
52.546	-	-	6.675	7.921	51.276	7.782	9.136	7.029	5.039	3.291	2.399
55.087	-	-	10.422	14.382	53.816	14.532	13.987	9.482	7.068	4.036	3.274
57.627	-	-	13.320	21.328	56.357	21.294	10.143	6.470	8.304	5.183	3.487
60.167	-	-	17.001	18.623	58.897	34.543	17.095	3.457	2.096	5.389	4.453