Pre-Publication of Parts of this thesis.

Chapter 3 – a manuscript from this section has been accepted for publication as follows: Uchida, Y., Clough, T.J., Kelliher, F.M., Sherlock, R.R. 2010. Soil microbial respiration responses to changing temperature and substrate availability in fertile grassland. *Australian Journal of Soil Research* Vol. 48 No. 5 Pages 395-403.

Chapter 4 – a manuscript from this section has been accepted for publication as follows: Uchida, Y., Hunt, J.E., Barbour, M.M., Clough, T.J., Kelliher, F.M., Sherlock, R.R. 2010. Soil properties and presence of plants affect the temperature sensitivity of carbon dioxide production by soils. *Plant and Soil* DOI: 10.1007/s11104-010-0533-9.

The Effects of Substrate, Temperature and Soil Fertility on Respiration and N_2O Production in Pastoral Soils

---

A thesis
submitted in partial fulfilment
of the requirements for the Degree of
Doctor of Philosophy

at
Lincoln University
by
Yoshitaka Uchida

Lincoln University
2010

Abstract of a thesis submitted in partial fulfilment of the requirements for the Degree of Doctor of Philosophy.

The Effects of Substrate, Temperature and Soil Fertility on Respiration and N_2O Production in Pastoral Soils

by

Yoshitaka Uchida

Soil respiration (R_S) and N_2O emissions from pastoral ecosystems are responsible for a substantial portion of global greenhouse gas budget. The soil processes responsible for R_S and N_2O emissions are sensitive to soil temperature (T_S). However, there are many points which are uncertain in this temperature sensitivity of soil processes, because of the complexity of the mechanisms controlling the processes. The temperature sensitivity is defined as a proportional increase of the rate of soil process or activity per a unit change of soil temperature. An important factor controlling the temperature response of the soil processes is substrate availability. Hence the objectives of this research were (1) to quantify the interaction between temperature and soil substrate availability on soil microbial activity in the absence of plant substrate inputs, (2) to determine the temperature sensitivity of respiration sourced from root-derived C (R_{RD}) and soil organic matter decomposition (R_{OM}) in two pasture soils of contrasting nutrient status, and (3) to investigate the effects of a urine deposition events on R_S and N_2O fluxes.

The first experiment measured the changes in soil microbial respiration (R_M) without plants present at 3°, 9°, and 24°C. At 9° and 24°C, R_M was significantly reduced within 2 days while R_M remained constant for 14 days at 3°C. The decrease in R_M at higher T_S was caused by substrate depletion but the substrate depletion was not indicated by the soil's water soluble and hot-water soluble C. The first experiment showed that at higher T_S, soil microbes could access soil C that was not accessible to soil microbes at lower T_S. The second experiment focused on the temperature sensitivity of R_S with plants present in two soils with contrasting nutrient status (fertility). The components of R_S; R_{RD} and R_{OM} were separatedly measured using a natural ^{13}C abundance technique. The results suggested the temperature sensitivity of R_{OM} was significantly reduced in the low fertility soil only when plants were actively

growing, while the temperature sensitivity of R_{RD} was unaffected by soil nutrient status. Finally the third experiment investigated the changes in soil N_2O emissions and R_S following a urine deposition event on a pasture soil at various T_S (11°, 19°, and 23°C) with or without plants present. Soil moisture was increased from 50% to 70% water-filled pore space at 21 days following the urine deposition event. Soil-N contributed to soil N_2O emissions only at the early phase of the experiment, especially at higher T_S, and the contribution was lesser when plants were present. The presence of plants increased N_2O flux particularly when soil moisture contents were high, and when T_S was > 19°C. Urine application primed soil C and increased the rate of R_S. The magnitude of urine-induced priming of soil C was relatively larger at lower T_S, and was larger when plants were absent based on the estimation from cumulative R_S. Based on the results obtained using a natural ^{13}C abundance, when plants were present, urine application increased the contribution of R_{OM} to R_S particularly at 19°C.

This study indicates that the responses of soil N_2O fluxes and R_S to temperature changes are markedly affected by plant presence and soil nutrient status. Both urine addition and plant activity primed R_S but the magnitude of the priming effect was influenced by other factors (e.g. T_S). To accurately predict the global warming feedback of belowground soil processes, the factors affecting the aboveground plant activity (e.g. soil nutrient status) have to be taken into account.

Keywords: Carbon, pasture, water soluble C, soil temperature, soil respiration, atmospheric CO_2, organic matter, ^{13}C, root exudate C.

Acknowledgements

I would like to sincerely thank my supervisors, Professor Francis Kelliher and Associate Professor Timothy Clough, for their great supervision. I would like to thank Dr John Hunt, Dr Margaret Barbour and Associate Professor Rob Sherlock for their advice and help for this work.

Thanks to all the technicians in the department.

Many thanks to Lincoln University for providing me with a stipend and to Manaaki Whenua Landcare Research for funding this project from their capability fund. This study was funded by and was performed in Manaaki Whenua Landcare Research, Lincoln, New Zealand. I thank Graeme Rogers, J. Richard Sedcole and Takayoshi Ikeda for their technical support and advice on the statistical analysis.

To Aya, Tom, Yukiko, Rika, Risa, Jolon, Yumi, Tomohiro, Connie, Shizuka, and everyone else who encouraged me all the time, thanks all of you.

I thank my God.

Finally I wish to thank my parents and my sister for their love and support.

Table of Contents

List of Tables

List of Figures

List of Abbreviations

C	Carbon	
CO_2	Carbon dioxide EGM	Environmental gas monitor
F_{CO_2}	Soil carbon dioxide flux	
GPP	Gross primary production	
N	Nitrogen	
N_2O	Nitrous oxide	
NEP	Net ecosystem respiration	
NPP	Net primary production	
R_E	Ecosystem respiration	
R_H	Heterotrophic respiration	
R_{LEAF}	Leaf respiration	
R_M	Soil microbial respiration	
R_{OM}	Soil organic matter decomposition	
R_{RD}	Root derived carbon respiration	
R_{RHIZ}	Rhizosphere respiration	
R_{ROOT}	Root respiration	
R_S	Soil respiration	
TDL	Tunable diode laser	
T_S	Soil temperature	

Chapter 1
Introduction

1.1 Background

This thesis reports the outcomes of a PhD research project where the overall theme of the study was to identify the effects of substrate, temperature and soil fertility on soil respiration (R_S) and N_2O emissions from pastoral soils. Soil respiration occurs as a result of microbial or plant processes. The substrate supply for R_S is affected by soil temperature (T_S) (Jacobson and Alexander, 1980; Kirschbaum, 2006). Carbon (C) substrates for soil microbes are provided by plants but there is considerable debate around the potential interactions that occur between C substrate inputs from plants, soil microbial activity, and T_S (Pendall et al., 2008).

Rates of R_S are very sensitive to changes in T_S. One model suggests that a 1°C increase of T_S at 10°C causes a 10% increase in R_S (Lloyd and Taylor, 1994). It is hypothesised that a small change in R_S could have a significant impact on atmospheric CO_2 concentrations since terrestrial ecosystems, including vegetation and soils, are one of main reservoirs of C on Earth, containing 2200 Gt C, or approximately three times the C in the atmosphere (Schimel, 1995).

According to the Intergovernmental Panel on Climate Change (IPCC), the global average surface temperature has been increasing and this trend is likely to continue (IPCC, 2007a). Future climate scenarios have been assessed and a warming of 0.2°C per decade is projected. It is considered likely that global warming will influence R_S and soil N_2O emissions but uncertainty remains high with regards to the magnitude of the influences caused by global warming (Rustad et al., 2000).

Soil N_2O emissions are generated by microbial nitrification and denitrification processes and these processes are regulated by temperature. Soil N_2O emissions from pastoral ecosystems are an important contributor to the global N_2O budget (Oenema et al., 1997) and global warming is also expected to impact upon N_2O emissions from soils (Smith, 1997). Despite this there are very few studies that have examined the effects of temperature on N_2O fluxes in pastoral ecosystems and none that have examined the effect of T_S changes on urine-N derived N_2O fluxes.

If the uncertainty surrounding the prediction of future changes in R_S and soil N_2O emissions, with respect to global warming, is to be reduced then the C substrate supplying processes

from the plants must also be considered. The availability and the amount of the C substrate required to perform R_S may be reduced or depleted when plants are not actively growing and plant activity is influenced by temperature. Carbon substrate is also required for soil N_2O emissions when N_2O is produced via heterotrophic processes. Hence, the temperature response of R_S and soil N_2O emissions is controlled by the complex interaction of soil/plant/animal processes and the interaction is not well understood.

1.2 Research objectives

The objectives of this project were to:

- Quantify the interaction between T_S and soil substrate availability on soil microbial activity in the absence of plant substrate inputs; Chapter 3.
- Determine the temperature response of respiration sourced from root-derived C (R_{RD}) and soil organic matter decomposition (R_{OM}) in two pasture soils of contrasting nutrient status, with and without plants present; Chapter 4.
- Investigate the effects of urine application, T_S, and plants on soil N_2O and carbon dioxide (CO_2) fluxes; Chapter 5.

1.3 Thesis structure

This thesis is divided into 6 chapters. The first and second chapters provide a general introduction and a review of the relevant literature, respectively. The following three chapters (3, 4 and 5) contain short introductions, materials and methods, results and discussion sections. Finally chapter six provides an overall discussion, summary and recommendations for future work. In brief each chapter contains the following:

Chapter 1 This chapter briefly introduces information on processes pertaining to R_S and N_2O emissions in pasture soils and the global importance of these processes.

Chapter 2 This chapter is a literature review that describes the current knowledge of R_S and soil N_2O emissions from pasture soils. It has been suggested that increasing global temperature will exert a positive feedback with respect to global warming. However, there are many sources of uncertainty in the prediction of future R_S as the review shows. To the best of my knowledge there are very few if any studies that have examined the implications of global warming with respect to N cycling in pasture soils and associated N_2O

emissions. This review discusses these aspects and states a case for the research performed.

Chapter 3 This chapter discusses the responses of soil microbial respiration to changes in T_S and substrate availability. The experiment was performed using a fertile pasture soil without plants present.

Chapter 4 This chapter describes the responses of root-derived C respiration (R_{RD}) and soil organic matter decomposition (R_{OM}) to changing T_S, in two pasture soils of contrasting nutrient status. The experiment was performed using soils with plants, and R_{RD} and R_{OM} were separated using a natural ^{13}C stable isotope abundance technique.

Chapter 5 This chapter reports a laboratory experiment that focused on N_2O emissions and R_S following a bovine urine deposition event at different T_S in the presence of plants. A natural ^{13}C abundance technique was used in this experiment to determine CO_2 sources while the bovine urine was ^{15}N-enriched in order to assess the N source of the N_2O emissions.

Chapter 6 This chapter summarizes the major findings reported in this thesis and contains recommendations for future research.

Chapter 2
Literature Review

2.1 Introduction

Pastoral ecosystems provide most of the milk, wool and much of the meat for human consumption (Humphreys, 1997). The increasing global population has been placing pressure on pastoral ecosystems to increase their production efficiency. At the same time, there is an increasing demand for pastoral ecosystem production to be performed in an environmentally sustainable way (White, 2000).

Pastoral ecosystems play a key role in regulating global carbon (C) and nitrogen (N) budgets because pastoral soils are an important reservoir of both organic C and N (Amundson, 2001). Unlike forest ecosystems, pastoral ecosystems are dominated by fast-growing plant species and they are disturbed by grazing animals (Bardgett and Wardle, 2003). In pastoral ecosystems plants assimilate C via photosynthesis. Plants also need inorganic forms of N for their growth. The ruminant grazing animal subsequently disaggregates the C and N combined in the herbage pool, with N redistributed predominantly in the urine stream while C dominates in the dung stream. There is also a considerable loss of C in the form of methane (CH_4) during enteric fermentation (Johnson and Johnson, 1995). The latter is not considered hereafter. Carbon and N may also be returned to the pasture soil via plant litter (Schuman et al., 1999). Subsequently some of the C and N deposited onto the pasture soil, in the various organic pools, may be returned to the atmosphere as carbon dioxide (CO_2) via soil respiration (R_S) processes or as nitrogenous gases such as ammonia and nitrous oxide (N_2O) via N transformation processes (Flessa et al., 2002).

Nitrous oxide is a greenhouse gas and a precursor to compounds responsible for stratospheric ozone depletion (Ravishankara et al., 2009). Currently atmospheric concentrations of N_2O are increasing at 0.26% yr^{-1} (Forster et al., 2007). Worldwide, grazed pastures are responsible for more than 10% of the global N_2O emissions (Oenema et al., 1997). Concentrations of atmospheric CO_2 are also increasing at a rate of 1.4 µl l^{-1} yr^{-1} mainly due to anthropogenic activities such as the combustion of fossil fuels (Forster et al., 2007).

Despite the global efforts of governments and organizations to reduce greenhouse gas emissions, the Intergovernmental Panel on Climate Change (IPCC) concluded in its fourth Assessment Report (2007b) that both greenhouse gas emissions and the global average

surface temperature have been increasing and that these trends are likely to continue. Future atmospheric CO_2 concentrations, simulated by the coupled climate-carbon cycle models, range between 730 and 1020 µl l^{-1} by 2100, the former value is approximately twice the atmospheric CO_2 concentration in 2005 (Meehl et al., 2007). An expert assessment performed by the IPCC concluded that if the atmospheric concentrations of greenhouse gases continue to increase at the current rate then average global surface temperatures by 2090 to 2099 are predicted to increase by as much as 4.0°C (range of 2.4°C to 6.4°C) compared to the period 1980-1999. (Meehl et al., 2007).

Such an increase in the global surface temperature could potentially alter many enzymatic activities in pastoral ecosystems, e.g. photosynthesis and soil microbial activities. For example, studies have reported that global warming will reduce the efficiency of terrestrial ecosystems to absorb atmospheric CO_2 (e.g. Cox et al., 2000) because under a warmer climate, R_S is more likely to increase compared to net primary productivity. As a result, the IPCC has predicted that global warming will increase the fraction of anthropogenic derived CO_2 that remains in the atmosphere thus leading to an additional increase in the atmospheric CO_2 concentration (Denman et al., 2007).

However, the response of soil processes to changes in soil temperature (T_S) depends on many factors (Luo, 2007). For example, the temperature sensitivity of R_S, the relative increase in R_S per unit increase in T_S, decreases with increasing T_S (Lloyd and Taylor, 1994). Hence, the effect of global warming on soil processes may depend on the average value of T_S at the site (Lloyd and Taylor, 1994; Tjoelker et al., 2001). Heterotrophic R_S and N_2O emissions depend on C substrate availability and this availability influences the response of soil heterotrophic processes to T_S. For example, R_S has been shown to be less sensitive to T_S when R_S utilises the more readily available C substrates (Davidson and Janssens, 2006) or when substrate concentration is low (Davidson et al., 2006). Other factors such as plant activity can also influence the substrate supply and availability in soils and the relationship between aboveground plant processes and belowground soil processes are still not fully understood (Atkin et al., 2005; Pendall et al., 2008).

Currently there are no studies that have simultaneously examined the temperature sensitivity of both C and N cycling in grazed pasture ecosystems and very few studies have been performed to investigate the temperature sensitivity of soil processes in pastoral ecosystems. Undoubtedly this is a result of the complexity of the issues. In this literature review, the current understanding of the temperature sensitivities of both C and N dynamics in pastoral ecosystems are reviewed.

2.2 Soil respiration in pastoral ecosystems

2.2.1 Carbon cycling in pastoral ecosystems

In pastoral ecosystems, the largest C input occurs via photosynthesis and is subsequently termed gross primary productivity (*GPP*). Gross primary productivity was estimated to range from 100 to 120 Pg C yr^{-1} (Houghton and Woodwell, 1989). Plants fix CO_2 from the air and assimilate it forming organic C compounds through photosynthesis. A portion of the organic C fixed by plants is utilised to supply the plants with energy. This process is called plant respiration and CO_2 is released back into the atmosphere through this process. Plant respiration is also called autotrophic respiration and can be separated into aboveground (R_{LEAF}) and belowground plant respiration (R_{ROOT}). A pasture plant (*Lolium perenne*) allocates about 50% of the total assimilated C to the belowground pool (Domanski et al., 2001). Photosynthetically assimilated C is translocated rapidly to the soil. For example, within 24 h, approximately 12% of the total assimilated C in annual ryegrass plants (*Lolium multiflorum* Lam.) was found in the soil (Butler et al., 2004). Similarly, a study performed on a dairy farm pasture reported that 1.2 – 4.0% of the C uptake by plant leaves was transferred to the soil within 4 h (Saggar and Hedley, 2001).

Pastoral ecosystems also produce CO_2 during the microbial decomposition of litter and soil organic matter, a process called 'heterotrophic respiration (R_H)'. Soil microbial respiration also occurs in the rhizosphere, a zone immediately adjacent to the root surface (Richards, 1987), and this respiration process is called microbial rhizosphere respiration (R_{RHIZ}). Thus, the total CO_2 emission from a pastoral ecosystem (R_E) can be estimated by eqn. 2.1:

$$R_E = R_{LEAF} + R_{ROOT} + R_{RHIZ} + R_H \quad (2.1)$$

The total CO_2 emission from the soil surface, total soil respiration (R_S), is the sum of the belowground plant respiration (R_{ROOT}), microbial rhizosphere respiration (R_{RHIZ}) and the heterotrophic respiration (R_H) components (eqn. 2.2):

$$R_S = R_{ROOT} + R_{RHIZ} + R_H \quad (2.2)$$

Net primary production (*NPP*) is defined as *GPP* less the cost of autotrophic respiration and is a measure of the rate at which energy is stored by plants in the form of organic substances (Redmann, 1992) thus it is defined as (eqn. 2.3):

$$NPP = GPP - R_{LEAF} - R_{ROOT} \quad (2.3)$$

Net ecosystem production (*NEP*) is classified as the total CO_2 exchange at an ecosystem level and is the *GPP* less ecosystem respiration (R_E) and is defined as eqn. 2.4:

$$NEP = GPP - R_E \quad (2.4)$$

It has long been known that aboveground processes, for example, *GPP* strongly control belowground C inputs and R_S (Raich and Nadelhoffer, 1989). However, specific mechanisms of belowground CO_2 production processes in soil are less clear (Pendall et al., 2008). Continuous studies on the belowground processes in pastoral ecosystems are still required because pastoral soils contain approximately 30% of global soil C stocks (White et al., 2000) and are considered as a sink or source for CO_2 (Johnson et al., 1995). A review article on R_S in pastoral ecosystems has shown that reported values of R_S in temperate grassland vary from 52 to 1004 g C m^{-2} yr^{-1} and that the factors controlling R_S (e.g. temperature and grazing) vary among sites, with the review concluding that the reasons for the variability were poorly understood (Wang and Fang, 2009).

2.2.2 Soil respiration processes

Total soil respiration (R_S) is the sum of the respiration processes that occur in belowground plant tissues and soil microbial activity (eqn. 2.2). Physiologically, respiration is a series of metabolic processes that break down organic C containing compounds to liberate energy, water, and CO_2 in a cell (Luo and Zhou, 2006). Carbon-containing compounds are used as the substrate for R_S. The C substrates in soils are derived from organic material and these can be separated into fresh, incompletely decomposed material, and well decomposed material known as 'humus' (Kononova, 1966). Since these C compounds decay and accumulate at different rates (Schlesinger, 1977), R_S can be delineated according to sources of C substrate supply. This section of the literature review outlines each of the R_S processes according to the C substrate source utilised.

2.2.2.1 Root respiration

Root respiration utilises relatively labile C substrates, for example, sugars, proteins, lipids, and other materials produced within the plant (Thornley, 1970). In a laboratory experiment using ryegrass plants, R_{ROOT} accounted for between 1.5 and 6.5% of the photosynthetically assimilated C over an 8 day period (Kuzyakov et al., 1999). Photosynthetically fixed C has been shown to be respired by roots within 30 minutes, thus the shoot to root transport of C in plants is a very rapid process (Weixin et al., 1993). Total plant respiration, including R_{LEAF} and R_{ROOT}, is therefore strongly related to photosynthesis and plant growth (Lloyd and Farquhar, 2008). The maintenance respiration rate of roots is linearly correlated to the root

weight (Veen, 1981). In general, root production in pastoral ecosystems is proportional to the aboveground production but the relationship between shoot and root growth is dynamic due to seasononal and plant growth stage effects and grazing events (Garwood, 1967; Kuzyakov et al., 1999; Mattew and Yang, 1998; Meharg and Killham, 1990; Schuman et al., 1999).

2.2.2.2 Rhizosphere respiration and root-derived C respiration

The release of substances into the soil from root surfaces is termed rhizodeposition, and because of this process the soil adjacent to the root presents a favourable habitat for soil microorganisms and is termed the rhizosphere (Shamroot et al., 1968). In the rhizosphere, the population density of soil microbes is higher when compared to that of the bulk soil (Buyer et al., 2002; Waldrop and Firestone, 2004a). The microbes in the rhizosphere respire using the substrates released from the roots (R_{RHIZ}). A previous study using ryegrass plants has reported that 2.0 to 8.0% of the photosynthetically assimilated C was respired in the rhizosphere within 8 days, and these values were similar to those of R_{ROOT} from the same study noted above (Kuzyakov et al., 1999).

Both the plant roots and the microbial rhizosphere respiration processes utilise the recently produced C substrates from plants (Lynch and Whipps, 1990). Many previous studies have termed the sum of the respiration that occurs as a result of R_{ROOT} and R_{RHIZ}, autotrophic respiration, R_A (e.g. Olsson et al., 2005; Tang and Baldocchi, 2005). However, this frequently used term is incorrect because microbes in the rhizosphere decomposing the recently added C substrates are heterotrophic microbes (Bond-Lamberty et al., 2004; Kuzyakov, 2006).

Hence, in this thesis, the terms 'root-derived C respiration (R_{RD})' and 'soil organic matter C decomposition (R_{OM})' will be used. The root-derived C respiration is defined as the sum of R_{ROOT} and R_{RHIZ} since they utilise the recently produced soil C substrates from plants. Similar to this approach, Kirschbaum (2004) modelled soil C substrate dynamics using a system consisting of just two substrate pools, a fast and a slow pool and the same approach has been taken in other previous studies (e.g. Melillo et al., 2002; Trumbore, 2000). Soil organic matter C decomposition is defined below (section 2.2.2.3).

According to a review article, the contributions of R_{RD} to R_S can be as high as 90% hence R_{RD} is a very important component of R_S (Hanson et al., 2000). A large percentage (64 to 86%) of the C substrates added to soils are rapidly respired but about 2 to 5% of the net C assimilated by plants remains in the soil (Hütsch et al., 2002) and plays a critical role in the formation of soil organic matter. Soil organic matter is relatively more stable and less microbially available compared to the root-derived C. Despite this fact the less labile soil organic matter can be

utilised for respiration although the breakdown of soil organic matter involves complex processes.

2.2.2.3 Decomposition of soil organic matter and 'R_{OM}'

A large proportion of the soil organic matter pool is stabilised and protected from physical, chemical, and/or biochemical decomposition (Six et al., 2002). Hence, soil organic matter is decomposed very slowly when compared to the root-derived C pool. The size of the soil organic matter C pool is much larger than the size of the root-derived C pool in the soil (Gaudinski et al., 2000). Hence, in the absence of rhizodeposition soil microbes can only utilise the existing soil organic matter pool and they produce CO_2 at a relatively low but constant rate, over long periods (Joergensen et al., 1990). In forest soils the average age of soil organic matter C, based on ^{14}C measurements, ranged from 200 to 1200 yr, but the age of the C respired averaged 7 yr because the predomiant C source for R_S was R_{RD} (Trumbore, 2000). Similarly, the age of soil organic matter C in pasture soils can be up to 12,000 years in deep (80 cm depth) soils at high altitude but the age of C measured in R_S from pastoral soils has been reported to range from 15 to 31 yr (Townsend and Vitousek, 1995). Hence, the contribution of R_{OM} to R_S is relatively low when plants are present. However, the soil organic matter C pool is considerably larger than the root-derived C pool and so interest in changes in the rate of soil organic matter decomposition, as a consequence of changes in T_S, has increased because of its importance to the global C cycle (Trumbore, 2000).

2.2.2.4 Priming effect

The presence of living plants may increase R_{OM} by 3- to 5-fold, or decrease its rates by 10% to 30% (Kuzyakov, 2002). This change in R_{OM} is due to a process termed the 'priming effect' (Parnas, 1976). In a review paper by Kuzyakov (2002), seven possible mechanisms to explain priming effects were suggested: 1) the presence of plants results in faster drying-rewetting cycles in soil, thus increasing R_{OM}: 2) breaking down of soil aggregates by growing roots exposes a portion of the physically protected soil organic matter pool to soil microbial attack: 3) plant root uptake of organic substances reduces the size of available soil organic matter pool for microbes, thus reducing R_{OM}: 4) increasing microbial activity in the rhizosphere enhances microbial turnover and consequently increases the release of mineral N and CO_2: 5) the presence of plants induces competition for mineral N between plant roots and soil microbes, thereby depressing R_{OM}: 6) soil microbes prefer to respire the root-derived C compared with soil organic matter C, resulting in a decrease in R_{OM} due to the presence of plant root exudation: 7) soil microbial activity increases in the rhizosphere and this leads to an

increase in R_{OM} in order to obtain limiting N that has become limiting as a result of the enhanced microbial activity.

It has been concluded that although all of the mechanisms above are involved in priming, the biological factors are considered to be more important than the physical factors (Dormaar, 1990). A reason for this is that the growth stage of plants and the intensity of photosynthesis both influence the magnitude of the priming effect (Kuzyakov and Cheng, 2001a; Kuzyakov et al., 2001). A previous study has suggested that the absence of light caused a reduction in the priming effect because root-derived C inputs from the plant (wheat) decreased in the absence of photosynthesis (Kuzyakov and Cheng, 2001a). The priming effect may also be influenced by soil fertility because plants allocate a relatively larger percentage of their net C assimilation to the belowground C pool in low fertility soils compared to high fertility soils (Warembourg and Esterlich, 2001). In addition, the mineral N status in soils, which is influenced by soil fertility, may influence priming effects because R_{OM} is enhanced when soil mineral N is low (Bremer and Kuikman, 1997).

However, very few studies have investigated the combined effects of different plants or soils on R_{OM} in one experiment (Kuzyakov, 2002) and further studies are required to elucidate the role of plants.

2.2.3 Factors controlling soil respiration

The components of R_S discussed above involve many different chemical, physical, and biological processes and these are influenced by various biotic and abiotic factors. These factors include substrate quantity and availability, soil fertility and temperature.

2.2.3.1 Substrate quantity and availability

In soils, respiration sourced from root-derived C is influenced by plant photosynthesis and plant growth rates since the root-derived C substrates are exuded by or sloughed off plant roots. The residence times of root-derived C substrates are relatively short. In pasture soils R_S was shown to decrease by 70% within a week following the application of clipping and shading treatments to aboveground vegetation (Craine et al., 1999; Wan and Luo, 2003). Clipping and shading treatments reduce photosynthesis by limiting the light received by the plants, thus there is a clear link between whole plant C gain and R_S. In pastoral ecosystems, a large proportion of R_S originates from the decomposition of recently added and highly available root-derived C substrates from plants (Domanski et al., 2001).

Without plants present, R_S in pastoral soils decreases rapidly but can continue at a low rate for an extensive period of time. Feng and Simpson (2009) reported that without plants present, R_S

in a grassland soil was 12.6 µg CO_2 kg^{-1} s^{-1} at 1 d following the start of an incubation at 20°C, the R_S rate decreased 40% in the first week and then the R_S rate slowly decreased to be 1.3 µg CO_2 kg^{-1} s^{-1} after 370 d. This decline was due to soil microbes only having more recalcitrant C substrates available for respiration, compared to the root-derived C substrates. Plant residues are normally the last to start degrading and are decomposed relatively slowly (Berg et al., 1982). However, the decomposition of recalcitrant C is not limited by substrate supply because recalcitrant C is relatively abundant in soils. In a pasture soil, it was shown that only 1.4 – 3.8% of total soil C was available to microbes and the rest was relatively recalcitrant, when the soil was incubated at 20° - 40°C for 225 d (Townsend et al., 1997).

2.2.3.2 Temperature

The relationship between temperature and the biochemical respiration process can be described by an exponential equation (van't Hoff, 1884) or an Arrhenius equation (Arrhenius, 1898). To express the influence of temperature on R_S, the term "temperature sensitivity" has been recently used in studies of R_S (e.g. Davidson and Janssens, 2006). This term defines the relative change in R_S caused by a particular range of temperature change. Temperature sensitivity is often shown as a Q_{10} value (Singh and Gupta, 1977). The Q_{10} value is a factor that represents the increase in R_S rates when a temperature is raised by 10°C. If there is an exponential relationship between R_S and temperature, then the following equation (eqn. 2.5) can be used to calculate the Q_{10} value by assuming Q_{10} is a constant over the temperature range, where R_2 and R_1 are R_S observed at temperatures T_2 and T_1 respectively (Fang and Moncrieff, 2001).

$$Q_{10} = \left(\frac{R_2}{R_1}\right)^{10(T_2 - T_1)} \tag{2.5}$$

Early studies of R_S often expressed the relationship between R_S and temperature using a constant Q_{10} value of approximately 2 (Singh and Gupta, 1977). However, a more recent study suggested that the use of a constant Q_{10} value did not express the relationship very well. Lloyd and Taylor (1994) reported that the Q_{10} value changed widely over different temperature ranges and the Q_{10} value was larger at a lower temperature range. Hence, according to Lloyd and Taylor (1994), when no other factors are limiting R_S, the rate of R_S and T_S have an Arrhenius type relationship as follows (eqn. 2.6):

$$R = R_{10} e^{E_0\left(\frac{1}{283.15 - T_0} - \frac{1}{T_S - T_0}\right)} \tag{2.6}$$

where R_{10} is the rate of R_S at 10°C and E_0 is a parameter related to the activation energy. The parameter T_0 describes a temperature (K) when $R = 0$. Using the Lloyd and Taylor model (eqn. 2.6), a 1°C increase in temperature increases R_S by 10% at 10°C when no other factors are limiting R_S (Lloyd and Taylor, 1994). Other limiting factors of R_S can be nutrient supply, soil moisture, and the availability of soil organic matter. This is notable given the current predictions of future mean global surface temperature at 2090 to 2099 may be as much as 4°C higher compared to the mean global temperature over the period 1980 to 1999.

2.2.3.3 Effects of substrate supply and availability on the temperature sensitivity of soil respiration

Despite many experimental reports, there is still no scientific consensus on the temperature sensitivity of R_S (Kirschbaum, 2006). Kirschbaum (2006) concluded that substrate availability played a critical role in determing the temperature sensitivity of R_S, and that if the substrate availability changed during the period of measurement then this would confound the resulting temperature sensitivity of R_S. For example, when no factors but temperature are affecting R_S, the faster R_S that occurs at a warmer temperature depletes the available C pool relatively quicker, thus reducing the R_S rate. All things being equal substrate depletion is less likely to occur under cooler conditions. Hence, changes in C substrate pools at different temperature may bias the inferred temperature sensitivity (Gu et al., 2004).

An increase in T_S can also increase plant photosynthesis and subsequent root-derived C substrate supply. Substrate depletion of the root-derived C pool can occur if the rate of C input is smaller than the R_S rate. For example, Piao et al. (2008) reported that, based on the analysis of the changes in the atmospheric CO_2 concentration over the past two decades, there was a trend towards an earlier autumn-to-winter CO_2 build-up in the atmosphere, suggesting increasing soil C losses in autumn. Piao et al. (2008) concluded that while both plant photosynthesis and R_S increased during autumn warming the increase in R_S was greater. Autumn temperatures over the research period focused on by Piao et al. (2008) rose by 0.8°C over two decades. Conversely, Piao et al. (2008) found spring warming enhanced C sequestration because of a longer growing season and greater photosynthetic activity due to an increase in the average spring T_S (1.1°C) over the two decade study period. Piao et al. (2008) concluded that the increase in R_S during autumn warming was offsetting 90% of the increased CO_2 uptake during spring and that the predicted increase in global surface temperature may change this balance between R_S and CO_2 uptake. Hence, the relationship between substrate supply and R_S can contribute significant uncertainty to future projections for the effects of global warming on terrestrial ecosystems (Luo, 2007).

The temperature sensitivity of R_S also increases with the increasing molecular complexity of the C substrate (Bosatta and Ågren, 1999; Davidson and Janssens, 2006). Hence, in soils, organic matter decomposition is theoretically more temperature sensitive compared to respiration sourced from root-derived C because the molecular complexity of root-derived C is less than that of organic C. Work by Hartley and Ineson (2008), supporting this theory, showed that without plant C inputs, the temperature sensitivity of R_S increased significantly as incubation time increased. Hartley and Ineson (2008) incubated a garden soil for 124 d without plants over a temperature range of 10° to 20°C. The Q_{10} values increased from 3.0 at day 7 to 3.2 at day 124, as the recalcitrance of the soil C increased. However, equal temperature sensitivities have also been reported for the decomposition of soil C with variable recalcitrance (Bååth and Wallander, 2003; Conen et al., 2006). Davidson et al. (2006) have proposed that the variability in the temperature sensitivities of R_S are due to the multiplicative effects of several temperature-sensitive processes, namely, diffusion of oxygen and soluble C substrates through the air and water phases of the soil, diffusion of C substrates across cellular membranes, growth of microbial populations and root tissues, and enzyme activity. Bradford et al. (2008) also reported, using a > 15 year soil warming experiment, that in the long-term, the temperature sensitivity of R_S was influenced not only by the substrate depletion but also by the thermal adaptation of microbial respiration. Further research is required in this area to improve the understanding of the variability in temperature sensitivities of R_S.

2.2.3.4 Soil fertility and microbial biomass

Soil respiration is strongly related to *NPP* of plants (section 2.2.1) and soil fertility is a key factor determining the *NPP* (Robertson et al., 1997). In this thesis, soil fertility is defined as the soil's nutrient availability. Soil fertility controls the decomposition rate of soil C substrates (Melillo et al., 1982). For example, an experiment performed in Hawaiian forests showed that N and phosphorus (P) availability controlled the decomposition of litter in soil (Hobbie and Vitousek, 2000). A similar study performed in a grassland ecosystem has concluded that soil microbial respiration was limited by P availability, whereas N limited soil microbial respiration in soils with high levels of available P (Amador and Jones, 1993). A study by Hobbie and Vitousek (2000) found that other factors in addition to nutrient availability, for example, the abundance of soil fauna (i.e. earthworms and insects), also controlled the rates of R_S.

Soil fertility influences soil microbial community structure and population (Mawdsley and Bardgett, 1997; Orchard et al., 1992) and in pastoral ecosystems, R_S has been shown to be related to soil N status. For example, Lovell et al. (1995) reported that soils receiving

continuous inputs of N fertiliser over a long term had a reduced root mass compared to unfertilised soils which subsequently resulted in lower microbial biomass and lower R_S. Bardgett et al. (1999) also observed a biomass effect when they measured higher R_S from low-input, unfertilised grasslands than from intensively managed systems and they concluded this was because of the reduced size of the active microbial biomass in intensively managed systems. It was shown by Bardgett et al. (1999) that the proportion of fungi relative to bacteria increased in the unfertilised grasslands, suggesting the microbial community structure also plays a key role in determining R_S response. Further studies are required to determine the significance of soil fertility in pastoral ecosystems with respect to R_S.

2.2.3.5 Urine effects on soil respiration and C substrate supply

Soil organic matter decomposition rates (R_{OM}) respond positively to urine applications. For example, Kelliher et al. (2005b) showed that following a urine application, R_{OM} in a sieved pasture soil increased for 9 d when compared to R_{OM} in a control soil. Plant growth and plant nutrient uptake have also been shown to be improved for approximately 2 months following urine deposition onto pasture (During and McNaught, 1961). Given the significance of the relationship between plant growth and R_S described above (section 2.2.1) it would be expected that this enhanced plant growth would enhance root-derived C supply. However, Vines and Wedding (1960) reported that the large concentrations of ammonia reduced the respiration sourced from root-derived C because of the ammonia's potential toxic effect, although plants were treated with gaseous ammonia rather than urine-derived ammonia in this study.

Urine deposition onto pasture ultimately leads to higher plant N content (Bol et al., 2004). As N content increases so too does the photosynthetic capacity of plants (Schulze et al., 1994). Thus this increases the supply of root-derived C. In fact Burton et al. (2000) stated that R_{RD} was more dependent on N availability than T_S. The study by Burton et al. (2000) proposed that greater metabolic activity of roots in N-rich zones lead to greater C allocation to those roots, and thus increases in R_{RD} (at a given temperature) occur when a soil's available N content is higher. Ruminant urine deposition also increases soil microbial utilisation of C substrates, based on a community level physiological profiles analysis, and increases the number of bacteria which in turn affects C substrate respiration (section 2.2.3.4) (Williams et al., 2000).

Shand et al. (2002) reported significant root scorching and the death of roots following urine deposition as a result of the ensuing high soil pH (~ 9.0). Approximately 6% of the live roots had been killed 56 d after urine treatment (Shand et al., 2002). Dead roots are readily

decomposed by soil microorganisms (Larionova et al., 2006). In addition, solubilisation of soil organic C has been shown to occur following urine application. Water-soluble C levels increased from 104 to 1090 mg C kg^{-1} within 5 h following a urine application when the urine was applied to a clipped pasture soil (Monaghan and Barraclough, 1993). Thus urine deposition has an indirect effect on R_S by stimulating the release of C substrate suitable for R_S. Contrary to this are the results of Ambus et al. (2007) who stated that R_S was unaffected by urine deposition and that the increase of soil CO_2 fluxes was mainly due to urea hydrolysis. However, Clough and Kelliher (2005) showed that, in a pasture soil at 20°C, CO_2 produced from R_S following a urine application was greater than the amount of CO_2 that could have been produced solely from the urine. Clough and Kelliher (2005) concluded that the priming effect was induced by the urine application and this lead to the increase (45%) in R_S.

2.2.4 Methods to investigate the factors controlling soil respiration in pasture soils

Ecosystem models predict an increase in R_S in response to increasing T_S but with high degrees of uncertainty (Schimel et al., 1994). One of the areas in which the models are not successful is predicting which of the C pools is utilised (soil organic matter or root-derived C) (Zobitz et al., 2008). As noted above this literature review does not emphasize the distinction between R_{ROOT} and R_{RHIZ} and the sum of the two respiration pathways is termed 'R_{RD}'. Theoretically, it is possible to separately quantify R_{ROOT} and R_{RHIZ} but currently available methods have difficulty in accurately quantifying the two components (Kuzyakov, 2004).

A review article (Hanson et al., 2000) concluded that the approaches for measuring R_{RD} and R_{OM} could be broadly separated into three categories: component integration, root exclusion, and isotopic approaches. Isotopic methods have an advantage over component integration and root exclusion methods because they avoid the disturbance effects and they can measure R_{RD} and R_{OM} concurrently. The isotopic method approach is described in section 2.2.4.1. Other methods involve the removal of roots from soils and are based on the assumption that the R_{OM} and R_{RD} rates are not influenced by the removal of roots and other disturbance to the ecosystem. When the component parts of R_S (i.e., roots and bulk soil) are separated, uncontrolled factors, e.g. soil moisture, may influence the respiration processes and create artefacts. The disadvantage of isotopic methods is the complexity of the experimental setup and the difficulty and cost of analytical measurements since isotopic methods often involve the artificial labelling of plants with a tracer. To overcome the disadvantage of isotopic methods natural ^{13}C abundance techniques have been developed and Hanson et al. (2000)

have concluded that natural ^{13}C abundance techniques will become an increasingly popular for determining R_{OM} and R_{RD}.

2.2.4.1 Natural ^{13}C abundance technique

There are two naturally occurring stable isotopes of C (^{12}C and ^{13}C) and the natural abundance of C in atmospheric CO_2 is 1.1%. The ratio of $^{13}CO_2$ to $^{12}CO_2$ is used to generate a value termed 'delta thirteen C' ($\delta^{13}C$ which is expressed as follows in units of ‰, pronounced "per mill" (eqn. 2.7).

$$\delta^{13}\mathrm{C} = \left[\frac{R(\mathrm{sample})}{R(\mathrm{standard})} - 1 \right] \times 1000 \qquad (2.7)$$

where R is the ratio of ^{13}C to ^{12}C in the sample or standard. The R (standard) is an established reference for ^{13}C and is called Pee Dee Belemnite (PDB) standard. The $\delta^{13}C$ value of atmospheric CO_2 recorded recently in 2009 was -8.28‰ based on the extended record derived from INSTAAR/NOAA $\delta^{13}C$ (CO_2) discrete measurements at Mauna Loa (Tans, 2009). Plant materials have a different $\delta^{13}C$ value because enzymes involving in photosynthesis discriminate against $^{13}CO_2$. The mechanisms of the discrimination differ between C_3 and C_4 plants because different enzymes operate in C_3 and C_4 photosynthesis pathways (Bender, 1968). The C_3 plants are generally temperate species which follow the Calvin cycle of photosynthesis. The C_4 plants are tropical plants and they have a relatively more efficient enzyme to fix CO_2 but the enzyme requires a relatively high temperature to efficiently operate. The name "C_4" comes from the fact that they initially fix CO_2 in four-carbon dicarboxylic acids (Kortschak et al., 1965).

The $\delta^{13}C$ abundance values in C_3 and C_4 plant tissues range from -25 to -32‰ and -12 to -15‰, respectively (Boutton et al., 1999). Plant residues are the dominant source of soil organic matter in pasture soils. Hence, soil with a long-term vegetative cover of either C_3 or C_4 plants will have soil $\delta^{13}C$ abundances that reflect those of the respective C_3 or C_4 plant material grown (Schönwitz et al., 1986). The difference between the C_3 and C_4 plants and soils can be utilised to discriminate soil CO_2 flux sources. For example, if C_3 plants are growing on a 'C_4 soil', where soil organic matter has been produced, over time, from C_4 plant organic matter, then it is possible to determine the CO_2 flux source(s).

The fraction of CO_2 derived from root-derived C (f_{RD}) can be calculated using the following mass balance equation (eqn. 2.8; Lin et al., 1999):

$$f_{RD} = \frac{\delta^{13}C_{SOIL} - \delta^{13}C_{OM}}{\delta^{13}C_{ROOT} - \delta^{13}C_{OM}} \quad (2.8)$$

where the subscripts 'SOIL', 'OM', and 'ROOT' refer to the CO_2 respired from the soil surface (i.e. R_S), the CO_2 respired from the background soil (R_{OM}), and the CO_2 respired from the roots (R_{RD}), respectively (Lin et al., 1999). Previous studies have successfully separated R_{OM} and R_{RD} using this technique (Millard et al., 2008; Rochette et al., 1999). The method has been used to show that R_{OM} and R_{RD} are influenced by season and soil moisture in a broad-leaved forest (Sakata et al., 2007). It has also been used in a boreal mixed coniferous forest where it was shown that weather conditions markedly influenced R_{RD} but not R_{OM} (Ekblad and Högberg, 2001). However, no previous studies have investigated the temperature sensitivity of R_{OM} and R_{RD} in pastoral soils using the natural ^{13}C abundance technique.

2.3 Soil N_2O emissions

2.3.1 The importance of soil N_2O emissions

The concentration of N_2O in the atmosphere has increased linearly by 0.26% yr^{-1} for the period 1998 – 2005 and equalled 319 ± 0.21 µl l^{-1} in 2005 (Forster et al., 2007). While uncertainty remains with respect to the total global sources of N_2O, agriculture is believed to contribute about 80% of the anthropogenic emissions of N_2O, and of this livestock production systems contribute about 30% of the emissions (Davidson and Mosier, 2004; Oenema et al., 2001). The total N_2O emissions from animal production systems were estimated to be 1.5 Tg N in 2000 (Oenema et al., 2005). Agricultural sources of N_2O include; synthetic fertilisers, animal excreta and crop residues (de Klein et al., 2001). Ruminant urine is considered to be an important source of N_2O (Oenema et al., 1997). In New Zealand, ruminant urine is the largest potential source of anthropogenic N_2O emissions accounting for about 50% of the total N_2O emissions (de Klein et al., 2001), with N_2O contributing 17% of New Zealand's greenhouse gas emissions in 2007 (Petrie et al., 2007). The N_2O molecule has a life time of approximately 114 years and has the warming potential of 298 times greater than that of CO_2 over a 100 year period (Forster et al., 2007). Currently N_2O is also the dominant ozone-depleting substance and it is expected to remain so throughout the 21st century (Ravishankara et al., 2009).

2.3.2 Processes of nitrous oxide production in pastoral soils

2.3.2.1 Nitrification

Ruminant urine is the major source of N_2O emissions from grazed pastoral soils (de Klein et al., 2001). The major N component in urine is urea ($CO(NH_2)_2$) and it makes up 50 to 90% of the N contained in urine (Bussink and Oenema, 1998; Doak, 1952). Following the deposition

of urine onto soil, urea is rapidly hydrolyzed by the enzyme urease and ammonium ions (NH_4^+) are formed (eqn. 2.9; Reynolds et al., 1985):

$$CO(NH_2)_2 + 2H_2O \rightarrow (NH_4)_2CO_3 \leftrightarrow 2NH_4^+ + CO_3^{2-} \quad (2.9)$$

Nitrification is the oxidation of ammonia (NH_3) to nitrate (NO_3^-) (Figure 2.1) and it is performed by both bacteria and archaea (Leininger et al., 2006). Nitrification occurs in soils with relatively high O_2 contents. Ammonia and NO_2^- are the electron donors in nitrification processes and the microbes obtain their C from CO_2, in a process called autotrophic nitrification (Wezermal and Gannon, 1967). Heterotrophic nitrification may also occur under acid conditions in pastoral soils (Islam et al., 2007). Nitrous oxide is produced during oxidation of NH_3 and hydroxyamine (NH_2OH) and in soils (Yoshida and Alexander, 1970), and occurs when the soil is well aerated (Blackmer and Bremner, 1977).

$$\begin{array}{c} N_2O \\ \uparrow \\ NH_3 \rightarrow NH_2OH \rightarrow NO_2^- \rightarrow NO_3^- \end{array}$$

Figure 2.1 Outline of the nitrification pathways (after Wrage et al., 2001).

2.3.2.2 Denitrification

Denitrification is the biological reduction of NO_3^- to N_2 (Figure 2.2). The organisms involved in the denitrification processes are predominatly heterotrophic microorganims hence they utilise organic C substrates. The microbes involved in denitrification are facultative anaerobes which mean they can use both O_2 and NO_3^- (or NO_2^-) as the electron acceptors (John, 1977). Hence, the presence of O_2 stops denitrification and denitrification occurs in soils under conditions of O_2 depletion (Bremner and Shaw, 1958; Russow et al., 2009). Both NO and N_2O are obligate intermediates of denitrification (Payne, 1973; Russow et al., 2009).

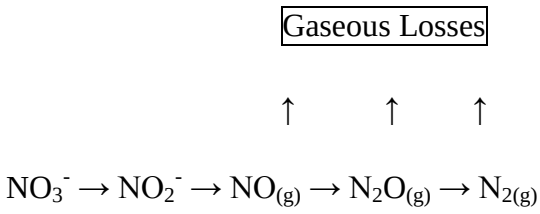

Figure 2.2 Outline of the denitrification pathway (after Hoschstein and Tomlinson, 1988).

2.3.2.3 Coupled nitrification-denitrification

In soils, coupled nitrification-denitrification occurs where there are anaerobic-aerobic interfaces (Wrage et al., 2001). Nitrite (NO_2^-) or NO_3^- produced during nitrification can be utilized by denitrifiers. Hence, coupled nitrification-denitrification occurs in soils where favourable conditions for both nitrification and denitrification are present (Figure 2.3). In terms of N_2O production, coupled nitrification-denitrification can be very important. For example, Khdyer and Cho (1983) observed that in a forest soil, N_2O following an addition of urea was mainly produced when the conditions were sub-obtimal for both nitrifiers and denitrifiers, and they concluded that the coupled nitrification-denitrification was the main source of N_2O when urea was applied on the surface of Wellwood soil samples at 20°C. When a cattle manure was applied to an aerobic soil, coupled nitrification-denitrification was again the predominant cause of N loss from the soil to the atmosphere (Nielsen and Revsbech, 1998).

2.3.2.4 Chemodenitrification

Chemodenitrification is the abiotic chemical decomposition of NO_2^- with organic or inorganic compounds (Wrage et al., 2001). Chemodenitrification occurs in soils of low pH (< 5) (van Cleemput and Baert, 1984) and it has been reported to be a significant source of N_2O when high concentrations of NO_3^- have accumulated (Anderson and Levine, 1986).

2.3.2.5 Nitrifier-denitrification

Nitrifier-denitrification is performed only by nitrifiers, whereas nitrifiers and denitrifers are involved in coupled nitrification-denitrification (Wrage et al., 2001). In nitrifier-denitrification, nitrifiers oxidize NH_3 to NO_2^- then reduce the NO_2^- to N_2O and N_2 (Figure 2.3). Nitrifier-denitrification can be the predominant source of soil N_2O fluxes when soil moisture contents are low (Webster and Hopkins, 1996). Using a dual-isotope labelling method (^{18}O and ^{15}N), Wrage et al. (2005) showed that nitrifier-denitrification contributed 37% of soil N_2O emissions 6 h following the addition of ^{15}N-labelled ammonium nitrate to an arable soil. Russow et al. (2009) reported that nitrifier-denitrification occurred when soil

oxygen contents were between 2 to 5%. However, studies focusing on nitrifier-denitrification and soil N_2O fluxes are sparse and further studies are required (Wrage et al., 2001).

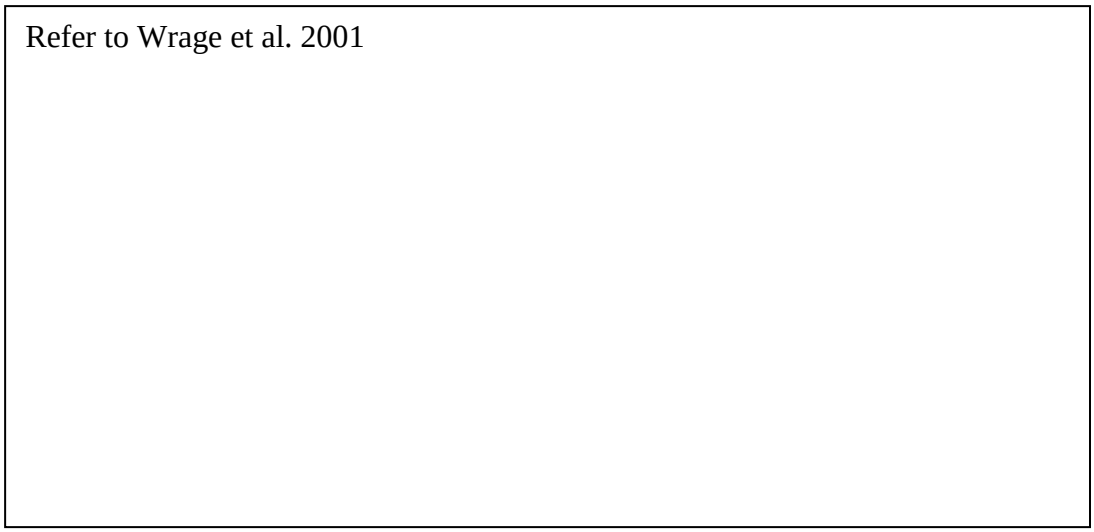

Figure 2.3. The pathways for coupled nitrification-denitrification and nitrifier denitrification. The overlapping boxes symbolize the possibility of a coupling between nitrification and denitrification. In the proposed pathway of nitrifier-denitrification, NO_2^- is reduced and NO_3^- is not formed (after Wrage et al., 2001).

2.3.3 Factors controlling soil N_2O emissions

The factors controlling soil N_2O emissions are complicated due to the many processes involved. The following literature review discusses the impact of the major factors controlling soil N_2O emissions, namely, soil pH, substrate supply and availability, temperature, and soil moisture. In addition, the importance of urine deposition events with respect to soil N_2O emissions from pastoral ecosystems is discussed.

2.3.3.1 Soil pH

Soil pH influences the rates of denitrification and the N_2O:N_2 ratio (Focht and Verstraete, 1977). The optimum pH range for denitrification has been reported as being between pH 7.0 and 8.2 (Delwiche and Bryan, 1976). However, Šimek et al. (2002) have reported that there was no simple relationship between denitrifying enzyme activity and soil pH and that soil denitrifiers could adapt to soils at different pH. The N_2O:N_2 ratio decreases as soil pH increases (Hauck and Melstead, 1956). During nitrification, an increase in soil pH generally increases nitrification activity but the optimum pH has varied from 3.0 to 9.5 and depends on the ecosystem (Kyveryga et al., 2004; Ste-Marie and Paré, 1999). In a pastoral ecosystem, the effect of soil pH on N_2O emissions is influenced by soil moisture contents, for example, Clough et al. (2004) reported that under saturated soil conditions, soil N_2O emissions from a

urine patch increased with soil pH between pH 4.7 to 6.6 but when soil moisture contents were at the field capacity, soil N_2O emissions decreased with increasing soil pH.

2.3.3.2 Substrate supply and availability

For the heterotrophic microbes involved in N_2O production, organic material acts both as a C source and as a hydrogen donor. Hence, N_2O production is normally greatly enhanced upon the addition of soil organic C. For example, the addition of glucose markedly enhances the denitrification rate in soils under anaerobic condition (Azam et al., 2002; Jacobson and Alexander, 1980). Bailey (1976) reported that, in pastoral ecosystems, readily available organic C is supplied from plant roots and that the presence of roots increased denitrification rates. Conversely, Sauer et al. (2009) concluded that soil inorganic N uptake by plants during the winter-spring period reduced N_2O emissions from a pasture soil. Thus, studies focusing on the effects of plants on soil N_2O emissions show contradicting results. Burford and Bremner (1975) published a classic study that demonstrated there was a very strong (r = 0.99) linear relationship between denitrification rate and the soil's water-soluble C content when there was enough NO_3^- present. Hence, factors that affect the magnitude and quality of the soil's various C pools (e.g. plant activity) also have the potential to control the C substrate supply available for heterotrophic induced N_2O emissions.

2.3.3.3 Temperature

The mechanisms controlling the effect of temperature on soil N_2O emissions are complex because multiple processes are responsible for soil N_2O emissions (Li et al., 1992). The optimum temperature for nitrification has been reported to be 35°C (Hadas et al., 1986) whereas for denitrification, the optimum may be as high as 65°C (Bremner and Shaw, 1958). However, soil N_2O emissions may not always respond positively to temperature. For example, Gödde and Conrad (1999) demonstrated that soil N_2O emissions can be greater at a low temperature (4°C) compared to a higher temperature (15°C) and their study concluded that soil microbes are able to adapt to low temperatures. The N_2O:N_2 ratio was shown to decrease with increasing temperature probably due to increasing denitrification rates at higher temperatures (Avalakki et al., 1995; Maag and Vinther, 1996). Conversely, Firestone (1982) reported that N_2O produced via denitrification could be enhanced relative to the other products at low temperatures. Temperature may indirectly influence soil N_2O emissions, for example, a T_S increase may increase R_S, causing O_2 concentration to decrease, thus creating favourable denitrification conditions and increasing denitrification (Parkin and Tiedje, 1984). Soil microbial populations or community structures may also influence the responses of soil N_2O production mechanisms to temperature because N_2O fluxes in soils sampled at the same

site, but in different seasons, have been shown to respond differently to soil temperature changes (Struwe and Kjøller, 1991).

2.3.3.4 Soil moisture

Soil moisture has a strong influence on soil N_2O emissions, since it controls the degree of aeration (Smith et al., 2003). When soil moisture contents are high, air permeability declines, reducing soil aeration, and denitrification is promoted under the low O_2 conditions (Grable, 1971). Generally the higher the soil moisture content, the more N_2O is emitted from soils because microbial activity is limited in dry soils (Weitz et al., 2001). Also, high N_2O emissions are commonly observed during a sudden and large change in soil moisture (Müller et al., 1997). However, when soils are completely saturated or are under completely anaerobic conditions, N_2O may further be reduced to N_2 via denitrification (Letey et al., 1980). Hence, soil N_2O emissions may peak at soil water-filled pore space (WFPS) values of 75% to 90% (Khalil and Baggs, 2005; Klemedtsson et al., 1988). Nitrous oxide production by nitrifiers is also affected by soil moisture. For example, Skopp et al. (1990) found that the optimal soil water content for nitrification was 60% WFPS. The effect of soil moisture on soil N_2O emissions may also vary according to soil structure and texture. Chalamat (1985) reported that the number of anaerobic sites was higher in fine structured soils. Hence, an increase in soil moisture content may have a more significant effect in fine textured soils if N_2O emissions are from denitrification. A sudden change in soil moisture, due to drying-wetting cycles, has an effect on soil N_2O emissions because a sudden wetting can solubilize soil C, feeding heterotrophic microbes (Rudaz et al., 1991).

2.3.3.5 The interaction between soil moisture and temperature

The temperature response of soil N_2O emissions is reported to be influenced by soil moisture (Schindlbacher et al., 2004). A review of soil N_2O emission data, defined as an emission factor (EF), for grassland systems across Europe, showed that the percentage of N_2O-N produced per unit of fertiliser N added, increased markedly with an increase in T_S, but only when WFPS was between 60 and 90% (Flechard et al., 2007). In Figure 2.4, it can be seen that EF values of up to 6.5% were predicted for a soil temperature at 25°C and a WFPS of between 70% and 80%, whereas at the same temperature and at a WFPS below 50% and above 90%, EF values of < 1% were predicted (Flechard et al., 2007). At lower T_S, changes in soil N_2O emissions with changes in soil moisture are reported to be relatively small (Maag and Vinther, 1996).

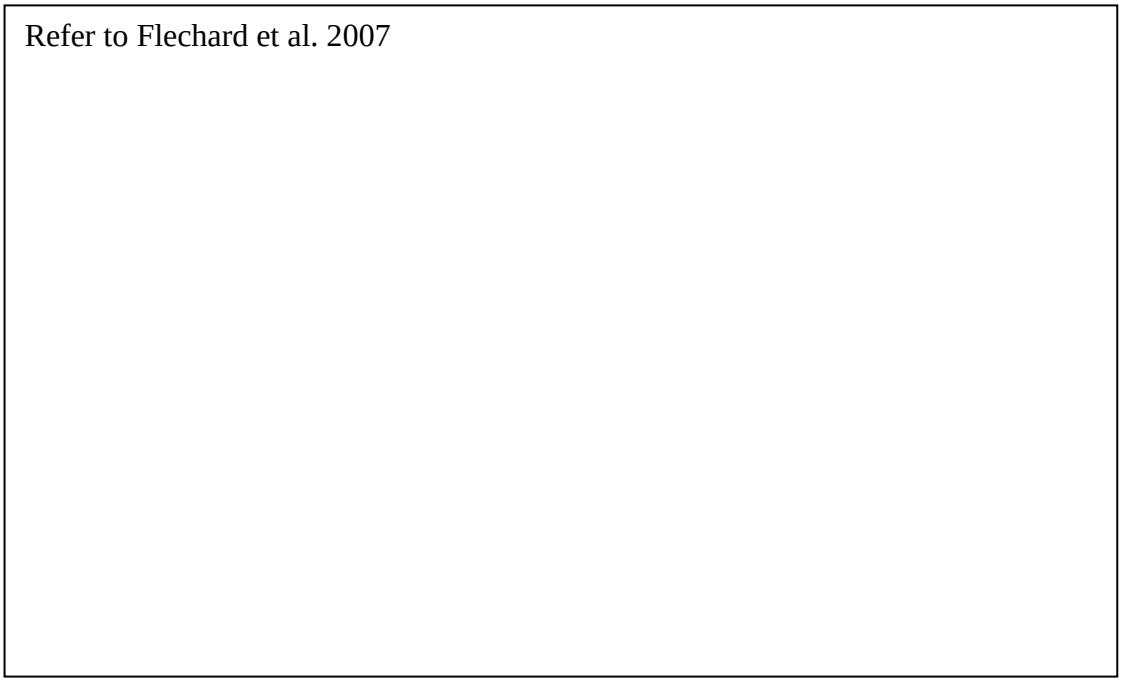

Figure 2.4 The modelled response of the emission factor (EF) from fertilizer to soil temperature and WFPS (after Flechard et al., 2007).

Hence, soil N_2O emissions can occur at high temperatures (~ 25°C) and high soil moisture contents but in practice, such optimum conditions seldom occur in pastoral systems and soil moisture contents are generally lower when temperatures are higher (McKenzie et al., 1999). A field study performed in a pastoral system in Switzerland has concluded that soil water content was the main factor controlling soil N_2O emissions (Rudaz et al., 1999). Despite this the rewetting of dry soil may also cause a marked increase in soil N_2O emissions as demonstrated during an incubation study at a constant T_S of 14°C, hence the interaction between soil moisture and temperature is a critical determinant of soil N_2O emissions (Ruser et al., 2006).

2.3.3.6 Urine deposition

In pastoral ecosystems, urine-N is the single most important source of soil N_2O emissions (de Klein et al., 2001). A brief description of soil N transformation processes which occur following the application of urine were described earlier (section 2.3.2). A portion of the N contained in the ingested pasture is returned to the soils as urine-N from the grazing animals. This N is predominantly in the form of urea. The urine-N is not evenly distributed onto the pasture but deposited in small concentrated patches, termed urine patches. The concentration of N under a urine patch can be as high as 1000 kg N ha^{-1} (Haynes and Williams, 1993). A previous study has estimated that between 0.1 and 3.8% of ruminant urine-N is emitted to the atmosphere as N_2O (Oenema et al., 1997).

The mineralization of soil organic N also increases under the soils treated with urine. The increase in the N mineralisation may occur due to an increase in microbial activity under the urine patch (Zaman et al., 1999). The increased plant activity under a urine patch, a consequence of the fertiliser effect of the urine constituents, may also lead to the higher rates of N mineralisation (Thompson and Fillery, 1997).

As noted above (section 2.2.3.5) the application of urine to soils leads to a rapid increase in soil pH due to the hydrolysis of urea (Doak, 1952). The increase in soil pH increases the solubility of soil organic C (Monaghan and Barraclough, 1993). In addition, C substrates can increase in urine-treated soils due to the root scorching events (Carter et al., 2006). Shand et al. (2002) observed in a field experiment that the increase of soil pH after the application of sheep urine on pasture was followed by an increase of dissolved organic C in the soil. Hence, urine provides both N and C substrates for subsequent nitrification and denitrication processes. Nitrous oxide is produced through both nitrification and denitrification and following a urine application, denitrification may be the main N_2O producing process, however, this depends greatly on soil water content (van Groenigen et al., 2005).

2.3.4 Methods to investigate the factors controlling soil N_2O emissions in pasture soils

2.3.4.1 Nitrogen isotope technique

To investigate the N_2O source following urine-N deposition, a ^{15}N isotope technique has commonly been used (Wachendorf et al., 2008). For example, Clough et al. (2004) studied the fate of ^{15}N-labelled urea mixed with synthetic urine applied to a pasture soil (500 kg N ha^{-1}). Over a 85 d incubation period following urine-N addition, < 0.1 to 1.7% of ^{15}N applied was recovered as N_2O-N. Clough et al. (2004) investigated the effects of soil pH and soil moisture contents on soil N_2O emissions and the ^{15}N isotope technique successfully allowed the treatment effects on soil N_2O emissions to be quantified. Bronson et al. (1999) also showed, using a ^{15}N labelled urine, that the short-term N dynamics under urine patches could be studied using a ^{15}N isotope technique. Bronson et al. (1999) concluded that following urine addition to a soil with plant litter on its surface, the major loss of urine-N due to NH_3 volatilization with negligible losses of N_2O from urine-N. The ^{15}N isotope technique is a standard technique used to investigate N transformations under urine patches.

2.4 Global warming and the pastoral ecosystem

The IPCC reported that global mean surface temperatures have risen by 0.13 ± 0.03°C per decade over the last 50 years (Trenberth, 2007) and it has been shown that *NPP* responds on a

global basis to small changes in temperature e.g. a drop of 0.5°C as a result of the Mount Pinatubo volcano eruption in 1991 decreased the *NPP* of the global boreal zone by 25 g C m^{-2} yr^{-1} in 1992 (Lucht et al., 2002). Understanding the effect of temperature on soil processes is critically important for predicting the responses of the pastoral ecosystem to global warming in terms of the role temperature will play in mediating either positive or negative feedbacks on R_S (Cox et al., 2000; Luo et al., 2001; Melillo et al., 2002) and N_2O emissions (Smith, 1997). In this section, recent literature is reviewed with respect to the current trends and predictions for global warming, in order to provide an overview of the complex relationships between global warming and soil processes in pastoral ecosystems.

2.4.1 Changes in global mean atmospheric temperature

The Fourth Assessment report (AR4) of the Intergovernmental Panel on Climate Change (IPCC, 2007b) states that over the last 100 years, the global mean surface temperatures have risen 0.74 ± 0.18°C (Fig. 2.5) and this is unequivocally attributed to anthropogenic activity. Future climate scenarios have been assessed and a warming of 0.2°C per decade is projected. However, if the atmospheric concentrations of greenhouse gases continue to increase at the current rate then average global surface temperature by 2090 to 2099 are predicted to increase by as much as 4.0°C (range of 2.4°C to 6.4°C) compared to the period 1980-1999 (Meehl et al., 2007).

Refer to IPCC AR4 report 2007

Figure 2.5 Observed changes in global average surface temperature based on the global average surface temperature in 1990. The shaded areas are the uncertainty intervals estimated from a comprehensive analysis of known uncertainties (after IPCC, 2007b).

For New Zealand, the predicted changes in climate, resulting from global warming, are predicted to vary with geographical region (Christensen, 2007) with the mean surface temperature increases for New Zealand predicted to be 0.9°C by 2040 and 2.1°C by 2090. New Zealand's Ministry for the Environment (2008) has stated that this predicted increase in temperature, along with other possible changes in climate, may change the frequency and intensity of existing risks and hazards, but may also bring opportunities in agricultural sectors (e.g. the potential to grow new crops).

2.4.2 Potential effects of global warming on soil processes in pastoral ecosystems

2.4.2.1 Potential effects of global warming on soil respiration

In pastoral ecosystems, almost every aspect of the soil-plant continuum, for example, plant growth, soil microbial activity, and soil moisture characteristics have been shown to be affected by changes in soil and atmospheric temperature (Thornley and Cannell, 1997). The study by Thornley and Cannell (1997) analysed the effects of increasing both annual mean atmospheric and T_S by 5°C, where the 5°C increase in atmospheric and T_S represented an extreme in order to demonstrate the response of soil processes. As noted above (section 2.2.3.2) R_S is very sensitive to T_S hence, it is considered likely that global warming will increase R_S but uncertainty remains high with respect to the magnitude of any changes in R_S as a result of global warming (Rustad et al., 2000).

A small change in R_S could potentially have a significant impact on atmospheric CO_2 concentrations because on a global scale, terrestrial ecosystems, including vegetation and soils, are one of the main reservoirs of C on Earth, containing 2200 Gt C, or approximately three times the C in the atmosphere (Schimel, 1995). Hence a small increase in the rate of R_S as a consequence of global warming, could have a significant impact on atmospheric CO_2 concentration, if all other factors remained the same.

2.4.2.2 Acceleration in carbon loss from soil and soil carbon cycle feedback

Global warming can potentially increase C loss from soil to atmosphere due to enhanced R_S and this phenomenon could further accelerate global warming (Cox et al. 2000), resulting in a positive feedback. Global warming directly influences factors controlling R_S such as soil microbial community function via processes such as soil enzyme activity (shown as a 'direct feedback' in Fig. 2.6). Further indirect effects occur as secondary processes. For example, elevated temperatures may enhance *NPP* and rhizodeposition (Fig. 2.6). The increase in *NPP* increases root-derived C substrate supply for R_{RD}, and R_{RD} can prime R_{OM}. The relationship between *NPP* and R_S is stronger in grassland compared to forest sites, possibly because there

is no allocation of C to wood production in grasslands, thus grasses may have more photosynthate available for R_S (Raich and Tufekciogul, 2000). It was reported that in long-term pasture ecosystems, losses from respiration exceeded the inputs from photosynthate and resulted in a long term (17 – 30 years) C loss from soils (Schipper et al., 2007). Hence, the better understanding of the relationship between C substrates and respiration in grassland ecosystems is critical to accurately predict the effect of global warming on soil C in grassland ecosystems. Bardgett et al. (2008) have concluded that the consideration of both direct and indirect impacts of global warming on soil microbes and R_S processes is critical in order to make progress in understanding the soil C cycle feedback.

There are considerable gaps in our understanding of belowground processes (e.g. R_S) when compared to our knowledge of aboveground processes (e.g. photosynthesis) (Pendall et al., 2008). However, the issue is complicated further by the knowledge that aboveground and belowground processes strongly interact (Bardgett et al., 2008). For example, actively growing plants are reported to enhance or supress R_{OM} (Kuzyakov et al., 2000). Soil microbes are also reported to determine plant community and productivity (Wardle et al., 2004). Hence, there is an urgent need for greater understanding of how R_S directly and indirectly responds to global warming.

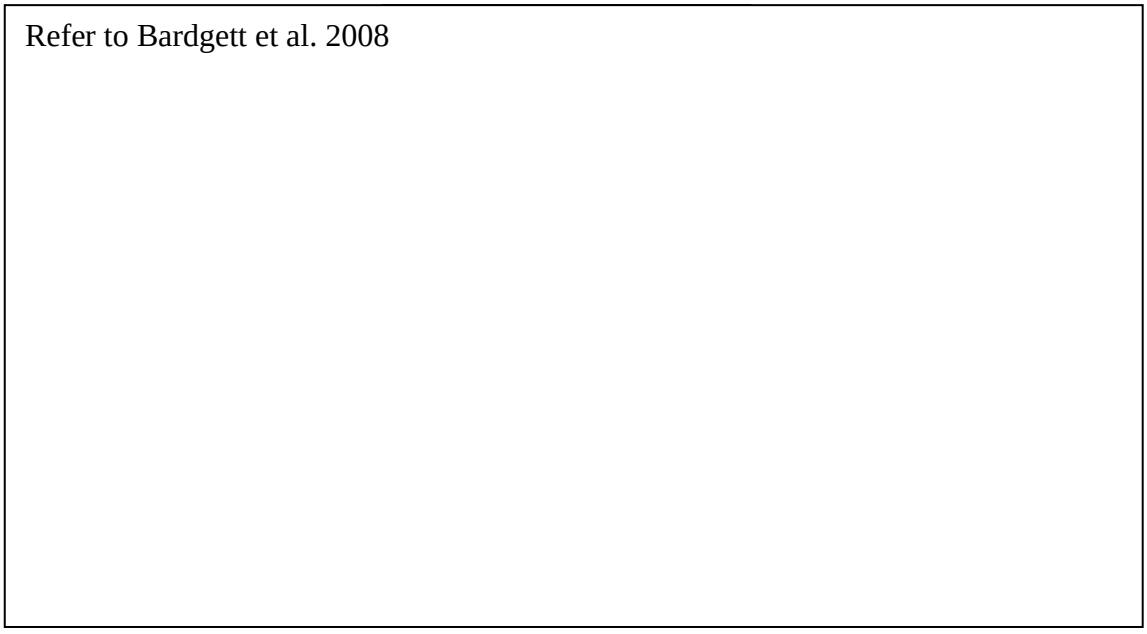

Figure 2.6 Direct and indirect effects of climate change on soil microbial communities and routes of feedback to global warming through soil respiration (R_S) (After Bardgett et al. 2008).

2.4.2.3 Potential effects of global warming on soil N_2O emissions

As noted above, N_2O production from soils is positively correlated to T_S (section 2.3.3.3) hence, a positive feedback in response to global warming is predicted for soil N_2O emissions (Smith, 1997). A mathematical model, the Denitrification-Decomposition (DNDC) model (Li et al., 1992), has predicted that in a maize field under a humid climate, soil N_2O emissions are very sensitive to T_S, and annual N_2O fluxes will increase by 13% if annual mean T_S increases by 1°C (Li et al., 1996). The increase in soil N_2O emissions may be partially due to increased enzymatic activity during N_2O production and the increased release of soil N due to enhanced mineralisation (Lükewille and Wright, 1997). The model (Li et al., 1992) also predicted an increase in mineralisation when T_S increased. The increase in mineralisation of N and T_S may also increase *NPP* (Rustad et al., 2001), leading to increased root exudation and C inputs into the soil which may further increase soil N_2O emissions (Song and Zhang, 2009).

In complete contrast, using another mathmatical model for soil N_2O emissions (Carnegie-Ames-Stanford or CASA model), Potter et al. (1996) have concluded that a 20% increase in T_S was likely to reduce soil N_2O emissions by between 1 to 21% in many different types of ecosystems including pastoral ecosystems because reduced soil moisture contents and increased plant N uptake, influenced by the increase in T_S, will negatively influenc soil N_2O emissions. Similar to Potter et al. (1996), Hantschel et al. (1995) conducted a soil warming experiment (+ 3°C T_S) and showed that soil N_2O emissions from a cropping farm over a 3-month winter period decreased as a result of soil warming.

Further investigation of the factors controlling soil N_2O emissions is therefore critical since 70% of global N_2O emissions originate from soils (Conrad, 1996). New Zealand's agricultural greenhouse gas inventory has a significant soil derived N_2O component, as discussed above (section 2.3.3.3), hence a small change in T_S could have a marked effect on soil N_2O emissions and New Zealand's agricultural greenhouse gas inventory.

2.5 Recommendation for research

A very complex interaction of soil/plant/animal processes and environmental factors controls R_S and N_2O emissions. These complex interactions need to be investigated to improve our knowledge of the temperature response of R_S and N_2O emissions.

Firstly the influence of readily available C supply on the relationship between T_S and R_S must be studied. Soils contain a small amount of readily available C substrates, which deplete within days without plants, and a large amount of less available or non available C substrates. It is known that soil microbes preferably utilise readily available C substrates but they also

slowly utilise less available C substrates. However, uncertainty remains high with respect to microbes ability to decompose less available C at different T_S.

To further improve our understanding of the interaction between plants and readily available C substrate supply to soils, further studies on R_S substrates are critically important. Current models for the temperature sensitivity of R_S fail to accurately describe the relationship between R_{OM} and R_{RD} (Zobitz et al., 2008). The relationship between R_{OM} and R_{RD} at different T_S needs to be studied using stable isotope techniques. The predicted acceleration of C loss from soils due to global warming (section 2.4.2.2) is strongly related to R_{OM} whereas R_{RD} may indirectly be related to the acceleration of soil C loss because plant activity can also increase R_{OM} (priming effect). It is unclear how soil nutrient availability affects the priming effect and the temperature response of R_{OM} and R_{RD}. This needs to be examined.

In pastoral ecosystems, the high concentration of N deposited in ruminant urine patches increases both plant production and readily available C substrate supply to soils resulting in high soil CO_2 and N_2O fluxes. Because of an increase in soil pH, which solubilises soil organic matter and the high N loading under urine patches, the factors controlling the temperature response of soil CO_2 and N_2O fluxes from urine patches could be different when compared to other ecosystems (e.g. forests). Thus, studies of soil CO_2 and N_2O fluxes from urine patches at varying T_S are needed to increase our knowledge of the pastoral ecosystem's response to potential increases in global mean surface temperatures.

Chapter 3
Soil microbial respiration responses to changing temperature and substrate availability in fertile grassland

A manuscript from this study has been accepted for publication in the Australian Journal of Soil Research: Uchida, Y., Clough, T.J., Kelliher, F.M., and Sherlock, R.R. Soil microbial respiration responses to changing temperature and substrate availability in fertile grassland. This is refered to in later chapters as Uchida et al. (2010).

3.1 Abstract

A relationship between soil respiration rate (R_S) and temperature (T_S), has been understood to be predicated on carbon (C) substrate availability. However, unlike T_S, C availability in soils is not a state variable that can be readily measured. The C in soils has come from plants, so the C supply rate can be affected by the weather and nutrient supply. I studied a fertile soil beneath pasture, measuring R_S across a range of temperate-climate T_S values. The objectives for the study were to: (1) quantify the synchrony of diurnal changes in T_S and R_S beneath pasture under conditions favourable for plants, (2) quantify responses of microbial respiration (R_M) to the removal of plants and depletion of C supply over time at various T_S and (3) determine if R_M was related to water (20ºC) soluble (WSC) and hot-water (80ºC) soluble C (HWSC) contents of the soil. At a grassland site, R_S increased with T_S as predicted by an Arrhenius type relationship. Sampled soil was incubated at 3°, 9° and 24°C and R_M was measured over 14 d. In addition soil samples were pre-incubated at 3° or 9°C for both 5 and 14 d, then incubated at 24°C for 1 d and R_M was measured. On day 2, R_M was less than predicted at 24° and 9°C, respectively, suggesting a C availability limitation. The time courses of R_M, revealed that at 24°C, R_M utilised C that was not utilised at lower T_S, indicating that evidently recalcitrant C was available to microbes at a warmer temperature. The responses of R_M at 24°C after the pre-incubation treatments were identical for the 3°C and 9°C pre-incubation treatments although significantly more C was respired during pre-incubation at 9°C. The WSC and HWSC contents were unaffected by T_S and did not provide useful measures of the C substrate available for R_M.

.

3.2 Introduction

In soils, the relationship between microbial respiration (R_M) and temperature (T_S) is of heightened interest (e.g. Cox et al., 2000). The mean global surface temperature has been predicted to increase by 2.4° to 6.4°C over the next 90 years (Meehl et al., 2007). Microbial respiration can be very sensitive to T_S and soils contain a large reservoir of C (approximately 1500 Gt C - Amundson, 2001). Thus warmer soils could accelerate the rate of increase in the atmospheric CO_2 concentration and further induce global warming (Amundson, 2001; Davidson and Janssens, 2006; Kirschbaum, 1995). According to Lloyd and Taylor (1994), a 1°C increase in T_S may increase R_M by 10% at 10°C. The effect of a 1°C increase in T_S on R_M varies inversely to T_S; hence R_M is more temperature sensitive at lower T_S.

Soil microbial respiration (R_M) utilises C substrates of varying availabilities. Low molecular weight C substances such as sugars and organic acids are more readily utilised by microbes, so called 'readily available C substrates' (Sikora and McCoy, 1990; Townsend et al., 1997). Their residence time in soils can be measured in terms of days (Domanski et al., 2001). Soils also contain C substrates that are either not available or less readily available to microbes because they are physically and/or chemically protected. Their residence time in soil can be > 1500 yr (Parton et al., 1987) and these protected C substrates have been called 'recalcitrant C substrates' (Rovira and Vallejo, 2002; Six et al., 2002). The recalcitrant C substrate fraction in a soil is normally much larger than the readily available C substrate fraction because soil microbes preferably consume readily available C substrates at faster rates (Koepf, 1953; Townsend et al., 1997).

The rate of readily available C substrate supply is related to plant photosynthesis and growth. The relationship can be expressed as;

$$NPP = GPP\ (1 - \varphi) \qquad \text{eqn. 3.1}$$

where *NPP*, *GPP*, φ are net primary production, the average rate of photosynthesis, and the proportion of assimilated C lost through plant respiration as well as other processes such as exudation of readily available C substrate from plants to soil, respectively (Lloyd and Farquhar, 2008). The study of Lloyd and Farquhar (2008) has reported that the parameter φ was relatively constant. Hence, I can infer that soil fertility, which strongly controls *NPP*, also affects readily available C substrate supply to soils. The C assimilated by pastoral plants can

be made available for R_M within 24 h and the turnover of recently assimilated C was estimated to have been completed in 3 – 4 d according to Butler et al. (Butler et al., 2004). Saggar and Hedley (2001) also reported that within 4 h of pulse ^{14}C application, 7 – 12% of the ^{14}C was detected in roots. Hence soil fertility, influences readily available C supply to soils from plants, and R_M can be strongly correlated on a short-term basis.

The decomposition of recalcitrant C substrates is not normally limited by substrate supply from plants. Soil microbes consume recalcitrant C substrates at much slower rates (van Hees et al., 2005). Hence, R_M can be limited during microbially favourable temperature conditions if the readily available C substrate supply is exhausted and soil microbes have only recalcitrant C substrates to obtain their energy. This is called substrate limitation (Kirschbaum, 2006). The response of R_M to T_S can be suppressed by substrate limitation (Hartley et al., 2008). It should be noted that the predicted increase in mean global temperature does not occur in isolation, but that it is predicated on the increasing concentration of atmospheric CO_2. This may confound the relationship between R_M and T_S. In fact a meta-analysis of 117 free air carbon dioxide enrichment (FACE) and open top chamber studies by de Graaff et al. (2006) reported soil respiration increased by 17.7% although the C dynamics were highly dependant on soil nutrient supply. Experiments performed in a grazed pasture FACE facility located in a New Zealand grazed temperate grassland showed that while root growth and root turnover rates increased under elevated CO_2 (Allard et al., 2005) the uncertainty of the effect of increased atmospheric CO_2 concentration on microbial activity in a grazed temperate grassland remained high (Allard et al., 2006; Allard et al., 2005). It has also been suggested that substrate limitation will become more common in soils if global temperatures increase, as soil microbes are forced to utilise more recalcitrant C substrates because an increase in T_S may cause a greater increase in R_M compared with the increase of C inputs to soils from plants, although this may depend on soil types and substrate availability (Christensen et al., 1999; de Graaff et al., 2006; Piao et al., 2008).

Hence more studies are required to investigate the relationship between C substrate limitation and the response of R_M to T_S (Kirschbaum 2006). Carbon substrate availability has received the least attention as a controller of R_M, probably because it is very difficult to measure (Davidson et al., 2006). Simple and commonly reported methods for estimating readily available C substrates include water soluble C (WSC) and hot-water soluble C (HWSC) analyses. These have been used as indicators of substrate availability in soils (Davidson et al., 1987; Ghani et al., 2003; Haynes, 2000). Grazing intensity, land use types, denitrification activity, root activity and microbial biomass C have been shown to be closely related to WSC

or HWSC in previous studies (Burford and Bremner, 1975; Cheng, 1996; Ghani et al., 2003; Sparling et al., 1998). Microbial availability and molecular weights of substances in WSC are influenced by land use, management and sampling depth of the soil profile (Boyer and Groffman, 1996; Jandl and Sollins, 1997). Kelliher et al. (2005b) concluded that R_M was not proportional to WSC at constant T_S. However, the relationships between WSC, HWSC, and short-term changes in R_M at varying temperatures remain uncertain.

The first objective of this study was to measure the response of total soil respiration (R_S) to T_S at a grassland site. This recognized that the most rapid and largest predicted changes in T_S can also happen within a daily timeframe. The second objective was to change T_S of the sieved soil and measure the response of R_M without plants. The third objective was to change T_S and the quantity of C available to the microbial community and measure the response of R_M, while the fourth object was to determine if R_M responded to changes in WSC and HWSC that have been proposed to indicate available C in soils. I hypothesised that at higher temperatures, readily available C in a pasture soil would be depleted more rapidly by R_M and that this would be indicated by the WSC and HWSC measurements.

3.3 Materials and Methods

3.3.1 Field measurements of soil respiration rates

To measure the response of R_S to changing T_S an irrigated and regularly fertilised grassland site, producing in excess of 18 tonnes ha^{-1} of herbage dry matter yr^{-1}, at Lincoln, New Zealand (43° 39'S, 172° 29'E, 8 masl) was selected. The soil is a poorly drained Temuka silt loam (Soil Survey Staff, 1998). In this study, the term R_S includes root and microbial respiration. Soil microbial respiration, which was measured from sieved soils (0 – 50 mm depth) without plants, was termed R_M (see below). I measured R_S at the field site at intervals over 16 h during a sunny day in autumn (3rd April 2009). Sunrise and sunset on the day were at 7:40 a.m. and at 7:08 p.m., New Zealand Standard Time (NZST), respectively. It was assumed that T_S and R_S would change in synchrony over the day indicating no substrate limitation of R_S because the grass would supply C substrates to soils as required. The soil was well-watered, with measurements conducted one day after rain. The soil's water-filled pore space (WFPS) was 59 ± 6% and the bulk density (0 – 5 cm depth) was 980 ± 80 kg m^{-3} (± SEM, n = 3). To measure R_S, 5 PVC collars (5 cm depth and 10 cm in diameter) were inserted 24 h beforehand with vegetation clipped. A portable chamber with an infrared gas analyser (SRC-1 and EGM-1, PP Systems, Hitchin, UK) was used to measure R_S. At a depth of 1 cm, T_S was

continuously measured with a thermocouple probe and data logged using a HOBO data logger (Onset Computer Co.).

To quantify the response of R_S to changes in T_S the model developed by Lloyd and Taylor (1994) (eqn. 3.2) was used. The parameter R_{10} is the respiration rate at 10°C, T_S is expressed in Kelvin (K), and 308.56 (K) is a parameter analogous to the activation energy derived from the soils data analysed by Lloyd and Taylor. R_x is either R_S or R_M (the latter measured in laboratory experiments described below). This equation yields a decreasing response of R_x to changing T_S. The model may be written following 'eqn 11' of Lloyd and Taylor's paper (1994).

$$R_x = R_{10} e^{308.56\left(\frac{1}{56.02} - \frac{1}{T_s - 227.13}\right)} \qquad \text{eqn. 3.2}$$

3.3.2 Preparation of soil for laboratory experiments

Soil samples (0 – 5 cm depth) were collected from a local dairy farm at Lincoln, New Zealand in early autumn 2009. The soil is a poorly drained Temuka silt loam (Soil Survey Staff, 1998). At sampling, the vegetation was principally perennial ryegrass (*Lolium perenne* L.) and white clover (*Trifolium repens* L.). Chemical tests showed the soil to have a pH, organic matter, total C, and total N contents of 5.9, 15.5%, 9.0%, and 0.90%, respectively. At sampling the soil moisture content was 64% (WFPS). The pasture was grazed by dairy cattle every 21 – 28 d. Sample processing included the removal of roots and litter by hand and passage through a 5.6 mm mesh sieve. The sieved soil was packed to a depth of 5 cm into PVC containers (6 cm deep and 10 cm in diameter) and water was added to bring the soil moisture content up to 0.54 g H_2O g^{-1} dry soil (66% water-filled pore space), an optimum level for R_M (Howard and Howard, 1993; Rey et al., 2005). The soil had been sieved and placed in the PVC containers within 4 h of sampling to minimize the loss of readily available C substrate during processing. The containers were maintained at the same moisture content throughout the experiment by daily spraying water onto the soil surface. This was achieved by maintaining the weight of soil plus PVC containers, at the beginning of the experiment, during the whole incubation period.

3.3.3 Incubation procedure and soil respiration rate measurements

To measure the responses of R_M over time at varying T_S without plant C inputs, the soil samples were separated into three groups of eight cores and incubated, in temperature-controlled incubators, at either 3°C (3.23 ± 0.39°C), 9°C (9.05 ± 0.05°C), or 24°C (24.2 ± 0.15°C) (± SEM). The T_S shown in brackets was the mean of measured values from randomly chosen soil containers, every other day. The chosen temperature treatments were based on an hourly T_S (10 cm depth) frequency distribution over years 2006 – 2008 measured beneath grass that was mown regularly at a weather station near the soil sampling site (Fig. 3.1a). On average, the hourly T_S changed 7.2 ± 3.3°C (± standard deviation) within a day. The daily T_S range varied from zero to 15°C (Fig. 3.1b).

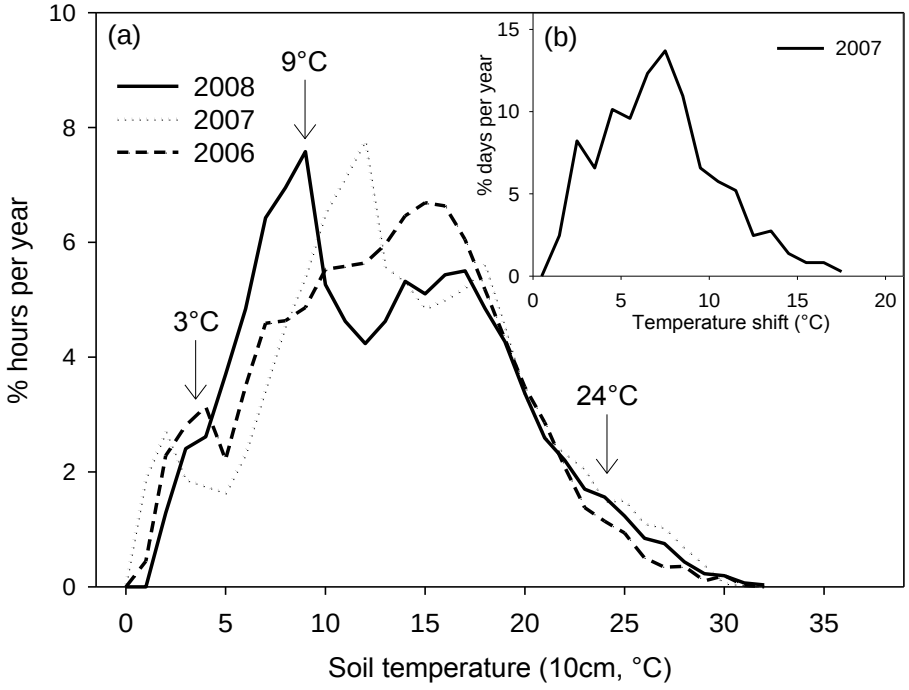

Figure 3.1 (a) Hourly average soil temperature (10 cm depth) frequency distributions for years 2006 - 2008 at Lincoln, New Zealand, beneath mown grass. The arrows are the three treatment temperatures in the laboratory study. (b) Frequency distribution of daily temperature range during year 2007 calculated using the hourly measurements. These data were obtained from New Zealand's National Climate Database (cliflo.niwa.co.nz).

The first measurement of R_M was started within 24 h following the start of the incubation so that the microbial activity associated with the decomposition of readily available C substrates could be evaluated (Feng and Simpson, 2009). The R_M was measured at 24, 48, 72, 96, 120, 144, 192, and 336 h following the start of the incubation. A portable chamber with an infrared

gas analyser was used to measure R_M as described above. Values for R_M are expressed as a mass of CO_2 (µg CO_2) per unit mass of contained soil (kg^{-1}) per unit time (s^{-1}). I hypothesised that R_M would decrease over time as readily available C substrates in the soil samples decreased. To quantify the decrease in R_M over time a model (eqn. 3.3) was fitted to the data with three parameters (*a*, *b*, and *r*).

$$R_m = a + br^t \qquad \text{eqn. 3.3}$$

An asymptote was determined by parameter *a* (µg CO_2 kg^{-1} s^{-1}), *b* was a scaling factor, and *t* was time (h) following the start of the incubation. The parameter *r* is a decay factor and the exponential time constant (see below) is related to *r*. I chose this model since this has been successfully used to describe the changes in R_M over time during laboratory incubation (Kelliher et al., 2005b; Paul et al., 1999). The parameters were fitted using a non-linear least squares method, the errors for the parameters were calculated based on the sum of squared residuals, with the assumption that the errors were normally distributed. Statistics were performed using GENSTAT (Release 11, Lawes Agricultural Trust, Rothamsted, UK). The areas below the fitted curves for each treatment temperature were determined by integration to estimate the amount of CO_2-C lost to the atmosphere during the incubation period.

To further investigate the differences in the parameters for eqn. 3.3 time constants were estimated for the decreases in R_M. A time constant is a period (h) over which a variable (R_M) would have decayed to an asymptote if the initial rate of decay (the initial slope) had continued. The time constants were obtained by using the parameter *a* as an asymptote and calculating a 63% step change based on the change from the first R_M measurement (at 24 h) to the asymptote.

3.3.4 Effects of pre-incubating temperature on soil respiration rate

An additional sixteen soil cores were prepared, and were separated into two groups of eight cores. These groups were pre-incubated at either 3° or 9°C. The experimental design for this part of the experiment was a completely randomized block with four replicates. After 72 h in the incubators at either 3° or 9°C, four of the eight cores in each incubator were removed and placed in another incubator at 24°C. Then R_M was measured 24 h after incubation at 24°C (96 h following the start of the incubation). This procedure was repeated for the remaining soil cores after 336 h in the incubators at either 3° or 9°C followed by 24 h incubation at 24°C (i.e.

360 h following the start of the incubation). The effects of pre-incubation temperatures (3° and 9°C) and pre-incubation length (70 h and 336 h) on R_M at 24°C were compared with R_M from the soil cores continuously incubated at 24°C.

3.3.5 Water soluble C and hot water soluble C measurements

The water soluble carbon (WSC) and the hot-water soluble carbon (HWSC) were determined using a procedure described by Ghani et al. (2003). The experimental design for this part of the experiment was a completely randomized block with three replications. Soil cores described above were randomly assigned to one of the three incubators at either 3°, 9°, or 24°C and fresh soil samples (equivalent of 25 g dry soil) were removed from three randomly chosen soil cores from each incubator at 24, 72, 192, and 336 h following the start of the incubation. Then the soil samples were placed in 300 ml centrifuge bottles and shaken with 150 ml of distilled water at 20°C for 1 h. The samples were then centrifuged for 30 min at 4000 rev min^{-1}, the supernatant filtered through a 0.45 µm cellulose membrane filter, and the amount of soluble organic C determined using a total C analyser (TOC-5000A, Shimadzu Oceania Pty Ltd, NSW, Australia). This was the WSC fraction of the soil organic C. A further 150 ml of distilled water was added to the sediments remaining in the centrifuge tubes after the supernatants (WSC) were decanted and the tubes were capped and left for 16 h in a hot-water bath at 80°C. The samples were then centrifuged, filtered, and analysed for soluble organic C contents using the same methods as for the WSC analysis. This was the HWSC fraction of the soil organic C. Values of WSC and HWSC are expressed here as a mass of C (mg C) per unit mass of the contained soil (kg^{-1}).

3.3.6 Statistical analysis

One-way analysis of variance tests were performed on R_M, WSC, and HWSC for each day of measurement to determine significant differences between the temperature treatments from 24 h to 336 h. Each data set was tested for normality according to the Anderson-Darling normality test and the data set was normally distributed (Anderson and Darling, 1952).

3.4 Results

3.4.1 Field measurements of soil respiration

Over the day T_S varied from 9° to 15°C while R_S increased from 116 ± 3 µg CO_2 m^{-2} s^{-1} just before sunrise to 182 ± 21µg CO_2 m^{-2} s^{-1} late in the afternoon (Fig. 3.2a). Substrate limitation was not indicated because R_S and T_S data corresponded to the Lloyd and Taylor model (r^2 = 0.92, Fig. 3.2b). The response of R_S to changes in T_S was rapid, for example, the largest

increase in T_S occurred between 14:20 a.m. and 15:40 a.m. (NZST), when T_S and R_S increased by 2.3°C and 30 µg CO_2 m^{-2} s^{-1} (22%), respectively. Measurements of R_S taken late in the afternoon onwards were relatively variable. Some of the R_S measurement locations were shaded due to nearby trees while T_S was measured at one location, the variable shading affected R_S. Based on the Lloyd and Taylor equation (eqn. 3.2), the parameter R_{10} was estimated as 114 µg CO_2 m^{-2} s^{-1}.

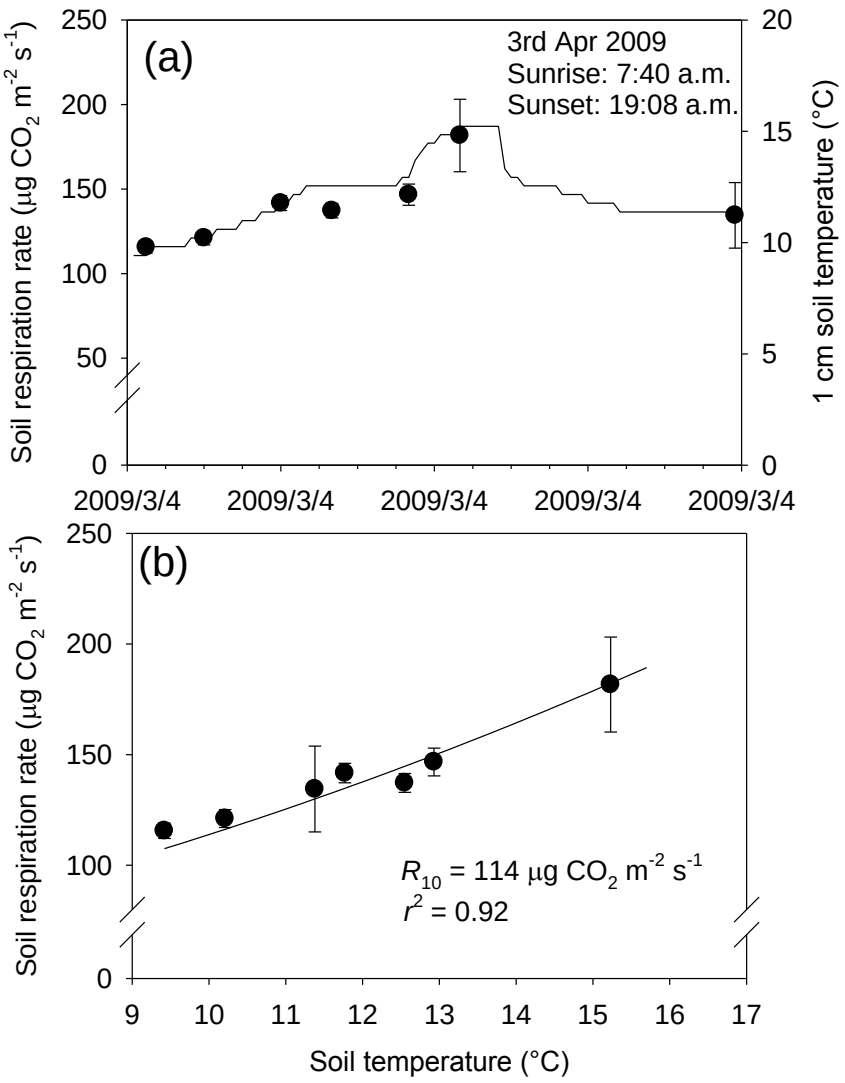

Figure 3.2 Soil respiration rate (R_S, µg CO_2 m^{-2} s^{-1}) and continuously measured soil temperature (1 cm depth) during a fine day, 3rd April 2009, at Lincoln, New Zealand. Diel courses of the soil temperature (solid line, °C) and R_S (●) over time are shown in (a) and the curve in (b) was fitted to the data by regression, as described in the text. The Lloyd and Taylor model was fitted according to R_{10}, R_S at 10°C, and accounted for 92% of the variance.

3.4.2 Effects of temperature on soil CO_2 flux

At 3°C, R_M did not significantly change over the 336 h incubation period. However, R_M declined significantly over time for soils at 9° and 24°C (Fig. 3.3). At 9°C and 24°C, R_M was approximately halved after an incubation period of approximately 48 h but the amount of CO_2 respired was significantly greater at 24°C. The exponential decay model (eqn. 3.3) fitted the data well and the parameters are shown in Table 3.1. The mass of C emitted as CO_2 to the atmosphere from 24 h until 336 h was 295 ± 25, 354 ± 25, and 726 ± 18 mg C kg^{-1} soil at 3°, 9°, and 24°C, respectively. There was a significant effect of T_S on the amount of CO_2-C emitted during the incubation period ($p < 0.01$). The time constants (h) for R_M at 24°, 9°, and 3°C were 76 ± 4, 53 ± 3, and 185 ± 55 h, respectively. The time constants for R_M at 24° and 9°C, which were not significantly different from each other, were significantly shorter than the time constant for R_M at 3°C based on the Fisher's individual confidence interval test (p = 0.05).

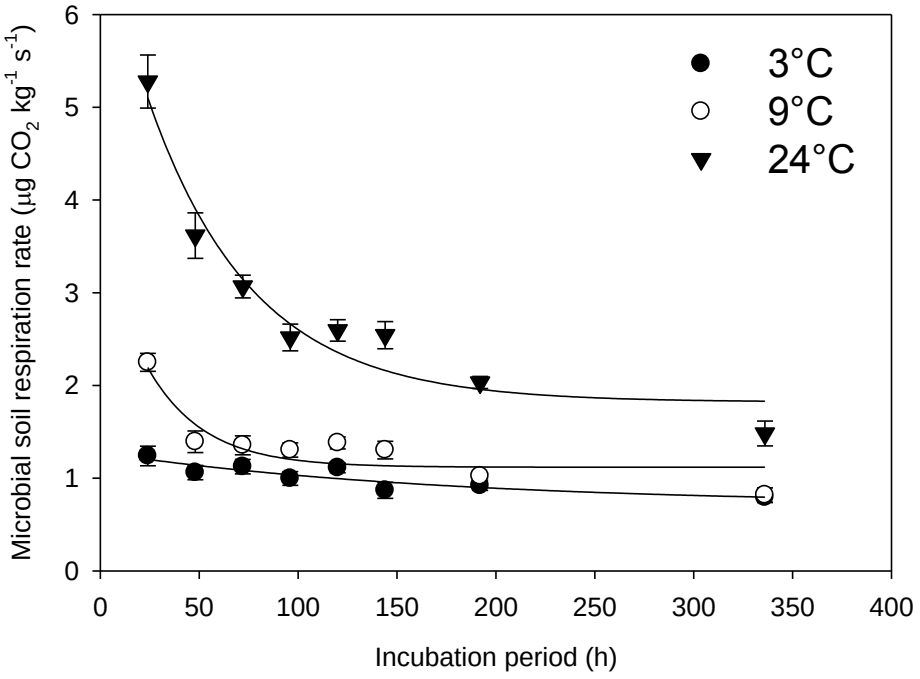

Figure 3.3 Soil microbial respiration rates (R_M) for samples incubated at three temperatures. Error bars are SEM. The curves were fitted to the data by regression using a model of the form $R_M(t) = a + br^t$. An asymptote was determined by parameter *a*, *b* was a scaling factor, and *t* was time (h) elapsed during the incubation. The values for parameters *a*, *b*, and *r* are shown in Table 3.1.

Table 3.1 The parameters describing the responses of soil respiration rate over time at different temperatures. The model used was $R_M = a + br^t$ and standard errors for each parameters are shown in the "s.e." columns. For each parameter, different letters denote significant differences.

	3°C		9°C		24°C	
parameter	estimate	s.e.	estimate	s.e.	estimate	s.e.
r	0.9943[a]	0.0139	0.9629[a]	0.0199	0.9813[a]	0.0033
b	0.561[c]	0.493	2.660[b]	1.460	5.167[a]	0.528
a	0.714[b]	0.607	1.128[b]	0.118	1.823[a]	0.175

The response of R_M to T_S was predicted by the Lloyd and Taylor model (eqn. 3.2) on the first day of incubation (Fig. 3.4). The parameter R_{10} was 2.3 μg CO_2 kg^{-1} s^{-1}. To compare this R_{10} value with the R_{10} obtained from the field study, the R_{10} from the laboratory study was converted to μg CO_2 per unit area (m^{-2}) per second using the soil bulk density (980 ± 80 kg m^{-3}) and a depth of 5 cm, thus the estimated R_{10} for the current laboratory study was 113 μg CO_2 m^{-2} s^{-1}.

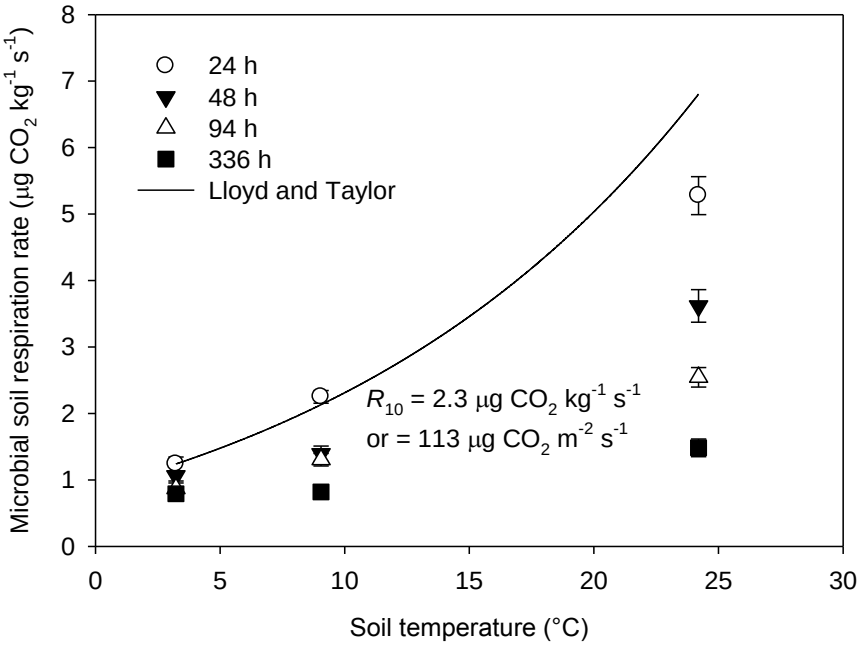

Figure 3.4 The responses of soil microbial respiration to soil temperature (symbols) and the Lloyd and Taylor model (solid line). Error bars are SEM (n = 8). The Lloyd and Taylor model was fitted to the soil microbial respiration rate at 3°C, 24 h into the incubation. The parameter R_{10} (respiration rate at 10°C) is noted on the figure using different units, μg CO_2 kg^{-1} s^{-1} and μg CO_2 m^{-2} s^{-1}. The latter units included a bulk density of 980 kg m^{-3} and depth of 0.05 m.

3.4.3 The effect of temperature alteration on soil respiration rates

Soil samples were pre-incubated at 3° or 9°C for 72 h or 336 h, and then incubated at 24°C for 24 h. The R_M flux was measured immediately following the 24 h incubation at 24°C. The R_M fluxes from soil samples at 3°C with 72 h pre-incubation, and at 3°C with 336 h pre-incubation, at 9°C with 72 h pre-incubation, at 9°C with 336 h pre-incubation were 3.8 ± 0.4 µg CO_2 kg^{-1} s^{-1}, 3.1 ± 0.1 µg CO_2 kg^{-1} s^{-1}, 3.6 ± 0.3 CO_2 µg kg^{-1} s^{-1} and 2.9 ± 0.2 CO_2 kg^{-1} s^{-1}, respectively (Fig. 3.5). Overall, pre-incubation temperature (3° and 9°C) did not significantly change R_M at 24°C, but the longer pre-incubation period (336 h) reduced R_M ($p < 0.05$) at 24°C, when compared to R_M at 24°C from soils incubated for 72 h.

The values for R_M at 24°C after 72 h and 336 h of pre-incubations at lower temperatures (averaged over pre-incubation temperatures) were, on average, 38 ± 8% and 62 ± 12% higher than the R_M of soil samples continuously incubated at 24°C for the same periods but the two proportional increases were not significantly different.

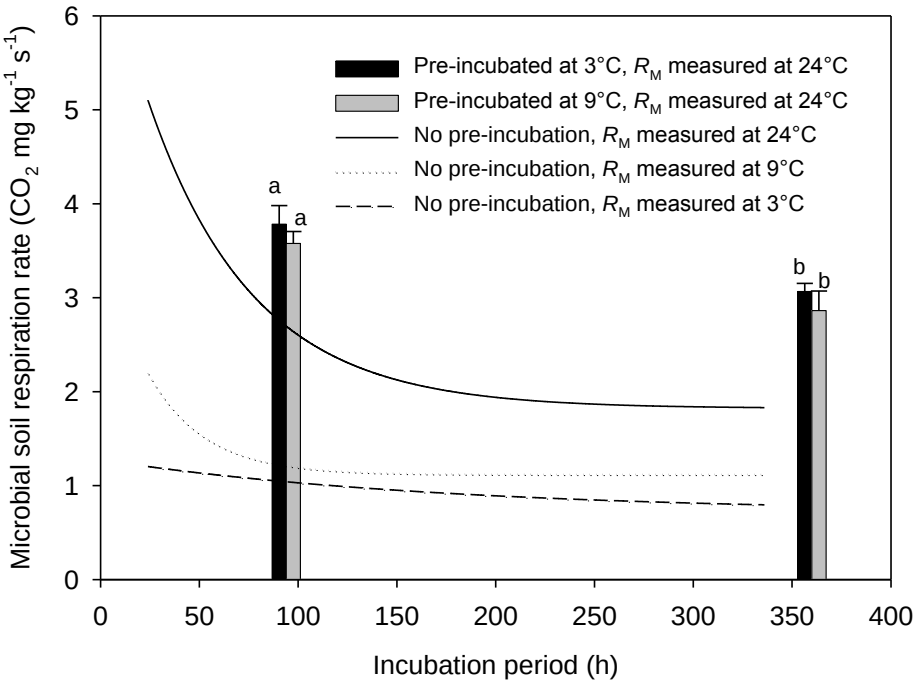

Figure 3.5 The effects of pre-incubation temperature and period on soil microbial respiration (R_M) at 24°C. The bars at 96 h are R_M after a 24 h incubation at 24°C for samples that were pre-incubated at 3° (black bar) and 9°C (silver bar) for 72 h. The bars at 360 h are R_M after a 24 h incubation at 24°C for samples that were pre-incubated at 3° (black bar) and 9°C (silver bar) for 336 h. Error bars are SEM (n = 4). The curves shown in Fig. 3.3 portray R_M over time for samples that were continuously incubated at 24°, 9°, or 3°C.

3.4.4 Effects of temperature on WSC and HWSC

The concentrations of WSC and HWSC extracted from the incubated soils did not change significantly over time (Fig. 3.6). No clear relationship was observed between R_M and either WSC or HWSC. There was also no clear effect of T_S on WSC and HWSC. On average, the amounts of WSC and HWSC varied from approximately 150 to 350 mg C kg^{-1} dry soil and 3600 to 4200 mg C kg^{-1} dry soil, respectively, during 336 h of incubation.

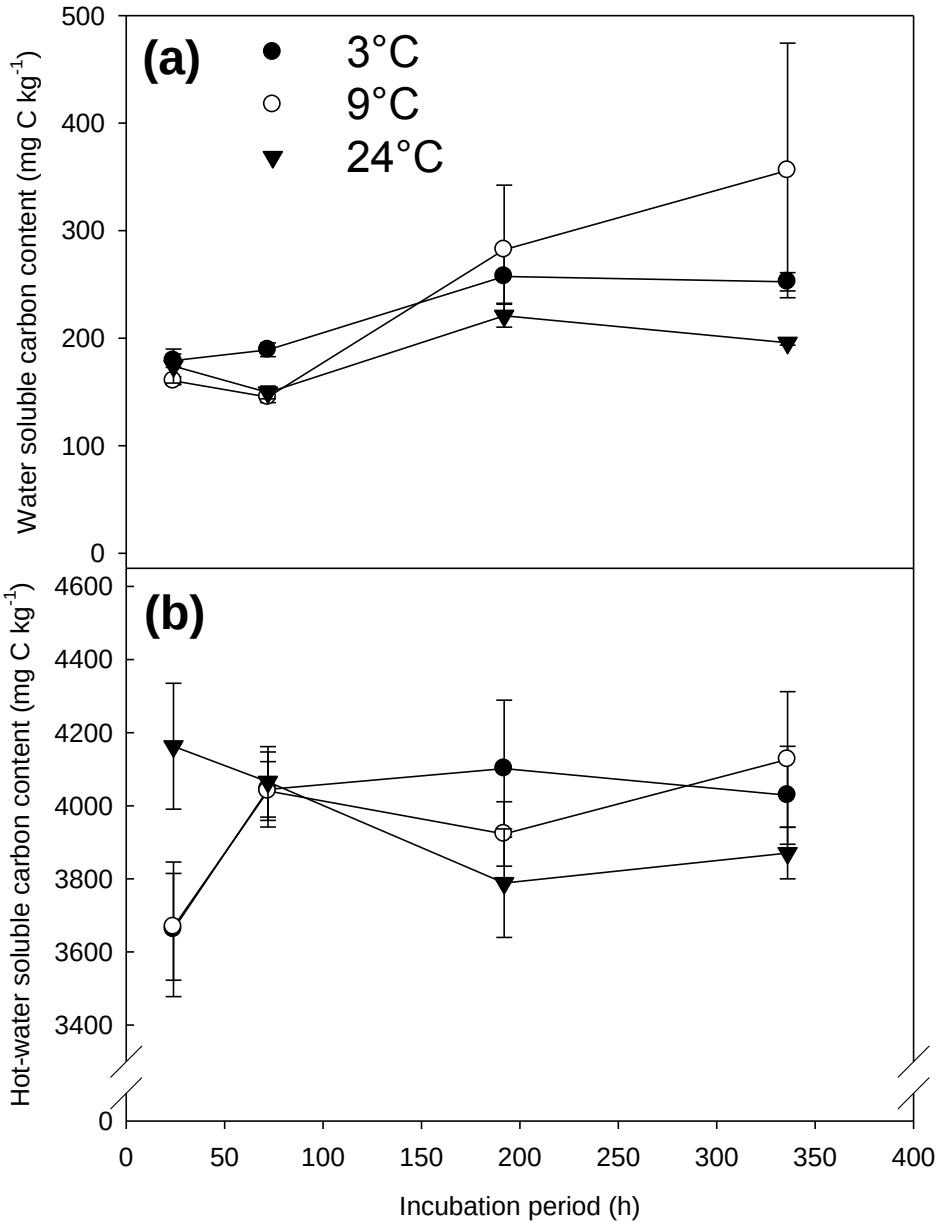

Figure 3.6 Water soluble C content (a) and hot-water soluble C content (b) of soil samples incubated at 3°, 9°, and 24°C. The error bars were SEM (*n* = 3).

3.5 Discussion

3.5.1 Responses of soil respiration to changing temperatures

In the field, R_S responded in synchrony with T_S which changed significantly and rapidly during the day. The responses of R_S to T_S were predicted by the Lloyd and Taylor model. Thus, I concluded that the pasture plants had supplied the required readily available C for R_S. However, the laboratory study showed that C substrate limitation of R_M occurred within 1 d at 24°C. The parameter R_{10} for R_S from the field study was 114 μg CO_2 m^{-2} s^{-1} including root and microbial respiration. Unexpectedly, this R_{10} was similar to R_{10} for R_M from the laboratory studies that included only microbes (113 μg CO_2 m^{-2} s^{-1}, Fig. 3.4). This suggested R_M measured in the laboratory had been unduly large. This was interpreted to reflect a microbial stress response to disturbance caused by sieving of the samples prior to incubation and measurement. Earlier field studies showed that for intensively managed grassland soils, R_{10} for R_S was ~200 μg CO_2 m^{-2} s^{-1} (Kelliher et al., 2002; Tate et al., 2000). The C supply rate can be affected by the weather and nutrient supply, and different soils having different R_{10} for R_S may have different availabilities of C substrates (Paul et al., 1999; Singh et al., 2009). In a fallow field without plants, R_S also responded in synchrony with T_S which changed significantly and rapidly during the day but R_{10} for R_S was only ~10 μg CO_2 m^{-2} s^{-1} (Nakadai et al., 2002).

During the laboratory incubations, R_M decreased significantly at the initial stage of the incubation for the soil samples at 9° and 24°C. However, R_M from the samples at 3°C was relatively constant (Fig. 3.3). The rates of decrease in R_M at 9° and 24°C were similar to values from earlier studies where R_M decreased by approximately 50%, within 3 d after clipping vegetation (Craine et al., 1999; Wan and Luo, 2003). The decrease of R_M was caused due to C substrate limitation. A previous study showed that when T_S was held constant at 20°C, applying readily available C to soil, in the form of dried ground pasture leaves, corresponded with R_M increasing nearly immediately and significantly for 17 days (Kelliher et al., 2007).

According to the time constants, R_M at 9°C and 24°C decayed at a similar rate but the amount of C evolved was significantly larger at 24°C, suggesting that the microbial community's ability to access the more recalcitrant soil C fraction(s) was positively related to T_S. Towards the end of the incubation period, R_M was clearly substrate limited at 9°C and 24°C but the soils at 24°C had a significantly higher R_M (Fig. 3.3). The amount of C emitted before R_M showed signs of substrate limitation was also much larger for soil samples at 24°C as

indicated by higher R_M in soil samples at 24°C compared to samples at 9°C and by the fact that the time constants calculated from the eqn. 3.3 were not significantly different between 9° and 24°C. While these results support earlier studies (Koch et al., 2007; Kuzyakov, 2002; Mikan et al., 2002), where microbes decomposed more recalcitrant C at higher temperatures, this study was done at much lower temperatures corresponding to a temperate climate.

The apparently reduced temperature sensitivity of R_M from soil, after C substrate supply ceased (Fig. 3.4), has been reported for other soils, although these were forest soils or pastoral soils at a much higher temperature range (20° to 40°C) (Kirschbaum, 2006; Townsend et al., 1997; Winkler et al., 1996). The information on the relationship between the C substrate limitation and R_M in pasture soils at field temperatures is particularly important because, when T_S was held constant, the response of R_M to readily available C application was significantly faster and greater for soil sampled at a highly productive dairy farm pasture compared to soil sampled at a pristine forest that had never been fertilized (Kelliher et al., 2005a). During the plant growing season, it has been reported R_M responded to T_S according to the Arrhenius type relation but the response lessened significantly when the plants were dormant or absent (Kirschbaum, 2006; Rustad et al., 2001). Kirschbaum (2006) emphasized the importance of seasonal changes in the apparent temperature sensitivity of R_M caused by different levels of C substrate limitation. The current study supports this idea but I also note that the apparent temperature sensitivity of R_M in pasture systems could change significantly on a daily basis if the rate of readily available C substrate inputs from the vegetation did not change in synchrony. Pasture plants can transfer C belowground rapidly. An earlier study showed that, in high fertile pastoral soils, more than 10% of the C uptake by ryegrass was transferred to the soil within 24 h (Butler et al. 2004). Similarly, Saggar and Hedley (2001) reported that 1.2 – 4.0% of the C uptake by dairy farm pasture was transferred to the soil within 4 h. It is also important to note that temperature influenced the efficiency of C utilization (Steinweg et al., 2008). Steinweg et al. (2008) reported that the microbial community was more efficient, released less CO_2 per unit of assimilated C, at lower temperatures. Hence, further study is needed to investigate the interaction between the efficiency of C utilization, C substrate availability, and T_S.

3.5.2 WSC and HWC

I tested two measures proposed to determine the amount of readily available C substrates in soils namely, WSC and HWSC analyses. Neither indicated the occurrence of substrate limitation with respect to R_M. At 24°C, for example, R_M decreased approximately 70% during the 336 h incubation and the soil emitted 726 ± 18 mg C kg^{-1} soil, as CO_2, to the atmosphere.

During the same period of incubation at 24°C, WSC and HWSC were constant at approximately 30% (~ 200 mg C kg^{-1} soil) and 550% (~ 4000 mg C kg^{-1} soil) of the amount of C emitted to the atmosphere (Fig. 3.6). An earlier treatment that had significantly changed R_M over time was not related to WSC at constant T_S of 20°C for soil sampled from the same dairy farm (Kelliher et al., 2005b). However, the current study encompassed a wide range of temperature and highlighted the fact that neither WSC nor HWSC were related to R_M. Two studies reported a strong linear relationship between WSC and R_M when different soil types were compared and when soils were incubated for a longer period (Liu et al., 2006; Wang et al., 2003). Wang et al. (2003) compared soils sampled from 25 different locations and their WSC contents varied from 50 to 1000 mg C kg^{-1} soil. The WSC contents were linearly correlated to CO_2-C evolved in 7 d of incubation at 35°C. Liu et al. (2006) incubated two soils at 30° and 10°C for 35 d and corresponding values of WSC contents were significantly different at the end of the experiment. The difference between the current study and previous studies suggested that WSC and HWSC may not be useful for indicating short-term changes in R_M over time from one type of soil.

3.5.3 The effect of pre-incubation treatments

The pre-incubation experiment indicated that soils maintained readily available C for a longer period at the lower T_S. The experimental design was similar to a study by Koepf (1953), who reported that the pre-incubating temperature controlled the responses of R_M to T_S. The current study had a much lower pre-incubating temperature range (3° – 9°C) compared to Koepf (1953) (10° – 30°C). At the soil sampling site, T_S was < 10°C for 50% of the time between 2006 to 2008. The two pre-incubating T_S (3° and 9°C) did not differ in their effect on R_M when samples were shifted to the higher T_S (24°C). This was different than Koepf (1953) who reported that different pre-incubating temperatures (10° to 30°C) had different effects on R_M when samples were shifted to 30°C but results from the current study agree with Bekku et al. (2003) who reported that the pre-incubation of soil samples from an abandoned field at 8° and 12°C for 17 d did not affect the response of R_M to T_S. I expected different responses of R_M at 24°C when samples were pre-incubated at 3° and 9°C since the amount of C lost during the pre-incubation was significantly larger at 9°C. At the sampling site's average T_S of 9°C, the microbial community structure might have been able to respond more efficiently to the later increased T_S. Pre-incubation at the minimum T_S of 3°C may have set the community in a state of dormancy and the 24 h period at a higher T_S (24°C) may not have been long enough for a complete recovery to the potential microbial activity. The alteration of soil microbial community structure due to an increase in T_S has been previously reported but for much

longer periods of incubation (Feng and Simpson, 2009). The responses of R_M from soils pre-incubated for 72 h was higher overall than in those pre-incubated for 336 h. Therefore readily available C substrates were slowly lost during the pre-incubations even at lower T_S. This type of information is potentially useful for accurately predicting increases in R_M when T_S increases rapidly.

3.6 Conclusions

At the field site, R_S responded rapidly to changes in T_S, increasing 20% in one hour when T_S increased by 2°C as predicted by an Arrhenius type relation. Soil incubated in the laboratory in the absence of plants showed that R_M became C substrate limited within a few days at 9° and 24°C. At 3°C, R_M was relatively constant at 1.05 ± 0.03 μg CO_2 kg^{-1} s^{-1} for 14 d, suggesting a T_S limitation. The T_S values chosen for the incubation experiments were the maximum, average, and minimum based on the frequency analysis of T_S at the field site. At 9° and 24°C, R_M declined by one time constant (63% decrease within 53 ± 3 and 76 ± 4 h, respectively) but R_M at 24°C declined to a significantly higher asymptote value than that for R_M at 9°C. This suggested that microbial ability to access the more recalcitrant C fraction(s) in soils was temperature dependent. The values of WSC and HWSC contents were unexpectedly constant for 14 d at all of the treatment temperatures and were not proportional to changes in R_M. Thus, measuring WSC and HWSC did not indicate the microbially available C substrate. The non-significant differences in R_M at 24°C, after samples were pre-incubated for 14 d at 3° or 9°C, suggested that more recalcitrant C substrate was respired at the warmer pre-incubation temperature. This study has shown that R_S beneath high fertility pasture responded rapidly to T_S change when the C supply was sufficient. When the C supply was removed, R_M was dependent on both T_S and C substrate availability. Further research is required on the mechanisms and effects of C supply in relation to the microbial community of soils, respiration, temperature, and soil fertility.

Chapter 4

Fertility and plants affect the temperature sensitivity of carbon dioxide production in soils

A manuscript from this study has been submitted to Plant and Soil: Uchida, Y., Hunt, J.E., Barbour, M.M., Clough, T.J., Kelliher, F.M., Sherlock, R.R. Fertility and plants affect the temperature sensitivity of carbon dioxide production in soils.

4.1 Abstract

Soil temperature can strongly regulate soil respiration (R_S). However, despite abundant literature, the predicted response of R_S to possible climate change remains uncertain. I hypothesized that some of the variability in results to date was due to the influence of soil fertility and absence or presence of plants. Using dairy farm pastoral soils of contrasting nutrient status, e.g. Olsen P, C: N ratio, and available N, the temperature sensitivity of R_S was measured. Measurements made in soil samples incubated at 13 – 27ºC for several days without plants showed respiration derived from organic matter (R_{OM}) averaged ~3 times greater in the high fertility soil. In the previous chapter, the term "R_M" was used to express soil microbial respiration in the absence of plants. However, R_M included root-derived C respiration because R_M was measured immediately following the removal of plants from the soil. In this chapter, the term "R_{OM}" was used to express soil microbial respiration solely from soil organic matter decomposition and the utilisation of root-derived C is termed R_{RD}. For both soils, without plants present, R_{OM} increased similarly by 3 – 4 times over this temperature range. By growing C_4 or C_3 grass species in soils with a different isotope signature, and making measurements of the natural ^{13}C abundance of CO_2 derived from R_S, the rate of root-derived respiration (R_{RD}) and R_{OM} without disturbance were determined. The temperature sensitivity of R_{RD} was not affected by soil fertility and R_{RD} contributed more than 70% of R_S, on average. However, with plants, R_{OM} was constant in the low fertility soil in contrast to the soil-only incubations at different temperature. In the high fertility soil, with plants, R_{OM} doubled as soil temperature increased from 17 – 27ºC. The results did not support the idea of a universal nor simple relationship between temperature and carbon dioxide production in soils.

4.2 Introduction

Soil respiration (R_S) plays a critical role in the carbon (C) cycle of terrestrial ecosystems with approximately 20 – 40% of all non-anthropogenic CO_2 inputs to the atmosphere produced from soils (Raich and Schlesinger, 1992; Raich and Potter, 1995). Soil respiration rates are positively related to plant productivity (Raich and Schlesinger, 1992) and are negatively related to C:N ratio (Thomsen et al., 2008). In recent years, many studies have focused on partitioning R_S into its components to understand the relationship between sources of CO_2 and the environmental factors controlling them. A natural ^{13}C abundance method enabled the measurements of R_S components without any disturbance to the system (Hanson et al., 2000; Millard et al., 2008; Robinson and Scrimgeour, 1995), by exploiting the difference in $\delta^{13}C$ signatures of C fixed by C_3 and C_4 photosynthetic pathways (O'Leary, 1988). With this method, a C_4 plant was grown in a soil developed under C_3 vegetation (C_3 soil), and vice versa. A linear mixing model was used with the $\delta^{13}C$ abundances and CO_2 concentrations of the two naturally labelled contributing sources of R_S.

The two contributing sources of R_S were root-derived C and soil C (Trumbore, 2000). Carbon that is respired from root-derived C includes microbial rhizosphere activity, as well as root respiration, and is strongly coupled to photosynthetic activity because it utilizes C substrates recently added to soil by plants (Heilmeier et al., 1997). Hereafter, root-derived C respiration is defined as R_{RD}. The respiration of soil C originates from soil organic matter decomposition. The soil organic matter pool is considerably larger than the root-derived C pool and interest in soil organic matter decomposition has increased recently because of its importance to the global C cycle (Trumbore, 2000). Hereafter respiration from the soil organic matter pool is defined as R_{OM}. Soil organic matter is heterogeneous with respect to decomposition because soil organic matter consists of several pools, with characteristic turnover times of less than a year to over several hundred years (Parton et al., 1987). The turnover time of soil organic matter depends on the recalcitrance of soil organic matter (Trumbore, 2000).

Many studies have shown that increases in soil temperature (T_S) increase R_{OM} (e.g. Kirschbaum, 1995). It has been predicted that global warming may cause a significant increase in R_{OM}, resulting in an increase in atmospheric CO_2 and an acceleration of climate change (Cox et al., 2000). Most of CO_2 produced during R_{OM} is derived from relatively short-lived soil organic matter but the age of soil C utilized by R_{OM} varies. For example, in boreal forest sites, the age of soil C utilized by R_{OM} was 2 – 30 years but in tropical soils, it was <1 year. The age of soil C is related to the recalcitrance of soil C. The effect of T_S on R_{OM} may vary depending on the relative size of the soil organic matter pools and their respective

recalcitrance. The decomposition of relatively more recalcitrant soil organic matter may be more temperature sensitive (Bol et al., 2003; Hartley and Ineson, 2008; Vanhala et al., 2007). Thermodynamically, the more enzymatic steps that are required for decomposition, the more temperature sensitive the entire process (Bosatta and Ågren, 1999).

Soils have rarely been studied with and without plants, with regards to R_{OM}. However, Bader and Cheng (2007) showed that, using a soil sampled at a cottonwood tree forest, the temperature sensitivity of R_{OM} markedly changed when plants were present, when compared to R_{OM} without plants. This is because plants can significantly influence R_{OM}, a phenomenon commonly termed the "priming effect" (Bingeman et al., 1953). Plants can either accelerate R_{OM} or suppress it (i.e. a negative priming effect) (Cheng et al., 2003; Cheng, 1996).

The relative magnitude of the priming effect may depend on the size of the soil organic C pool (Kuzyakov et al., 2000), plant species and phenology (Cheng et al., 2003), photosynthetic intensity (Kuzyakov and Cheng, 2001b), plant biomass (Dijkstra et al., 2006), and soil nutrient status such as soil-N content (Liljeroth et al., 1994). Soil temperature may also indirectly affect the magnitude of the priming effect by controlling plant activity (Scott-Denton et al., 2006). Thus, the temperature sensitivity of R_{OM} may be influenced by both the presence of plants and soil fertility. Currently there are no studies that have observed the influences of plant presence and soil fertility on the temperature sensitivity of R_S, R_{OM} and R_{RD} in pastoral soils.

In this study, a natural ^{13}C abundance method was used to measure the contribution of R_{OM} and R_{RD} to total R_S. This technique permitted the distinction between R_{OM} and R_{RD}, rather than strictly microbial versus root respiration. However, I argue that the root-derived C pool will dominate the root and associated microbial rhizosphere respiration, while CO_2 released carrying a soil C isotope signal must derive from microbial activity in the bulk soil. There was no contribution to R_S from litter decomposition due to the lack of litter production over the time period of the study.

To investigate the effect of soil fertility on the temperature sensitivity of R_S, with respect to R_{OM} and R_{RD}, Two dairy farm pasture soils of contrasting nutrient status and plant productivity were sampled. It was hypothesised that soil nutrient status would influence the temperature sensitivity of R_{OM} in the absence of plants, but in the presence of plants, both photosynthetic activity and soil nutrient status would affect the temperature sensitivity of R_{OM}. This hypothesis was based on the assumption that R_{RD} is closely associated with plant

activity while R_{OM} is associated both with the recalcitrance of soil organic matter and nutrient availability in soils.

4.3 Materials and methods

4.3.1 Soil

Soils were sampled (0-5 cm depth) from two dairy-farm pasture sites; one from an extensively managed low productivity dairy farm at Kerikeri, Northland, New Zealand (35°16'S, 173°55'E, 79 masl) and the other from an intensively managed high productivity dairy farm at Lincoln, Canterbury, New Zealand (43°39'S, 172°29'E, 8 masl). The mean annual T_S (10 cm depth, in years 2006 to 2008) at the respective sampling sites were 15.0 ± 1.1°C and 10.5 ± 0.2°C, respectively (± SD based on the average T_S for the three years). The Lincoln soil had a high fertility (*HF*), indicated by nutrient contents significantly greater than the low fertility (*LF*) Kerikeri soil (Table 4.1). The *LF* soil was a well-drained Kerikeri friable clay loam (Mottled Orthic Granular Soil; Hewitt, 1993)(Typic Haplohumox; Soil Survey Staff, 1998) and the *HF* soil was a poorly drained Temuka silt loam (Typic Orthic Gley Soil; Hewitt, 1993) (Fluvaquentic Endoaquept; Soil Survey Staff, 1998). I recognize the fact that the two soils were of different parent material but the definition of fertility is based on measured variables (Table 4.1). The *LF* soil was dominated by a C_4 pasture grass (kikuyu, *Pennisetum clandestinum* Hochst.) and the *HF* soil was dominated by a C_3 perennial ryegrass (*Lolium perenne* L.). At both sites, these pasture species had been present for > 20 years. Soils were passed through a 5.6 mm sieve and were stored for approximately 2 months at < 4°C.

Table 4.1 Selected properties of the *LF* and *HF* soils. The soil test was performed by R J Hill Laboratories Limited, Hamilton, New Zealand. Total nutrient was measured using an EPA Aqua Regia digest method. Available N was measured using an anaerobically mineralizable N method. Water soluble C and hot-water soluble C were measured using a method described by Ghani et al. (2003) and are the mean ± SEM (*n* = 3). The soils were collected in April 2009.

Soil	*LF* soil	*HF* soil
pH	5.6	5.9
Olsen P (mg l^{-1})	18	33
Potassium (mmol kg^{-1})	2.4	5.7
Calcium (mmol kg^{-1})	58	103
Magnesium (mmol kg^{-1})	8.5	19.7
Sodium (mmol kg^{-1})	2.5	5.3
CEC (cmolc kg^{-1})	21	35
Base saturation (%)	64	74
Soil density (g cm^{-3})	0.92	0.70
Available N (15 cm depth, kg ha^{-1})	414	640
Organic matter (%)	13.3	15.5
Total C (%)	7.7	9.0
Total N (%)	0.66	0.90
C: N ratio	11.7	10.0
Anaerobically mineralisable N (kg ha^{-1})	299	606
Water soluble organic C (mg C kg^{-1})	161 ± 50	277 ± 24
Hot-water soluble organic C (mg C kg^{-1})	2424 ± 556	4658 ± 121

4.3.2 Preparation of pots

The sieved soils were packed to a 10 cm depth into funnel shaped pots (12 cm deep, 22 cm and 10 cm in diameter on the top and the bottom, respectively). Each pot contained the equivalent of 2200 g dry soil. The soil C in the *LF* and *HF* soils was derived from the original plant cover, kikuyu (C_4) and perennial ryegrass (C_3), respectively. Preliminary studies showed that the $\delta^{13}C$ signatures of CO_2 respired from R_{OM} in the *LF* and *HF* soils without plants at 15°C were -13.6 ± 0.2 and -27.4 ± 0.1‰ (expressed as $\delta^{13}C_{V\text{-}PDB}$), respectively (± SEM). Hence to create a difference in $\delta^{13}C$ signatures between R_{OM} and R_{RD}, C_3 perennial ryegrass (*L. perenne*) was planted into the previously C_4 *LF* soil and C_4 paspalum (*Paspalum dilatatum* Poir.) was planted into the previously C_3 *HF* soil. These species typify pastures grown in the temperate and sub-tropical climate regions of New Zealand. The seeds of each species were only planted on the edge of each pot and an aluminium collar (7 cm depth and 14 cm in diameter) was inserted into the middle of the soil surface of each pot to allow R_S

measurements to be made (see section 4.3.3). The pots were maintained at 20°C in a glasshouse and watered daily for 8 – 12 weeks until plants had established. Urea fertilizer was applied three times during the establishment of the plants at 1, 3, and 5 weeks following the germination of the seeds (60 kg N ha^{-1}), but no fertilizer was applied three weeks prior to R_S measurements. Pots without plants were also prepared to obtain the $\delta^{13}C$ signatures of the bulk soil. These were used to obtain the $\delta^{13}C$ signatures of CO_2 respired from R_{OM} (see section 4.3.4).

4.3.3 Total soil respiration measurements

Four and six replicate pots of the *LF* and *HF* soil, respectively, with plants, were allocated to one of four nominal temperature treatments (10°, 15°, 20°, and 25°C). Temperature treatments were applied using controlled-environment growth cabinets at ambient CO_2 concentrations, 70% humidity with a 16 h day length. Soil temperature (T_S) was measured with a thermocouple (5 cm depth) during the collection of soil-respired CO_2. Although cabinet temperatures were stable to within 1°C of the set point, T_S was increased by radiation loading. Hence, the actual measured T_S are used in the analysis. There were three replicate pots of each soil type without plants. Photosynthetic photon flux density was 650 - 700 µmol m^{-2} s^{-1} at the height of the uppermost leaves (measured with a quantum sensor, L190SA; Li-Cor, Inc., Lincoln, NE, USA). One day before the application of the temperature treatments, pots were watered to field capacity, drained, and then weighed after 12 h to obtain a value for field capacity. The soil moisture was then maintained at field capacity throughout the experiment by daily watering onto the soil surface. Field capacity was chosen since the optimum soil moisture content for R_S has been frequently found at this level of soil moisture (Howard and Howard, 1993; Rey et al., 2005).

Pots were conditioned for at least 6 d in the growth cabinet at the appropriate treatment temperatures and measurements of R_S were performed between day 7 and 13 following the start of temperature treatment. A portable chamber with an infrared gas analyser (SRC-1 and EGM-1, PP Systems, Hitchin, UK) was used to measure R_S. The chamber was directly connected onto the aluminium collar on soil surface. The values of R_S were expressed as a mass of CO_2 (µg CO_2) per unit mass of dry soil (kg^{-1}) per unit time (s^{-1}) and also as a mass of CO_2 (µg CO_2) per unit mass of plant biomass (g^{-1}) per unit time (s^{-1}).

4.3.4 Collection of respired CO_2 from various sources and $\delta^{13}C$ measurements

Soil-respired CO_2 (R_S) was collected into evacuated 2 l Tedlar® bags immediately after the R_S rates were measured using an open chamber system, described by Midwood et al. (2008).

Midwood et al. (2008) used an open chamber with a CO_2 free air to sample soil-respired CO_2 without the influence of atmospheric CO_2. To collect CO_2 respired from R_{RD}, roots were separated from the soil after the measurement of R_S. The roots were then gently washed to remove soil, placed in Tedlar® bags, and the bags flushed with CO_2-free air. To collect CO_2 respired from R_{OM}, a sub-sample of the soils in the pots without plants was placed in Tedlar® bags and flushed with CO_2-free air. Bags with either roots or soils were incubated at the appropriate treatment temperatures until the concentration of CO_2 in the bags reached the calibrated concentration range of the tuneable diode laser (350 to 500 µmol mol^{-1}) where upon they were analysed for $\delta^{13}C$ values.

The $\delta^{13}C$ values of CO_2 sourced from R_S, R_{RD}, and R_{OM} were determined using a tuneable diode laser absorption spectrometer (TDL, TGA2000, Campbell Scientific Inc. Logan, UT, USA), with a precision of ± 0.1‰. The TDL system quantitatively assesses the concentrations of $^{12}CO_2$ and $^{13}CO_2$ at the same time using a laser absorption spectrometry. The TDL was described by Bowling et al. (2003). The $\delta^{13}C$ values of CO_2 sourced from R_S, R_{RD}, and R_{OM} were termed $\delta^{13}C_{Rs}$, $\delta^{13}C_{RD}$, and $\delta^{13}C_{OM}$, respectively.

4.3.5 Calculation of R_{OM} and R_{RD} rates

The ratio $R_{OM}/R_S = f_{OM}$ was calculated using a mixing model (eqn. 4.1) described by Kuzyakov (2004). The remainder was called f_{RD} (eqn. 4.2). The rates of R_{OM} and R_{RD} were obtained by multiplying f_{OM} or f_{RD}, respectively, with the plant biomass based values of R_S.

$$f_{OM} = \frac{\delta^{13}C_{R_S} - \delta^{13}C_{RD}}{\delta^{13}C_{OM} - \delta^{13}C_{RD}} \quad (4.1)$$

$$f_{RD} = 1 - f_{OM} \quad (4.2)$$

When there were plants, R_S was expressed on the basis of both plant biomass and soil weight, the later for comparison with R_S measured from the soil samples in the absence of plants.

The variances (i.e. the square of standard errors, σ) of f_{OM} were calculated as described by Phillips and Gregg (2001) (eqn.4.3):

$$\sigma^2(f_{OM}) = \frac{1}{(\delta^{13}C_{OM} - \delta^{13}C_{RD})^2}\left[\sigma^2(\delta^{13}C_{R_S}) + f_{OM}{}^2 \times \sigma^2(\delta^{13}C_{OM}) + (1 - f_{OM})^2 \times \sigma^2(\delta^{13}C_{RD})\right] \quad (4.3)$$

where $\sigma^2(\delta^{13}C_{Rs})$, $\sigma^2(\delta^{13}C_{OM})$, and $\sigma^2(\delta^{13}C_{RD})$ represent the variances of $\delta^{13}C_{Rs}$, $\delta^{13}C_{OM}$ and $\delta^{13}C_{RD}$, respectively. The variance of f_{RD} was also determined by switching the $\delta^{13}C_{OM}$ and $\delta^{13}C_{RD}$ subscripts in eqn. 4.3. All statistical analyses were performed in GenStat (Lawes Agricultural Trust, Rothamsted, UK).

4.3.6 Temperature sensitivity analysis

The Lloyd and Taylor model (eqn. 4.4) was used to describe the temperature sensitivity of R_S, R_{OM} and R_{RD} (Lloyd and Taylor, 1994).

$$R_x = R_{10} e^{E_0\left(\frac{1}{56.02} - \frac{1}{T_s + 237.15 - T_0}\right)} \quad (4.4)$$

The Arrhenius-type model had three parameters (R_{10}, E_0, and T_0). The variable T_S was measured simultaneously with the R_S measurements, and R_x was either R_S, R_{OM} or R_{RD}. The R_{10} was a scaling parameter and it equated to R_x when T_S = 10°C. The E_0 (K) was the activation energy divided by the gas constant and the T_0 determined the temperature minimum (K) at which predicted R_x reached zero. Recent studies used the Lloyd and Taylor model without constraining the parameters (Mäkiranta et al., 2008; Richardson and Hollinger, 2005). I did not constrain E_0 or R_{10} but T_0 was set at 227.13K, as described by Lloyd and Taylor (1994). The two parameters were fitted to the data set using non-linear regression using GenStat (Lawes Agricultural Trust, Rothamsted, UK).

The R_x data, based on soil weight, were used to fit the model. However, for the soils with plants, temperature sensitivity of R_x per unit of soil weight might have been biased since plant biomass (g) per pot, measured after the R_x measurements, was significantly different between temperature treatments because the plants grew faster at higher temperature during the pre-conditioning and CO_2 measurement periods. Thus, in order to fit the R_x data to the model without the bias, R_x data were expressed on a plant biomass basis when plants were present.

The E_0, obtained from the fitted Lloyd and Taylor model, were compared, based on the standard error of the difference (z) between two E_0 values. The significance of the difference

(p value) was then obtained by comparing the difference between the parameters, divided by z, and using standard normal curve areas ($z > 1.96$ corresponds to $p < 0.05$). The same approach was used to compare R_{10}.

4.3.7 Photosynthetic rate measurements and herbage sample analysis

Measurements of leaf photosynthesis (A) were made on fully expanded leaves using a portable photosynthesis system (LI6400; Li-Cor, Inc. Lincoln, NE, USA) fitted with a leaf chamber 1 – 3 d before R_S was measured. The light intensity within the leaf chamber was set to match growth photosynthetic photon flux density at the uppermost leaves. Relative humidity and CO_2 concentration were not controlled within the leaf chamber, and averaged 56 ± 2% and 396 ± 5 µl l^{-1} respectively. A was measured at the four treatment temperatures described above (10° to 25°C) and the temperature sensitivity of A was interpreted from measured leaf temperature. Photosynthetic rates for the whole pot were estimated by multiplying A (per unit leaf area) by the total live leaf area per pot. Pot leaf area was calculated from measured specific leaf mass (65 ± 9 and 41 ± 10 g m^{-2} for ryegrass and paspalum, respectively) and total leaf dry weight per pot. This estimate of total A per pot makes the simplifying assumption that all leaves are equally exposed to the same radiation and microclimate conditions. Even though this assumption is not strictly valid, it is argued that it is appropriate because it is the relative differences between pots that are important, rather than the absolute rate of C fixation.

After R_S measurements and plants were harvested then dried at 65°C, weighed and finely ground. The leaf and root samples were then separately analysed for N content using an automated mass spectrometer (PDZ-Europa 20-20, Crewe, UK). These N contents (%) were multiplied by leaf and root biomass data and the effect of temperature treatments and soil type were investigated based on the N contents in the leaf and root biomass per pot using a two-way ANOVA. Root biomass was obtained by separating and gently washing the roots from the soil after the measurement of R_S and drying them in an oven (65°C).

4.4 Results

4.4.1 Respiration sourced from soil C without plants

Without plants and at the lowest T_S, the rate of R_{OM} in the *LF* soil (0.15 ± 0.04 µg CO_2 kg^{-1} soil s^{-1}) was half the rate in the *HF* soil (0.31 ± 0.04 µg CO_2 kg^{-1} soil s^{-1}, $p < 0.05$, Fig. 4.1a). The rate of R_{OM} in the *HF* soil was significantly higher than in the *LF* soil at all temperature treatments except at 25°C (Fig. 4.1a). The Lloyd and Taylor model fitted the change in R_{OM} and T_S in the *LF* and *HF* soils well ($r^2 \geq 0.90$, Fig. 4.1a). The E_0 values for the *LF* soil (504 ± 103 K) and for the *HF* soil (273 ± 59 K) were not significantly different ($p = 0.06$) but R_{10} was significantly ($p < 0.05$) larger for the *HF* soil.

4.4.2 Soil respiration rates with plants present

When averaged across all levels of temperature, in the presence of plants, R_S in the *LF* and *HF* soil (1.52 ± 0.17 and 1.83 ± 0.16 µg CO_2 kg^{-1} soil s^{-1}, respectively) did not differ significantly (Fig. 4.1b). At the lowest T_S, R_S in the *HF* soil (0.53 ± 0.06 µg CO_2 kg^{-1} soil s^{-1}) was significantly lower than R_S in the *LF* soil (1.18 ± 0.09 µg CO_2 kg^{-1} soil s^{-1}) but R_S did not differ between the two soils at other levels of T_S. The Lloyd and Taylor model again described the relationship between R_S and T_S in the *LF* and *HF* soil well, when R_S was described based on plant biomass ($r^2 \geq 0.90$, Fig. 4.1c). The E_0 for R_S was significantly larger ($p < 0.05$) for the *HF* soil (462 ± 71K) than the *LF* soil (277 ± 62K) (Fig. 4.1c). For the *LF* soil, the R_{10} value was significantly larger ($p < 0.001$) than for the *HF* soil (Fig. 4.1c).

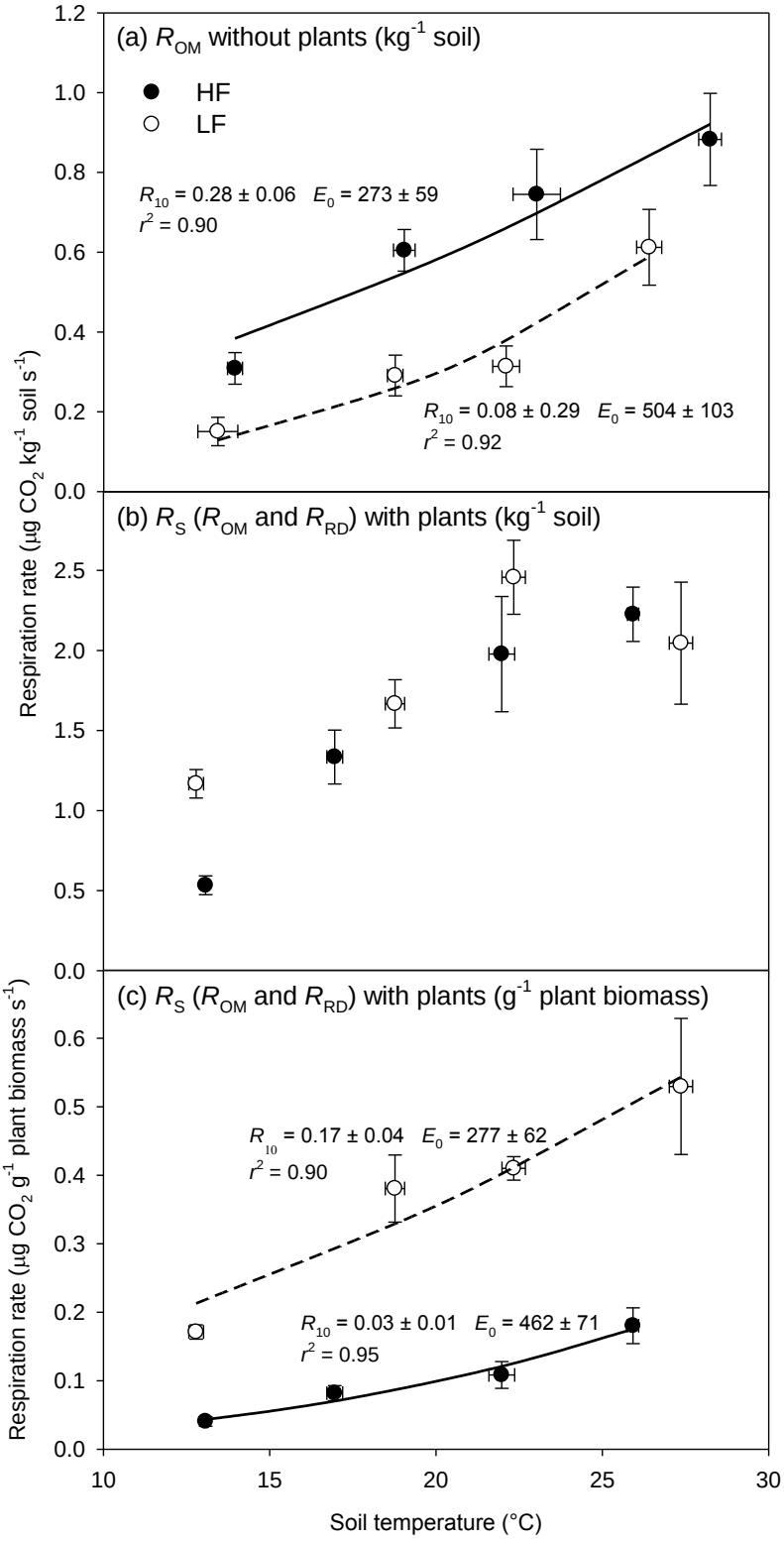

Figure 4.1 (a) R_{OM} without plants at different soil temperatures in the *LF* soil (○) and *HF* soil (●) without plants, and (b) R_S with plants present expressed as on a unit soil basis, or (c) expressed on a unit plant basis. Dashed and solid lines represent the best Lloyd and Taylor (1994) model fitted to R_{OM} or R_S, respectively. The E_0, R_{10}, and r^2 values are next to the corresponding curves, with the SE. Error bars are SEM (n = 3, 4, and 6 for soils without plants, the *LF* soil with plants, and the *HF* soil with plants, respectively). Note different y axis scales. Data in Fig. 4.1(c) was used to separate R_S into R_{OM} and R_{RD} and the results are shown in Fig. 4.2a and b.

4.4.3 Effects of soil fertility and temperature on R_{OM} and R_{RD}

The R_S data described in Fig. 4.1c was partitioned into the rates of R_{OM} and R_{RD} using the data in Table 4.2 (Fig. 4.2a and 4.2b). When T_S increased from 13° to 27°C, the R_{OM}/R_S ratio (= f_{OM}) decreased from 0.29 ± 0.02 to 0.08 ± 0.05 in the *LF* soil while the f_{OM} in the *HF* soil increased from 0.10 ± 0.03 to 0.43 ± 0.03 (Fig. 4.2c and 4.2d). In the *HF* soil, there was no significant difference between the temperature sensitivity (E_0) of R_{OM} with and without plants. For R_{RD}, there was no effect of soil fertility on the temperature sensitivity.

When R_{OM} in the presence of plants (Fig. 4.2a and 4.2b) was expressed based on the soil weight, R_{OM} at the lowest treatment T_S (~13°C) in the *LF* soil (0.29 ± 0.03 μg CO_2 kg^{-1} soil s^{-1}) was larger than the R_{OM} without plants at 13°C in the *LF* soil. For the *HF* soil, R_{OM} in the presence of plants at 13°C (0.05 ± 0.01 μg CO_2 kg^{-1} soil s^{-1}) was lower than R_{OM} without plants at 13°C. In the *HF* soil with plants, the R_{OM} rates increased to 0.76 ± 0.09 μg CO_2 kg^{-1} soil s^{-1} at the highest T_S (~26°C), while in the *LF* soil with plants, the R_{OM} rates remained constant at higher T_S.

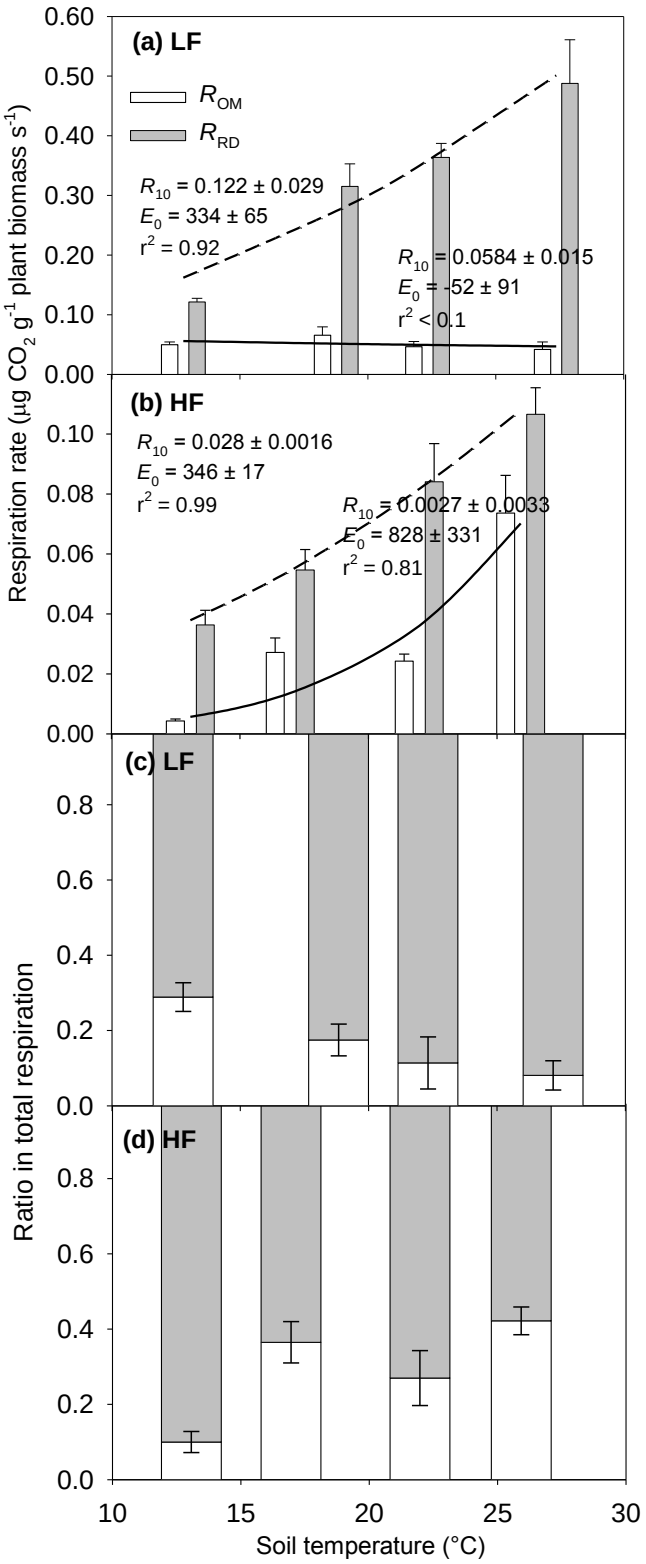

Figure 4.2 R_{OM} and R_{RD} and their contributions to R_S in the *LF* soil (a and c) and *HF* soil (b and d). Solid and dashed line in (a) and (b) represent the best Lloyd and Taylor (1994) model fitted to R_{OM} and R_{RD}, respectively. Parameters (E_0 and R_{10}) for the fitted Lloyd and Taylor model and r^2 values are next to the corresponding curves along with the SE. Error bars represent SEM (n = 4 and 6 for the *LF* soil and the *HF* soil, respectively). Error bars in (c) and (d) represent propagated SEM for the fraction of R_{OM} in R_S calculated according to Phillips and Gregg (2003) (eqn. 4.3).

Table 4.2 The $\delta^{13}C$ abundances of soil-respired CO_2 ($\delta^{13}C_{Rs}$), CO_2 sourced from R_{RD} ($\delta^{13}C_{RD}$), CO_2 sourced from R_{OM} ($\delta^{13}C_{OM}$), and calculated fractions of R_{OM} (f_{OM}) and R_{RD} (f_{RD}) in R_S from the (a) *LF* and (b) *HF* soil, respectively. Errors of the $\delta^{13}C$ abundances represent SEM ($n = 4$: $\delta^{13}C_{Rs}$ and $\delta^{13}C_{RD}$ for *LF*, $n = 6$: $\delta^{13}C_{Rs}$ and $\delta^{13}C_{RD}$ for *HF*, $n = 3$: $\delta^{13}C_{OM}$). The propagated errors for the fractions (f_{OM} and f_{RD}) were calculated based on the errors of the $\delta^{13}C$ abundances using a method described by Phillips and Gregg (2001). *, **, and ns denote the significant effect of treatment temperature with $p < 0.01$, $p < 0.05$, and $p \geq 0.05$, respectively.**

(a) *LF* soil (C_4 soil)

	$\delta^{13}C_{Rs}$(‰)	$\delta^{13}C_{RD}$(‰)	$\delta^{13}C_{OM}$(‰)	f_{OM}	f_{RD}
Treatment °C	***	***	ns	***	***
10°C	-26.7 ± 0.2	-30.8 ± 0.1	-16.7 ± 0.5	0.29 ± 0.02	0.71 ± 0.02
15°C	-26.2 ± 0.4	-28.2 ± 0.3	-17.1 ± 0.2	0.18 ± 0.05	0.82 ± 0.05
20°C	-27.0 ± 0.3	-28.3 ± 0.5	-17.1 ± 0.7	0.12 ± 0.06	0.88 ± 0.06
25°C	-27.8 ± 0.4	-28.6 ± 0.3	-18.2 ± 0.2	0.08 ± 0.05	0.92 ± 0.05

(a) *HF* soil (C_3 soil)

	$\delta^{13}C_{Rs}$(‰)	$\delta^{13}C_{RD}$(‰)	$\delta^{13}C_{OM}$(‰)	f_{OM}	f_{RD}
Treatment °C	**	***	ns	***	***
10°C	-15.0 ± 0.3	-13.7 ± 0.1	-26.8 ± 0.4	0.10 ± 0.03	0.90 ± 0.03
15°C	-17.2 ± 0.6	-12.5 ± 0.2	-25.3 ± 0.4	0.37 ± 0.07	0.63 ± 0.07
20°C	-17.1 ± 0.3	-13.4 ± 0.2	-27.2 ± 0.9	0.27 ± 0.04	0.73 ± 0.04
25°C	-18.1 ± 0.2	-12.6 ± 0.2	-25.5 ± 0.4	0.43 ± 0.03	0.57 ± 0.03

4.4.4 Carbon assimilation rates and R_{RD}

As expected, C assimilation rates (net photosynthetic rate, *A*) increased with increasing leaf temperature for both ryegrass and paspalum (Fig. 4.3). At < 15°C ryegrass in the *LF* soil had higher values of *A* per unit leaf biomass than paspalum in the *HF* soil (Fig. 4.3). For ryegrass, there was no further increase in C assimilation rates above 20°C while assimilation rates for paspalum continued to increase, reflecting higher thermal optima for C_4 photosynthesis (Long, 1999).

The calculated *A* per pot reached a maxima at 20° and 25°C for ryegrass (62 ± 4 µg CO_2 pot^{-1} s^{-1}) and paspalum (185 ± 15 µg CO_2 pot^{-1} s^{-1}), respectively (Table 4.3). The R_{RD} rates as a percentage of the net C assimilation rates (R_{RD}/A) were 6.4 ± 0.8 and 2.2 ± 0.4% for ryegrass and paspalum, respectively (Table 4.3), when averaged across all temperature treatments. Hence, R_{RD}/A was three times greater in the *LF* soil than in the *HF* soil. For the *HF* soil, R_{RD}/A increased as T_S increased. The differences in R_{RD}/A between temperature treatments were relatively small when compared to the differences between plant species (Table 4.3).

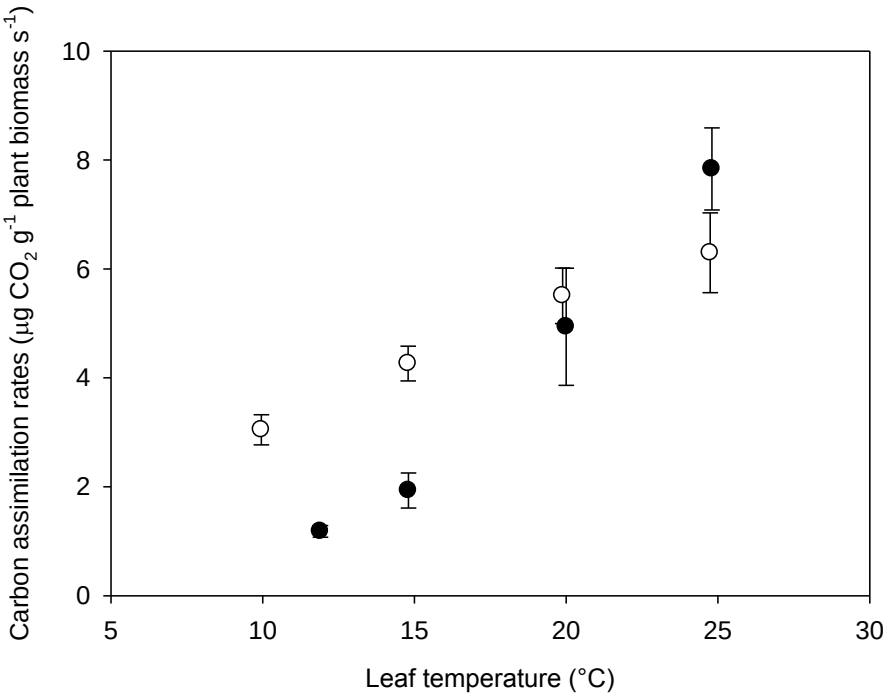

Figure 4.3 Temperature response of the carbon assimilation rates of ryegrass (○, *LF* soil) and paspalum (●, *HF* soil). Error bars represent SEM (*n* = 4).

Table 4.3 Leaf and root biomass per pot measured immediately following the R_S measurements and the estimated fractions of CO_2 respired via R_{RD} per unit weight of net CO_2 fixed by photosynthesis (A). The errors represent SEM (n = 4).

	Leaf biomass (g pot^{-1})	Root biomass (g pot^{-1})	A (μg CO_2 pot^{-1} s^{-1})	R_{RD} (μg CO_2 pot^{-1} s^{-1})	R_{RD}/A (%)
LF soil					
10°C	9.0 ± 0.6	3.7 ± 0.2	39 ± 2	1.5 ± 0.1	4.0 ± 0.9
15°C	6.1 ± 0.7	1.6 ± 0.3	36 ± 2	2.6 ± 0.4	7.4 ± 1.8
20°C	8.2 ± 0.8	3.0 ± 0.5	62 ± 4	4.1 ± 0.5	6.6 ± 1.2
25°C	5.7 ± 0.3	1.4 ± 0.2	45 ± 3	3.4 ± 0.6	7.7 ± 1.7
HF soil					
10°C	16.6 ± 1.2	5.5 ± 0.6	30 ± 3	0.9 ± 0.1	3.1 ± 1.3
15°C	27.2 ± 0.7	4.2 ± 0.4	59 ± 6	1.7 ± 0.2	2.8 ± 0.8
20°C	26.9 ± 5.5	7.6 ± 1.0	167 ± 15	2.8 ± 0.6	1.7 ± 0.4
25°C	19.5 ± 2.2	3.6 ± 0.1	185 ± 15	2.5 ± 0.3	1.4 ± 0.3

4.4.5 Herbage sample analysis

Paspalum leaves and roots in the *HF* soil always contained significantly ($p < 0.01$) more N than ryegrass leaves and roots in the *LF* soil (Fig. 4.4a and b). There was no significant trend in leaf or root N related to changes in temperatures for either species.

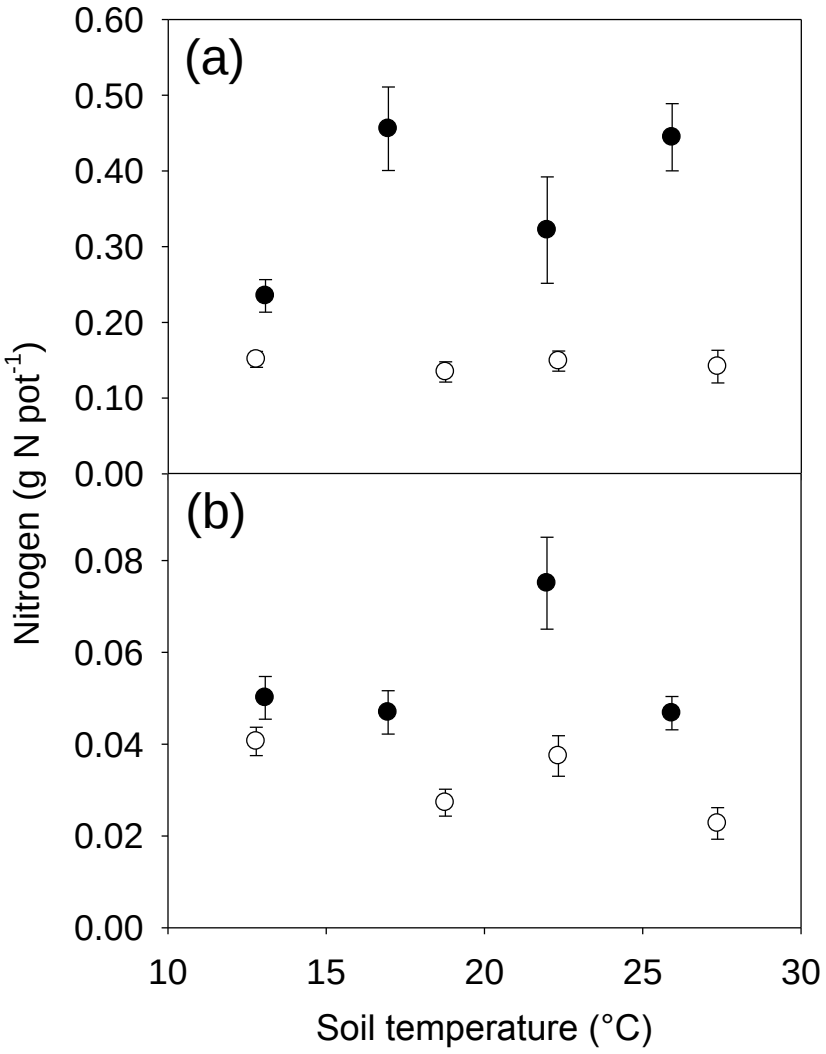

Figure 4.4 Total N contents of leaf (a) and root (b) biomass of ryegrass (○, *LF* soil) and paspalum (●, *HF* soil) grown at four temperatures. Error bars are SEM (*n* = 4 and 6 for the *LF* soil and the *HF* soil, respectively).

4.5 Discussion

4.5.1 Total soil respiration and R_{OM} with and without plants present

Without plants, greater nutrient (e.g. N and P) and C substrate availability (total C and water soluble C contents, Table 4.1) accounted for significantly larger R_{OM} in the *HF* soil. Previous work by Cleveland et al. (2006) also showed that soil nutrient availability was positively correlated to R_{OM} in a tropical rain forest soil. In the two soils, evidently, there was sufficiently available C for R_{OM} to more than double as T_S increased from 13 – 27°C. In the presence of plants, the same was true but in the *LF* soil, nearly all C emitted from the soil surface was from the roots i.e. R_{RD}. Unexpectedly, the R_{OM} from the *LF* soil surface was constant across the 14°C range of T_S in the presence of plants. As stated, indicative measurements suggested nutrient and C substrates were limited in the *LF* soil's organic matter. However, growing in the *LF* soil, ryegrass C assimilation rate doubled as T_S increased from 13 – 27°C. Thus, to meet the 'demand' of increased T_S on CO_2 production in the *LF* soil, microbes used the increasingly available root-derived C. In the *HF* soil, as T_S increased, microbes used broadly similar proportions of soil C (R_{OM}) and root-derived C (R_{RD}). The previous study in this thesis found that, using the *HF* soil, after the removal of roots, R_S rate halved within 4 d at the T_S range of 9 – 24°C due to C substrate limitation (Uchida et al., 2010). However, it was also concluded (Uchida et al. 2010) that, in the *HF* soil, R_S at higher T_S could remain relatively higher during the period of C substrate limitation, compared to R_S at lower T_S because microbes were able to access more recalcitrant C substrate as the temperature increased. The current study concurs with this previous finding as shown in Fig. 4.2b where the rate C respired from the old C pool (R_{OM}) increased with increasing temperature. I recognise that the current study was a short-term laboratory based and if this study was performed for a longer period, other factors such as soil microbial adaptation (Bradford et al., 2008) might have played an important role in relation to the temperature response of R_{OM}. However, I believe that although the current study was performed during a short-term period, the difference between the temperature responses of R_{OM} with and without plants present, and between the *HF* and *LF* soils, are extremely large and worth noting to improve the future prediction of R_S in pastoral soils.

Fertility affected R_{OM} in soils responding to changing T_S. Heightened interest in the temperature sensitivity of R_{OM} has been motivated by an implied and apparently appealing connection between the sustainability of soil C storage and the global warming. However, under carefully controlled, though short-term conditions, this hypothesis was not supported by *in situ* measurements in the *LF* soil with plants present. The results show that the situation is

more complex because of the effect of T_S on soil C respiration can also be affected by fertility and not just the 'availability' of soil C and root-derived C for microbial utilisation.

Despite the earlier results of chapter 3, a study by Wang et al. (2003) showed that water soluble C contents and R_{OM} were strongly correlated (linear relationship, $r^2 = 0.89$) based on the comparison of 25 different soils, without plants present. Hence the relationship between the rates of R_{OM} and soil fertility observed in the current study without plants present, agrees with this earlier work. However, the results with respect to the temperature sensitivities of R_{OM} in the *LF* and *HF* soils, in the absence of plants, contradict previous work (Bosatta and Ågren, 1999; Hartley and Ineson, 2008; Vanhala et al., 2007) that suggested the temperature sensitivity of R_{OM} was negatively correlated to the microbial availability of C. In the current study, the microbial availability of C was higher in the *HF* soil, when compared to the *LF* soil, based on the fact that R_{10} in the *HF* soil was higher than the *LF* soil without plants (Fig. 4.1a). However, R_{OM} in the two soils was equally temperature sensitive in the current study, although more replication might have changed these results because the difference between the temperature sensitivity of R_{OM} in the *LF* and *HF* soils without plants was marginally significant ($p = 0.06$, Fig. 4.1a). Hartley and Ineson (2008) showed that, in the absence of plants using a soil from an experimental garden, the temperature sensitivity of R_{OM} was positively related to the recalcitrance of soil organic matter. Vanhala et al. (2007) also showed that the decomposition of soil organic matter formed from recently (less than 5 years old) sown crops was more temperature sensitive than the decomposition of relatively older soil organic matter. However, previously reported studies have compared R_{OM} from soil organic matter pools of difference recalcitrance in the same soil, whereas the current study compared two soils sampled at two separate sites. Hence, a simple comparison between the current study and previous work may be difficult. The two soils used in the current study not only differed in their nutrient status and water soluble C contents, but also differed in soil types and the history of aboveground species, hence these factors may have influenced the temperature sensitivity of R_{OM} in the absence of plants.

In the presence of plants, the range in the E_0 of R_S in the *LF* and *HF* soils (Fig. 4.1c) encompassed the previously reported E_0 value obtained from a grazed pastoral soil (E_0 = 369.20 ± 8.50) (Brown et al., 2009). However, there was no statistically significant difference between the previously reported E_0 value and the E_0 values for R_S obtained in the current study.

The $\delta^{13}C$ values of the bulk soil-respired CO_2 for the *HF* and *LF* soils (Table 4.2) were similar to the previously reported $\delta^{13}C$ values of C_3 (-26‰, Balesdent et al., 1987) and C_4 (-14

to -19‰, Nissenbaum and Kaplan, 1972) soils, respectively. The $\delta^{13}C$ values of the ryegrass and paspalum roots in the current study (Table 4.2) were also similar to the previously reported $\delta^{13}C$ values of C_3 and C_4 plants, respectively (O'Leary, 1988).

The extremely low temperature sensitivity of R_{OM} in the *LF* soil, with plants present (Fig. 4.2a), indicated that soil microbes in the *LF* soil were more likely to preferentially utilise the root-derived C when it was available, as opposed to the bulk soil C (Kelliher et al., 2005a). It was probably because of low microbial availability of soil C in the *LF* soil. Conversely, for the *HF* soil, the temperature sensitivity of R_{OM} with plants (Fig. 4.2b) remained high, when compared to R_{OM} without plants (Fig. 4.1a). Dijkstra et al. (2006) reported that at an optimum T_S, the magnitude of the priming effect (the increase in R_{OM} when root-derived C was added to soil) was significantly higher in fertile soils (organic farm soils) when compared to less fertile soils (annually cultivated grassland soils) and the results from the current study agree well with this phenomenon. A priming effect occurs only when the microbial biomass increases or when it is activated by the addition of readily available C from plants (Kuzyakov and Bol, 2006). Hence, in the current study, R_{OM} in the *LF* soil was potentially suppressed due to a lower availability of soil C, while in the *HF* soil, microbial activity increased and primed more efficiently as a result of increased plant activity as evidenced by enhanced rates of photosynthesis, especially at higher T_S.

A further explanation for the difference in the temperature sensitivities of R_{OM} may have been the soils' N contents. In the current study, the N content in the plant biomass, on a per pot basis, was higher in the *HF* soil (Fig. 4.4). Thus, higher soil N availability in the *HF* soil probably increased the activities of soil enzymes related to R_{OM} especially at higher T_S, resulting in higher temperature sensitivity of R_{OM} in the *HF* soil. The positive relationship between soil N availability and R_{OM} was previously reported in a herb based ecosystem and in a tropical forest (Manning et al., 2008; Waldrop and Firestone, 2004b).

4.5.2 Root-derived C utilisation and C assimilation rates

Since the values of E_0 for R_{RD} were similar in both soils (Fig. 4.2a and b) and because they were also similar to the value for E_0 that had been determined by Lloyd and Taylor (1994), based on an analysis of R_S data from a wide range of ecosystems, it can be concluded that the C supply from plants was not limiting R_{RD} (Davidson et al., 2006). The results from the current study also agreed with Flanagan and Johnson (2005) who showed that, in a grassland ecosystem, aboveground activity was a good proxy for accounting for the variations in R_{RD}. Moyano et al. (2008) also showed that, in a forest ecosystem, microbial rhizosphere

respiration was strongly correlated to photosynthetic activity. However, results from the current study differed from those of Fitter et al. (1998) who reported that root respiration rates from plants adapted to warmer temperatures were more temperature sensitive. I found no increase in the temperature sensitivity of R_{RD} in paspalum, a C_4 plant which is adapted to a warmer climate, relative to ryegrass (C_3 plant).

The fact that the percentage of *A* subsequently respired via R_{RD} (R_{RD}/A, Table 4.3) was significantly lower for the paspalum in the *HF* soil compared to the ryegrass in the *LF* soil contradicts previous reports which showed that the percentage of assimilated C lost as R_{RD} was relatively higher in tropical plants when compared to temperate plants (Lloyd and Farquhar, 1996; Lloyd and Farquhar, 2008). However, in the previous works, tree species were the focus of the studies rather than pasture species, and also the R_{RD}/A values were estimated at the ecosystem level, rather than on a per pot basis as in the current study. Also, I made some assumptions when determining the C input into the soil from plants (see section 4.3.7) and these might have influenced the current results.

4.6 Conclusion

Without plants present, R_{OM} in the *HF* soil was double that in the *LF* soil. The response of R_{OM} to a change in T_S was significant, doubling for a temperature increase of 10° to 20°C, and statistically indistinguishable for the two soils. When plants were grown in the soils R_{RD} was similarly responsive to T_S, although R_{RD} was 4-fold greater for ryegrass plants growing in the *LF* soil. When plants were grown in the *HF* soil and T_S was doubled (13° to 26°C), R_{OM} increased 19-fold. However, when plants were grown in the *LF* soil and T_S was doubled, R_{OM} remained constant. This was attributed to preferential, microbial utilization of root-derived C and limited availability of soil C. The non-destructive measurement of R_{OM} with growing plants suggested nutrient and C availabilities regulated the soil's temperature sensitivity. This may not seem to bode well for the prospect of conserving C in highly fertile soils when growing pasture plants under warmer conditions, but recalcitrance of soil C in the low fertility soil seemed potentially promising from this perspective. The results of the current short-term study conducted under controlled conditions warrant further, longer-term study under field conditions.

Chapter 5
Effects of bovine urine, plants and temperature on nitrous oxide and carbon dioxide emissions from a low fertility soil

5.1 Abstract

Pastoral agriculture involves ruminant animals being fed by year-round grazing. Nitrogen (N) utilisation by ruminants is relatively low, and in urine patches up to 1000 kg ha^{-1} of N may be deposited, mostly in the form of urea. This N may be kept in the system through uptake by plants and immobilisation, or lost via ammonia volatilisation, leaching, or gaseous emission to the atmosphere as nitrous oxide (N_2O), nitric oxide (NO) and dinitrogen (N_2). These processes can be affected by temperature as can urea hydrolysis that significantly raises the soil pH, depending on the buffering capacity, liberating some of the soil's organic matter by solubilisation. Temperature and carbon substrate availability can also strongly affect carbon dioxide (CO_2) emissions from soils. I postulated that the response of soil CO_2 and N_2O emissions following bovine urine application would be affected by plants, temperature and soil fertility. Dairy cattle urine was collected near Lincoln, labelled with ^{15}N, and applied at 590 kg N ha^{-1} to a low fertility soil (previously studied in Chapter 4) that came from a dairy farm located about 1000 km north of Lincoln, near Kerikeri. At a soil temperature of 23ºC, with and without growing ryegrass plants, the urine application induced a significant increase in the N_2O emissions that was greater than that at 11ºC. At 23ºC, with and without plants, 0.7 and 2.2% of the applied N was directly emitted as N_2O, mostly over a 3 d period of incubation when the soil's water-filled pore space was at 70%. The corresponding recoveries of ^{15}N were, 0.6 and 1.5%. This suggested the plants had induced an almost 3-fold increase in the emissions and the emitted N_2O had mostly come from the applied urine. This was thought to have reflected the soil's low N availability. Urine application induced a significant carbon (C) priming effect because, without plants, the net CO_2 emissions (treated minus control) were significantly greater than zero. Thus, in contrast to the N_2O emissions, enhanced C mineralisation of the soil's organic matter was thought to have accounted for the increased CO_2 emissions. Without plants, urine application induced the CO_2 emissions at 23ºC to be 42% greater than that at 11ºC. Without a C substrate availability limitation, soil microbial CO_2 emissions at 23ºC should have been about 120% greater than that at 11ºC. Thus, following urine application to soil at 23ºC, the CO_2 emissions were evidently limited by

'insufficient' C substrate availability. This was thought to have reflected the soil's low C quality (WSC and HWSC, Table 4.1). With plants, CO_2 emissions also increased significantly in response to urine application but similarly at the two temperatures. This implied further effects of plant performance on soil CO_2 emissions at different temperatures.

5.2 Introduction

Ruminant urine deposition creates a significant nitrogen (N) input to grazed pasture soil ecosystems. The rate of N deposition during a dairy cattle urination event may range from 500 – 1000 kg N ha^{-1} (Haynes and Williams, 1993). These high rates of N are in excess of the pasture's immediate demand and as a result the urine patches contribute significantly to the global nitrous oxide (N_2O) budget (Oenema et al., 1997). Nitrous oxide is a greenhouse gas with a global warming potential 298 times that of CO_2 over a 100 year time frame, and the atmospheric concentration of N_2O continues to rise (Forster et al., 2007). Nitrogen oxides (NO_x = NO + NO_2) catalytically destroy stratospheric ozone (Crutzen, 1970) and the terrestrial emissions of N_2O are a significant source of stratospheric NO_x (Matson and Vitousek, 1990). Hence, Ravishankara et al. (2009) recently showed that N_2O emission is currently the single most important ozone-depleting gas.

The soil C pool (1580 Gt C) contains approximately twice as much C as the atmospheric C pool (750 Gt C) and it contributes to terrestrial CO_2 production (Schimel, 1995). Soil C substrates available for soil respiration (R_S) may comprise the original soil organic matter pool i.e. soil organic C, or recently deposited C such as root exudates i.e. root-derived C, the latter being associated with root and rhizosphere activity (Trumbore, 2000). The decomposition of soil organic C (R_{OM}) under urine patches has been shown to be enhanced following urine addition to soils and this phenomenon was interpreted as a urine-induced priming effect (Clough and Kelliher, 2005). Urine-induced priming possibly occurs because of the solubilisation of soil organic matter which is a result of the elevated soil pH that ensues during urea hydrolysis (Sherlock and Goh, 1984). Enhanced pasture production following urine deposition may also lead to greater utilisation of root-derived C (R_{RD}) within the urine patch, since increased plant production may increase root exudation per unit volume of soil (Ledgard et al., 1982). Conversely, a short-term (14 d) decrease in root-derived C utilisation, after urine deposition, has also been reported due to the fact that the high soil pH damaged pasture roots (Ambus et al., 2007).

Soil CO_2 fluxes (F_{CO_2}) following ruminant urine deposition are a combination of both abiotic processes, such as urea hydrolysis, and biological processes such as R_S (Follett et al., 2001;

Lovell and Jarvis, 1996). About 70 – 90% of urine-N is urea (Doak, 1952) and urea is rapidly hydrolysed in soils in accordance with eqn. 5.1:

$$CO(NH_2)_2 + 2H_2O \rightarrow 2NH_4^+ + CO_3^{2-} \quad (5.1)$$

Hydrolysis of the carbonate ions results in an increase in soil pH (eqn. 5.2):

$$CO_3^{2-} + H_2O \leftrightarrow HCO_3^- + OH^- \quad (5.2)$$

The high pH and presence of ammonium (NH_4^+) ions lead to the creation of ammonia (NH_3) gas (eqn. 5.3) and as this NH_3 is volatilized a decrease in pH occurs accompanied by the release of CO_2 according to Avnimelech and Laher (1977) (eqn. 5.4).

$$NH_4^+ \rightarrow NH_{3\,(g)} + H^+ \quad (5.3)$$

$$H^+ + HCO_3^- \leftrightarrow CO_2 + H_2O \quad (5.4)$$

Soil temperature (T_S) affects the mechanisms responsible for both N_2O and CO_2 production from soils (McKenney et al., 1980; Tiedje, 1988). During a field study, with plants (maize) present in a silty clay soil, an exponential relationship occurred between T_S and N_2O flux, when T_S ranged from 11° to 26°C (e.g. Song and Zhang, 2009). Soil moisture was not controlled in the study by Song and Zhang (2009), ranging from 24 to 78% WFPS.

With respect to R_S a 1°C increase in T_S may increase R_S by 10% at 10°C (Lloyd and Taylor, 1994) if other factors, such as soil moisture, are not limiting. The response of these N_2O and CO_2 producing processes, to changes in T_S, may also be influenced by other factors, for instance, an irrigation treatment (10 mm) reduced the relative responses of soil N_2O fluxes to T_S for 7 d, when T_S ranged from 10° to 20°C (de Klein and Van Logtestijn, 1996). The availability of soil C substrates, supplied from plants, has also been shown to influence the response of R_S to T_S (Hartley and Ineson, 2008). Similarly, the previous experiment (Chapter 4) showed that plants and soil nutrient status influenced the response of R_S to T_S with plants reducing the response of R_S to T_S under low fertility conditions. While under high fertility conditions, as would occur in a urine patch, R_S was sensitive to T_S both with and without plants.

Anthropogenically driven climate change is expected to result in an increase in the mean global surface temperature of between 1.8° – 4.0°C (IPCC, 2007a). This could potentially create a positive feedback for both soil N_2O and CO_2 fluxes because an increase in T_S tends to increase the rate of soil processes (Cox et al., 2000; Smith, 1997). Abdalla et al. (2009) reported that denitrification in grazed pastoral soils (in the absence of plants) was very

sensitive to temperature, with temperature ranging between 10 – 25°C, but they did not document how the temperature responses of soil N_2O and CO_2 fluxes would be influenced by the presence or absence of either plants or ruminant urine.

Many studies have studied soil N_2O and CO_2 emissions from urine patches in pastoral ecosystems. For example, Clough et al. (2003) studied both soil N_2O and CO_2 emissions following a urine addition but this study was performed without plants and at a constant temperature (20°C). However, despite the global significance of anthropogenically induced N_2O and CO_2 emissions from agriculture and land use change, there are no studies that have compared the soil N_2O and CO_2 emissions from urine patches with or without plants under varying T_S. This information is critically important because pastoral ecosystems play an important role in the global C and N cycles and the size and distribution of greenhouse gas sources and sinks remains uncertain (Fowler et al., 1997; Ojima et al., 1993) as does their response to increased T_S resulting from predicted climate change.

To determine the C sources contributing to the F_{CO_2} i.e. recently added C from roots and urine (R_{RDU}) or recalcitrant C from soil organic matter (R_{OM}), and their response to changes in T_S, a natural ^{13}C abundance technique was used (Millard et al., 2008; Robinson and Scrimgeour, 1995). This method was based on the difference in the $\delta^{13}C$ values of the C fixed by C_3 and C_4 plant photosynthetic pathways (O'Leary, 1988). A C_3 perennial ryegrass (*Lolium perenne*) was grown in a soil developed under C_4 vegetation (C_4 soil) with C_3 bovine urine. Similarly to determine the source of the N_2O-N and the response of the source to changes in T_S (soil versus urine) the urine was labelled with ^{15}N-enriched urea, thus enabling the distinction between soil-N and urine-N. The distinction between N_2O originating from soil-N and from urine-N is important because it is expected that as T_S increases soil-N mineralization will increase (Kladivko and Keeney, 1987; Stanford et al., 1973). The aim of this study was to investigate the effects of urine application, T_S, and plants on soil N_2O and CO_2 fluxes.

5.3 Materials and Methods

5.3.1 Soil collection and treatment structure

Soil samples (0 – 5 cm depth) were collected from a dairy pasture site at Kerikeri, Northland, New Zealand (35°18'S, 173°93'E, 79 masl). The primary vegetation on the soil was a C_4 Kikuyu grass (*Pennisetum clandestinum* Hochst.) and the soil was a well drained Kerikeri friable clay loam (Typic Haplohumox; Soil Survey Staff, 1998). The Kikuyu grass had been present for > 20 years. Soil samples were sieved to pass through a 5.6 mm mesh, plant root

material was removed, and the soil was stored at < 4°C. The mean annual T_S at the sampling site (10 cm depth in years 2006 to 2008) was 15.0 ± 1.1°C (± SD).

Sieved soils were packed to a depth of 10 cm into funnel shaped pots (12 cm deep, 22 cm and 10 cm in diameter on the top and the bottom, respectively). Each pot contained the equivalent of 2200 g dry soil. Soil-C in the sampled soil originated from C_4 plants and had a mean $\delta^{13}C$ value of -19.6 ± 0.01‰ (± SEM, all $\delta^{13}C$ values are expressed relative to Vienna PeeDee Belemnite; $\delta^{13}C_{V\text{-}PDB}$). Hence to create a $\delta^{13}C$ gradient between the soil-C and plant-C, a C_3 perennial ryegrass (*Lolium perenne* L.) was planted. The ryegrass seeds were planted on the edge of each pot (Plate 5.1) and an aluminium collar (7 cm depth and 14 cm in diameter) was inserted into the middle of the soil surface of each pot to allow N_2O and CO_2 flux measuring equipment to be placed (see below). The pots were maintained in a glasshouse at 20°C and were watered every day for 8 – 12 weeks until the ryegrass plants had established (Plate 5.1). Urea fertiliser was applied three times, 1, 3, and 5 weeks following seed germination, during plant establishment (60 kg N ha^{-1} in total as urea) but no fertiliser was applied three weeks immediately prior to treatment application and the subsequent N_2O and CO_2 flux measurements. Pots without plants were also prepared to act as controls and they were also placed in the glasshouse for 8 – 12 weeks and received the same inputs as the planted pots.

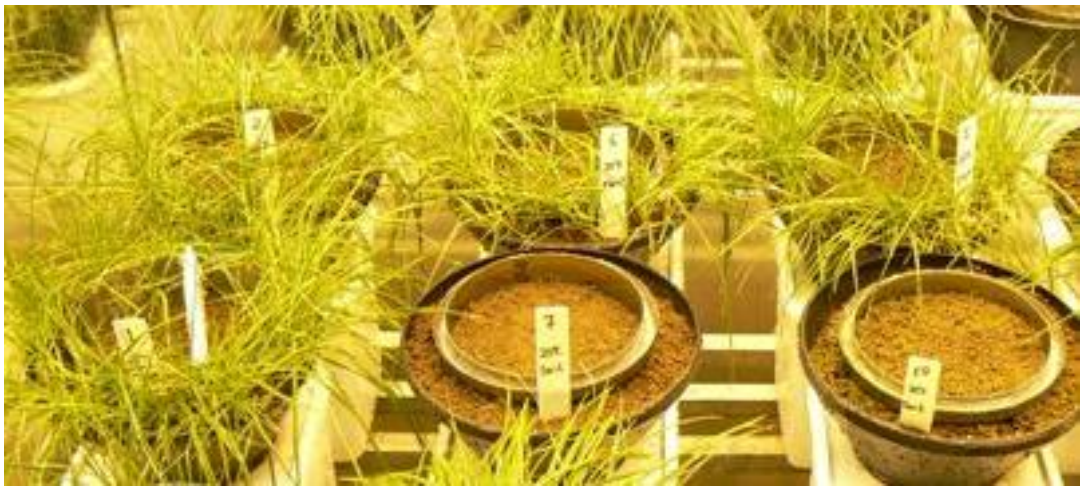

Plate 5.1 Pots with and without ryegrass plants immediately before soil N_2O and CO_2 fluxes were measured. The ryegrass were growing on the edge of each pot and an aluminium collar was inserted to a depth of 5 cm in the middle of the pot.

Pots were then allocated to air temperature treatments, at either 7°, 15° or 20°C which were applied using controlled-environment growth cabinets (PGV36, Controlled Environments Ltd, Winnipeg, Canada), with ambient CO_2 concentrations, a 70% humidity and a 16 h day length. Photosynthetically active radiation (PAR) was measured with a quantum sensor (L190SA; Li-Cor, Inc., Lincoln, NE, USA), giving a maximum midday irradiance of 400 µmol m^{-2} s^{-1} PAR at the surface of the uppermost leaves. Because of the radiation from the fluorescent tubes and incandescent lamps in the cabinets, T_S was approximately 3°C higher during soil CO_2 and N_2O flux measurement periods (between 1 to 3 p.m.), averaging 10.6 ± 0.8°, 18.9 ± 0.9°, and 23.2 ± 0.8°C in the cabinet at the air temperature of 7°, 15°, and 20°C, respectively. Hence, from here on, the three treatment temperatures are denoted as 11°, 19°, and 23°C.

The pots were pre-conditioned in the growth cabinets for 7 d at their allocated temperature treatments. Then pots had urine treatments applied. Pots contained either soil with ryegrass plus urine, soil with ryegrass minus urine, soil minus ryegrass plus urine, and soil minus ryegrass minus urine, and these are subsequently referred to as *ryegrass + urine*, *ryegrass control*, *soil + urine*, *soil control*, respectively. Three replicates of each treatment were allocated to each temperature treatments. The experimental design was a split plot design. Three temperature treatments formed the main plots, ryegrass the split plot and urine the split-split plot treatment.

The bovine urine was collected from Friesian dairy cows that had been grazing perennial ryegrass/white clover (*Trifolium repens*) pasture (C_3 plants) at the Lincoln University dairy farm during an afternoon milking. The urine contained 7.31 ± 0.05 g N l^{-1}. Immediately before the urine was applied, the urine was enriched with ^{15}N using ^{15}N enriched urea (98 atom % ^{15}N, Isotec^{TM}) to give a final total N concentration of 7.47 ± 0.06 g N l^{-1} with a ^{15}N enrichment of 0.996 ± 0.002 atom% ^{15}N. Then 300 ml (equivalent to 590 kg N ha^{-1}) of the urine was applied evenly to the surface of the soils on the *ryegrass + urine* and *soil + urine* pots. The *control* pots received the same volume of deionised water. The soils were then maintained at 50% WFPS for 408 h following the urine application by daily spraying water onto the soil surface (termed 'moderate moisture phase') then at 408 h the WFPS was increased to 70% and maintained at this WFPS until the end of the experiment (termed 'high moisture phase') which was 672 h following the urine application.

5.3.2 Measurement of soil N_2O fluxes and ^{15}N enrichment

Soil N_2O fluxes were measured at 24, 48, 72, 120, 192, 240, 288, 360, 432, 456, 504, 552, 600, and 672 h following the urine application. To measure the N_2O fluxes, a headspace (1.16

l) was created in the centre of each pot using a round plastic canister (PC9603, Milano) which was sealed by filling a trough in the aluminium collar, previously inserted, with water (Plate 5.2). Following checks for linearity of the N_2O flux, gas samples were taken after 30 minutes. Gas samples (8 ml) were taken from the headspace, via septa inserted in the canister, using a glass gas-tight syringe and placed in 6 ml Exetainers®. The over pressurization avoided the contamination of the sampled gas by ambient air through the septa. Duplicate ambient air samples were also taken and placed in 6 ml Exetainers® at each sampling.

Plate 5.2 Soil N_2O flux sampling. A headspace was created in the centre of each pot using a round plastic canister which was sealed by filling a trough in the aluminium collar, previously inserted, with water. Gas samples were taken from the headspace, via septa inserted in the canister, using a gas-tight syringe.

The 6 ml gas samples were first equilibrated to atmospheric pressure and then analysed for N_2O using a gas chromatograph (GC; 8610, SRI Instruments, CA), fitted with an electron capture detector, interfaced to a liquid auto sampler (Gilson 222XL, Middleton, WI, USA) which had been adapted to take a purpose-built double concentric injection needle (PDZ-Europa, Crewe, UK). This enabled the entire gas sample to be flushed rapidly from the

Exetainers® into the GC which had a configuration similar to that used by Mosier and Mack (1980).

The ^{15}N enrichment of the N_2O-N flux was determined by taking 15 ml gas samples at 24, 72, 120, 288, 456, and 672 h following the urine application and placing these in 12 ml Exetainers®. These 12 ml N_2O samples were also equilibrated to atmospheric pressure, immediately prior to analysis, and then analysed for ^{15}N enrichment, using an isotope-ratio mass spectrometer (IRMS; PDZ-Europa 20-20) as described by Steven et al. (1993).

5.3.3 Measurement of soil CO_2 fluxes and the calculation of the amount of soil-respired CO_2

Soil CO_2 fluxes (F_{CO_2}) were measured, every 48 h, using a portable chamber with an infrared gas analyser (SRC-1 and EGM-4, PP systems, Hitchin, UK). The chamber connected directly onto the circular aluminium soil respiration collar placed on each pot and the flux was determined over a 2 min period.

To compare the response of F_{CO_2} over time (*h*, hours after the urine application), the exponential decay model (eqn. 5.5) was fitted to the data by adapting the equation of Kelliher et al. (2007):

$$F_{CO_2} = a + br^h \qquad (5.5)$$

An asymptote is determined by the variable *a* (μg CO_2 kg^{-1} s^{-1}), *b* is a scaling factor, and *r* is the decay constant, related to the decay rate. Significant differences in the parameters between the treatments were obtained using the standard errors of the parameter estimation (see below).

The trapezoidal rule, based on the F_{CO_2} data plotted versus time, was used to calculate the amount of CO_2 emitted over the course of the incubation (0 – 672 h following the urine application). For the + *urine* treatments, the initial F_{CO_2} (at time zero) following the urine treatment was estimated by adding the *a* and *b* terms of the fitted model (eqn. 5.5). For the *control* treatments, R_S rate at 0 h was extrapolated to equal the R_S rate measured at 24 h.

In order to ascertain the potential urine-derived priming effect under the urine treatments, the maximum possible production of CO_2 from the applied urine was calculated. Based on the C content of the applied urine (1.90 g l^{-1}) and the volume applied (300 ml) it was calculated that 2.09 g of CO_2 was the maximum mass of CO_2 attributable to the urine addition, assuming that

all of the urine-C was released to the atmosphere as CO_2 within 672 h following the urine treatment.

5.3.4 The $\delta^{13}C$ values of soil-respired CO_2

The $\delta^{13}C$ values of the soil-respired CO_2 ($\delta^{13}C_{Rs}$) were calculated by adapting the method of Ohlsson et al. (2005). This involved sampling a headspace (see above) for soil-respired CO_2 after a period of 30 min, with plants absent. The CO_2 15 ml gas samples were then placed into 12 ml Exetainers® for $\delta^{13}C$ determination using IRMS (see above) at 24, 360, and 552 h following the urine treatment. The $\delta^{13}C_{Rs}$ values were calculated using the following equation (Ohlsson et al., 2005) (eqn. 5.6):

$$\delta^{13}C_{R_S} = \frac{[CO_2(\text{headspace})] \times \delta^{13}CO_2(\text{headspace}) - [CO_2(\text{air})] \times \delta^{13}CO_2(\text{air})}{[CO_2(\text{headspace})] - [CO_2(\text{air})]} \quad (5.6)$$

where $[CO_2(\text{headspace})]$, $[CO_2(\text{air})]$, $\delta^{13}CO_2(\text{headspace})$ and $\delta^{13}CO_2(\text{air})$ are the concentrations of CO_2 in the headspace of the chamber system, the concentration of CO_2 sampled in ambient air, the $\delta^{13}C$ value of the CO_2 in the headspace of the chamber system, and the $\delta^{13}C$ value of CO_2 in ambient air, respectively. The assumption was made that the $\delta^{13}C$ values in the ambient air were the same as the initial value of the $\delta^{13}C$ value in the headspace at time zero. Photosynthetic activity and leaf respiration did not influence the $\delta^{13}C$ value of the CO_2 in the headspace of the chamber system because leaves were excluded from the headspace (Plate 5.2).

5.3.5 Isotopic analysis of plant and soil and calculation of R_{OM} and R_{RDU}

In order to measure the contribution of soil organic matter decomposition (R_{OM}) or urine and root-derived C utilisation (R_{RDU}) to total R_S, the pots were destructively sampled following the last soil N_2O and CO_2 flux determinations at 672 h. The R_{RDU} originated from C fixed by C_3 plants. This estimate of the $\delta^{13}C_{RDU}$ value makes the simplifying assumption that the $\delta^{13}C$ value of CO_2 originating from the applied urine-C ($\delta^{13}C = -30.7 \pm 0.4$‰) and the root-respired C (-29.9 ± 0.7‰, when averaged across temperature treatment) were equal. Even though this assumption is not strictly valid due to the potential for fractionation of plant-C following harvest by the animal and the subsequent excretion of the urine-C, it is argued here that it is appropriate given that the difference in $\delta^{13}C$ values between urine-C and plant-C was not statistically significant when compared to the difference in the $\delta^{13}C$ values between the C_3 plant and C_4 soil (-19.6 ± 0.01‰). To obtain the $\delta^{13}C_{RDU}$ values, the roots in the treatments with plants were carefully removed from the soil, gently washed to remove the soil attached

to the roots, placed in Tedlar® bags, and then flushed with CO_2-free air. Then the bags containing the roots were incubated for 30 min at the appropriate treatment temperatures. Then 15 ml gas samples were taken and placed into 12 ml Extainers® using a gas-tight syringe via septa embedded on the bags. The CO_2 generated was analysed for $\delta^{13}C$ using IRMS as described above.

The CO_2 produced during R_{OM} originated from C originally fixed by C_4 plants. To obtain the value of $\delta^{13}C_{OM}$, a sub-sample of the soil in the *soil control* treatments was placed into separate Tedlar® bags and flushed with CO_2-free air with gas sampling and analyses performed as described above.

A natural stable isotope (^{13}C) abundance method was used to differentiate the F_{CO_2} components, based on the approach taken by Lin et al. (1999) where, the proportion of CO_2 originating from the soil organic C pool in the C_4 soil (f_{OM}) was calculated as follows (eqn. 5.7):

$$f_{OM} = \frac{\delta^{13}C_{R_S} - \delta^{13}C_{RDU}}{\delta^{13}C_{OM} - \delta^{13}C_{RDU}} \quad (5.7)$$

The variances (i.e. the square of standard errors, σ) of f_{OM} were calculated as described by Phillips and Gregg (2001) (eqn. 5.8):

$$\sigma^2(f_{OM}) = \frac{1}{(\delta^{13}C_{OM} - \delta^{13}C_{RDE})^2}\left[\sigma^2(\delta^{13}C_{R_S}) + f_{OM}{}^2 \times \sigma^2(\delta^{13}C_{OM}) + (1 - f_{OM})^2 \times \sigma^2(\delta^{13}C_{RDU})\right] \quad (5.8)$$

where $\sigma^2(\delta^{13}C_{Rs})$, $\sigma^2(\delta^{13}C_{OM})$, and $\sigma^2(\delta^{13}C_{RDU})$ represent the variances of $\delta^{13}C_{Rs}$, $\delta^{13}C_{OM}$ and $\delta^{13}C_{RDU}$, respectively. The variance of f_{RDU} was also determined by switching the $\delta^{13}C_{OM}$ and $\delta^{13}C_{RDU}$ subscripts in eqn. 5.8.

Separate sub-samples of soil from each pot were also analysed for soil inorganic-N (NH_4^+-N and NO_3^--N) concentrations by extracting these with 2 M KCl (1:10) for 1 h, and then filtering (Whatman 42) the extracts prior to colorimetric analysis on an auto-analyser. This had a detection limit for NO_3^--N and NH_4^+-N of 0.10 and 0.01 mg l^{-1}, respectively (Alpkem FS3000 twin channel analyser; application notes P/N A002380 and P/N A002423).

5.3.6 Photosynthesis rate measurements and herbage sample analysis

Leaf photosynthesis rates (*A*) were determined using a portable photosynthesis system (LI6400; Li-Cor, Inc. Lincoln, NE, USA), fitted with a leaf chamber, on three occasions; 96 h prior to urine application, and at 240 and 552 h post urine application. Light intensity within

the leaf chamber was the same as the photosynthetically active radiation within the growth chambers, measured with a quantum sensor as described above.

Photosynthetic rates for the whole pot at 552 h were estimated by multiplying *A* (per unit leaf area) by the total live leaf area per pot. Pot leaf area was calculated from measured specific leaf mass (65 ± 9 g m^{-2}) and total leaf dry weight per pot. This estimate of total *A* per pot made the simplifying assumption that all leaves were equally exposed to radiation and microclimate conditions. Even though this assumption is not strictly valid, it is argued that it is appropriate because it is the relative differences between treatments that are important, rather than the absolute rate of C fixation. Estimates of total *A* per pot were used to determine the percentage of root- and urine-derived CO_2–C (R_{RDU}) in photosynthetically fixed C.

All plants were harvested immediately after the last soil N_2O and CO_2 flux measurements (672 h). Aboveground and below ground vegetation was dried (65°C, 48 h), weighed, and finely ground prior to being analysed for N content and ^{15}N enrichment using Dumas combustion in conjunction with IRMS. Percentage recovery of ^{15}N in plant material was determined according to Cabrera and Kissel (1989).

5.3.7 Statistical and data analysis

The N_2O flux data were transformed ($\log_{10}$ (flux + 1)). Then two-way analysis of variance (ANOVA) was performed on the log transformed N_2O flux (GenStat; Lawes Agricultural Trust, Rothamsted, UK) and the F_{CO_2} data to determine the effect of the urine and ryegrass treatments at a given sampling time, and at each treatment temperature.

For the F_{CO_2} data, the parameters, obtained from the exponential decay model (eqn. 5.5), were compared based on the standard errors of the parameter estimation. The significance (*p* value) of any difference between the measured parameters, divided by the standard error of the difference (*z*), and using standard normal curve areas ($z > 1.96$ corresponds to $p < 0.05$).

The Lloyd and Taylor model was used to describe the temperature response of F_{CO_2} (Lloyd and Taylor, 1994). This model is an Arrhenius-type with three parameters (eqn. 5.9). The variable R_x is F_{CO_2}. The R_{10} is a scale parameter and it equates to R_x when $T_S = 10$°C. The E_0 (K) is the activation energy divided by the gas constant and the parameter T_0 determines the temperature minimum (K) at which predicted R_x reaches zero. The E_0, which determines the temperature sensitivity, was not constrained and neither was R_{10}, but T_0 was set at 227.13K, as described by Lloyd and Taylor (1994). The same approach was previously used by Mäkiranta

et al. (2009). The two parameters were fitted to the data set using non-linear regression using GenStat (Lawes Agricultural Trust, Rothamsted, UK).

$$R_X = R_{10} e^{E_0\left(\frac{1}{56.02} - \frac{1}{T_S + 237.15 - T_0}\right)} \quad (5.9)$$

5.4 Results

5.4.1 Basic information for soil and urine

The soil C:N ratio, organic matter, and water soluble C contents were 11.7, 13.3 g kg^{-1}, and 161 mg C kg^{-1}, respectively. Total C, total N, and atom% ^{15}N for the soil were 77 mg kg^{-1}, 6.6 mg kg^{-1} and 0.426 atom% ^{15}N, respectively. Other soil characteristics are described in the previous chapter (Table 4.1).

5.4.2 Soil N_2O fluxes over time

Under the moderate moisture phase, soil N_2O fluxes increased 24 h after urine application at 19° and 23°C and after 48 h at 11°C, and then N_2O fluxes declined (Fig. 5.1). Under the moderate moisture phase, the soil N_2O fluxes at 24 h were higher ($p < 0.05$) in the *soil + urine* treatment (23 ± 4 µg N_2O-N kg^{-1} h^{-1}) compared to the fluxes from the *ryegrass + urine* treatment (6 ± 2 µg N_2O-N kg^{-1} h^{-1}), when averaged across temperature treatments (Fig. 5.1).

Under the high moisture phase, the highest N_2O fluxes occurred 48 h after the increase in WFPS (456 h after urine application), when averaged over all temperature treatments (Fig. 5.1). At 48 h after the increase in WFPS, increasing temperature also significantly ($p < 0.05$) increased N_2O fluxes, when averaged over all urine and plant treatments. Also, at 48 h, urine treatment significantly ($p < 0.001$) increased N_2O fluxes. When compared to N_2O flux peaks under the moderate moisture phase, the N_2O flux peaks under the high moisture phase were markedly higher ($p < 0.05$). Only under the high moisture phase at 23°C did the presence of ryegrass significantly enhance N_2O fluxes ($p < 0.05$) (Fig. 5.1c).

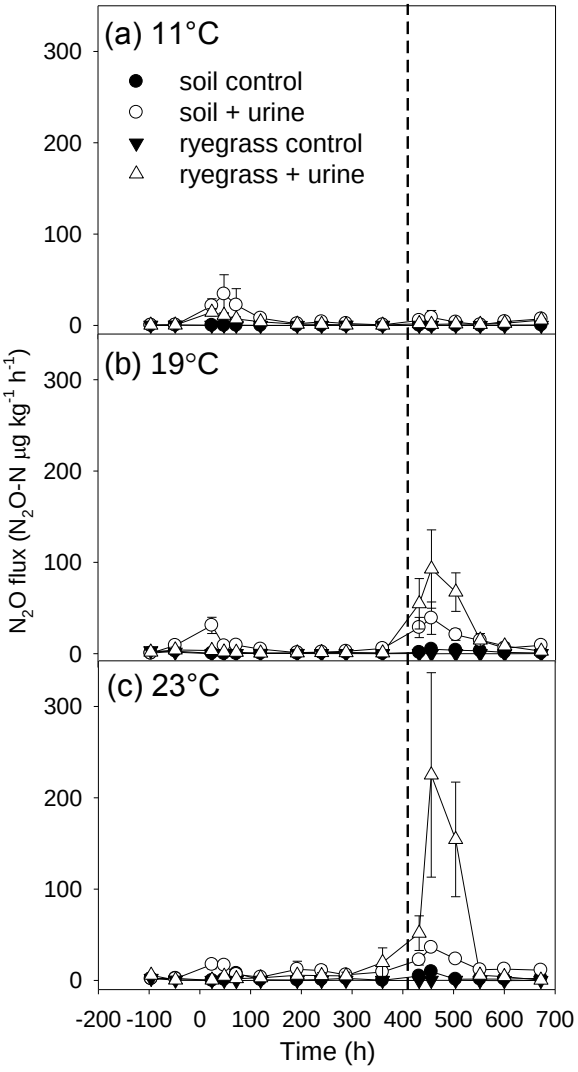

Figure 5.1 The effect of urine and ryegrass on soil N_2O fluxes over time (h) at (a) 11°C, (b) 19°C and (c) 23°C. The N_2O fluxes were measured twice before cow urine application at 0 h. Error bars are the SEM (n = 3). The vertical dashed line shows the timing of the increase in the soil moisture from 50% to 70% WFPS (at 408 h following the urine application).

5.4.3 Cumulative N_2O-N

During the moderate moisture phase (0 – 408 h), without urine, cumulative N_2O-N emissions were not influenced by plant presence, when averaged across temperature treatments. Despite the shorter period of the high moisture phase, at both 19° and 23°C, with urine present, cumulative N_2O-N emissions were higher ($p < 0.01$), when compared to the cumulative N_2O-N emissions in the moderate moisture phase (Table 5.1). When urine and plants were present, increasing T_S increased ($p < 0.05$) cumulative N_2O-N emissions during the high moisture phase by 2.1 and 4.1-fold at 19° and 23°C, respectively, when compared to cumulative N_2O emissions without plants. This was reflected in an increase ($p < 0.05$) in the percentage contribution of cumulative N_2O fluxes in the high moisture phase to the total cumulative N_2O fluxes when ryegrass was present (Table 5.1).

When considering the entire experimental period (672 h) the addition of urine increased ($p < 0.05$) the cumulative N_2O emissions (Table 5.1). The addition of urine and an increase in T_S from 11° to 23°C increased the cumulative N_2O fluxes by 21000 µg N_2O-N kg^{-1} with plants present. Without plants, the increase was only 4000 µg N_2O-N kg^{-1} when T_S increased from 11° to 23°C. Without plants, the cumulative N_2O-N flux as a % of N applied (EF) was not influenced by T_S, but with plants, EF increased with increasing T_S. The EF values with plants were significantly higher at 23°C but not at 11° and 19°C, when compared to the EF values without plants.

Table 5.1 Cumulative N_2O emissions (μg N_2O-N kg^{-1}) for the moderate moisture phase (0 to 408 h), the high moisture phase (408 to 672 h), and the total cumulative N_2O-N emission (0 to 672 h). The total cumulative N_2O emission is also expressed as a percentage of the urine-N applied, an emission factor (EF). Errors are ± SEM (*n* = 3). The values in brackets (high moisture phase) are the contribution of cumulative N_2O fluxes in the high moisture phase to the total cumulative N_2O emissions (%).

		Cumulative N_2O emissions (μg N_2O-N kg^{-1})		
	Treatment	11°C	19°C	23°C
Moderate moisture phase	soil control	34 ± 10	250 ± 97	822 ± 535
	soil + urine	3588 ± 2333	3186 ± 938	4145 ± 1639
	ryegrass control	52 ± 6	58 ± 12	36 ± 11
	ryegrass + urine	1454 ± 384	1796 ± 799	3935 ± 1578
High moisture phase	soil control	53 ± 18 (61%)	694 ± 428 (74%)	758 ± 167 (48%)
	soil + urine	1102 ± 784 (24%)	4215 ± 1371 (57%)	4587 ± 140 (53%)
	ryegrass control	0 ± 4 (5%)	8 ± 0 (13%)	11 ± 1 (24%)
	ryegrass + urine	500 ± 103 (26%)	9079 ± 3113 (83%)	18788 ± 8060 (83%)
Total	soil control	86 ± 24	944 ± 350	1580 ± 698
	soil + urine	4690 ± 3117	7402 ± 1754	8732 ± 1540
	ryegrass control	51 ± 10	67 ± 12	48 ± 11
	ryegrass + urine	1955 ± 296	10876 ± 3838	22744 ± 7230
Total cumulative N_2O-N / applied urine N (%)	soil + urine	0.45 ± 0.31%	0.63 ± 0.17%	0.70 ± 0.13%
	ryegrass + urine	0.19 ± 0.03%	1.06 ± 0.38%	2.23 ± 0.71%

5.4.4 ^{15}N atom% of N_2O-N and the ratio of N_2O-N derived from urine-N

Significant effects occurred due to temperature treatment ($p < 0.001$) and sampling time ($p < 0.001$) with respect to atom% ^{15}N values of the N_2O-N, with an interaction between these factors ($p < 0.05$). At 11°C, N_2O-N atom% ^{15}N values increased over time, peaking at 552 h; while at 19° and 23°C, the peak atom% ^{15}N values occurred at 288 h and 120 h, respectively (Fig. 5.2). Plant presence increased ($p < 0.05$) the ^{15}N enrichment of the N_2O-N. At 19° and 23°C this occurred between 0 and 120 h whereas it occurred later at 11°C at 288 to 456 h. At the last measurement (552 h), atom% ^{15}N values of N_2O-N averaged 0.85 ± 0.05 atom% ^{15}N, thus the atom% ^{15}N values for soil N_2O-N were very close to the value of the applied ^{15}N (1.00 atom% ^{15}N). At 552 h, there were no significant effects of T_S and plants on the atom% ^{15}N values of N_2O-N.

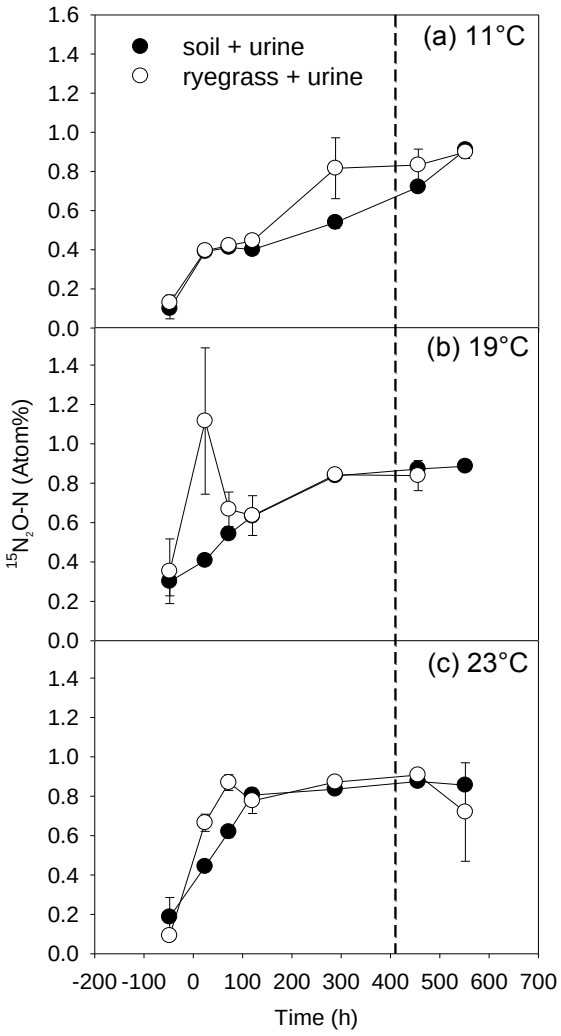

Figure 5.2 Atom% ^{15}N of N_2O-N fluxes following urine application for (a) 11°, (b) 19° and (c) 23°C with (○) and without (●) plants over time (h). The errors bars are SEM (*n* = 3). The vertical dashed line shows the timing of the increase in soil moisture from 50% to 70% WFPS (at 408 h following the urine application).

5.4.5 Nitrogen recovery as nitrous oxide

Cumulative recovery of the applied ^{15}N, as N_2O-N, varied from 0.10 ± 0.08 to 1.43 ± 0.40% after 672 h (Fig. 5.3). Temperature and ryegrass treatments significantly affected this ^{15}N recovery ($p < 0.05$). At 19° and 23°C, the recovery of applied ^{15}N was significantly higher when ryegrass was present, while at 11°C there was no significant difference in the recovery of ^{15}N as N_2O-N due to ryegrass presence.

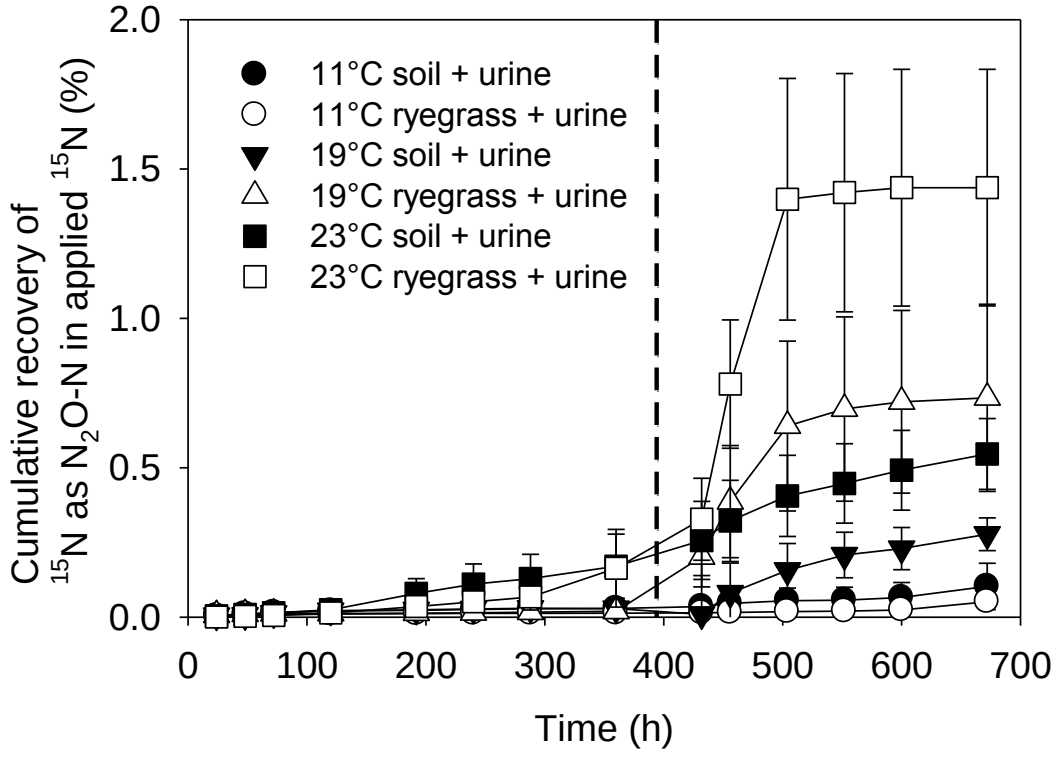

Figure 5.3 Cumulative recovery of ^{15}N applied as N_2O-N over time (h). Error bars represent SEM (n = 3). The vertical dashed line shows the timing of the increase in the soil moisture from 50% to 70% WFPS (at 408 h following the urine application).

5.4.6 Nitrogen contents in soils and plant N uptake

Without urine application, soil NO_3^--N and NH_4^+-N concentrations averaged 3 ± 1 and 47 ± 9 μg g^{-1}, respectively, when averaged across all temperature and plant treatments (data not presented). Without urine application, there were no significant effects of temperature and plant treatments on NH_4^+-N, but plant presence significantly ($p < 0.001$) reduced NO_3^--N. Urine application increased ($p < 0.001$) soil inorganic-N concentrations, measured at the end of the experiment, but the NH_4^+-N concentrations decreased with increasing T_S with no interaction due to the plant treatment (Table 5.2). Soil NO_3^--N concentrations decreased ($p <$

0.05) with increasing T_S and the presence of plants did not have effect on soil NO_3^--N content at 11° and 19°C (Table 5.2). However, at 23°C, the NO_3^--N concentration in the *soil + urine* treatment was higher ($p < 0.05$) than in the *ryegrass + urine* treatment (Table 5.2).

Table 5.2 Soil NH_4^+-N and NO_3^--N concentrations (µg N g^{-1} soil) measured at 672 h following urine treatment. Errors bars are SEM (n = 3).

Soil Temperature	Vegetation	NH_4^+	NO_3^-
11°C	soil + urine	11617 ± 1444	1192 ± 183
	ryegrass + urine	11583 ± 328	1372 ± 372
19°C	soil + urine	240 ± 56	1323 ± 511
	ryegrass + urine	347 ± 45	663 ± 158
23°C	soil + urine	132 ± 29	616 ± 37
	ryegrass + urine	142 ± 12	400 ± 13

The N contents of the leaf and root biomass in the *ryegrass + urine* treatments were higher ($p < 0.001$) than in the *ryegrass control* treatments (Table 5.3 and 5.4). Temperature treatment had no significant effect on leaf N contents in the *ryegrass + urine* treatments but increasing T_S increased ($p < 0.01$) atom% ^{15}N enrichment and ^{15}N recovery in the leaf (Table 5.4). In the *ryegrass + urine* treatment, the N concentrations in the root tissue were lower than in the leaves, with increasing T_S reducing ($p < 0.05$) the N concentration in the roots but increasing ($p < 0.05$) the percentage of added ^{15}N recovered in the roots (Table 5.4).

Table 5.3 Leaf and root %N contents at the end of the experiment (672 h following the urine treatment) for the *ryegrass control* pots. Error bars represent SEM (n = 3).

Soil Temperature	Vegetation	Leaf	Root
11°C	ryegrass control	2.33 ± 0.08	1.01 ± 0.07
19°C	ryegrass control	1.45 ± 0.05	0.66 ± 0.03
23°C	ryegrass control	1.37 ± 0.08	0.65 ± 0.08

Table 5.4 Ryegrass leaf and root N contents, atom% ^{15}N enrichments and ^{15}N recoveries (%) for the *ryegrass + urine* pots. Errors are SEM (*n* = 3).

		Leaf			Root		
Soil Temperature	Vegetation	N content %	Atom% ^{15}N	Recovery % ^{15}N	N content %	Atom% ^{15}N	Recovery % ^{15}N
11°C	ryegrass + urine	4.38 ± 0.20	0.648 ± 0.031	1.45 ± 0.52	1.98 ± 0.22	0.613 ± 0.068	0.18 ± 0.11
19°C	ryegrass + urine	4.70 ± 0.11	0.798 ± 0.001	6.43 ± 0.57	1.22 ± 0.02	0.684 ± 0.007	0.58 ± 0.14
23°C	ryegrass + urine	4.64 ± 0.11	0.812 ± 0.005	5.08 ± 0.52	1.28 ± 0.06	0.702 ± 0.009	0.88 ± 0.14
	Significance	ns	**	**	*	ns	*

Significance refers to the comparison between the temperature treatments. ** $p < 0.01$; * $p < 0.05$; ns (not significant)

5.4.7 Soil carbon dioxide fluxes over time

The change in soil moisture contents did not influence the soil CO_2 fluxes (F_{CO_2}) thus, the effects of soil moisture on F_{CO_2} are not considered hereafter. Within 24 h of urine application F_{CO_2} increased from 0.5 ± 0.1 to 10.0 ± 0.5 CO_2 kg^{-1} s^{-1}, when averaged across all temperature and ryegrass treatments (± SEM, Fig. 5.4). This increase ($p < 0.05$) in F_{CO_2}, as a result of urine application, continued until 432 h at 11°C, 192 h at 19°C but only for 120 h at 23°C (Fig. 5.4). The relationship between this period of enhanced F_{CO_2} and the accompanying temperature treatment was linear ($r^2 = 0.98$). The fitted model describing the changes in F_{CO_2} over time (eqn. 5.5), showed the *r* values decreased ($p < 0.001$) when T_S increased from 11° to 23°C (Table 5.5). The *a* values only increased significantly with T_S when ryegrass was present (Table 5.5). The *b* values were proportional to T_S.

There was an Arrhenius relationship between the initial F_{CO_2} response ($a + b$) to urine application and T_S (Fig. 5.5a). The temperature responses of the initial F_{CO_2} were not influenced by the ryegrass treatment. By 672 h after urine application, the effect of urine on F_{CO_2} had abated and was replaced by a ryegrass treatment effect with an Arrhenius relationship between T_S and F_{CO_2} with plants (Fig. 5.5b). When compared to the parameters for the Lloyd and Taylor model fitted to the F_{CO_2} data, for the immediate response of F_{CO_2} following urine application (Fig. 5.5a), the parameter R_{10} at 672 h was smaller but the parameter E_0 at 672 h was not significantly different (Fig. 5.5b).

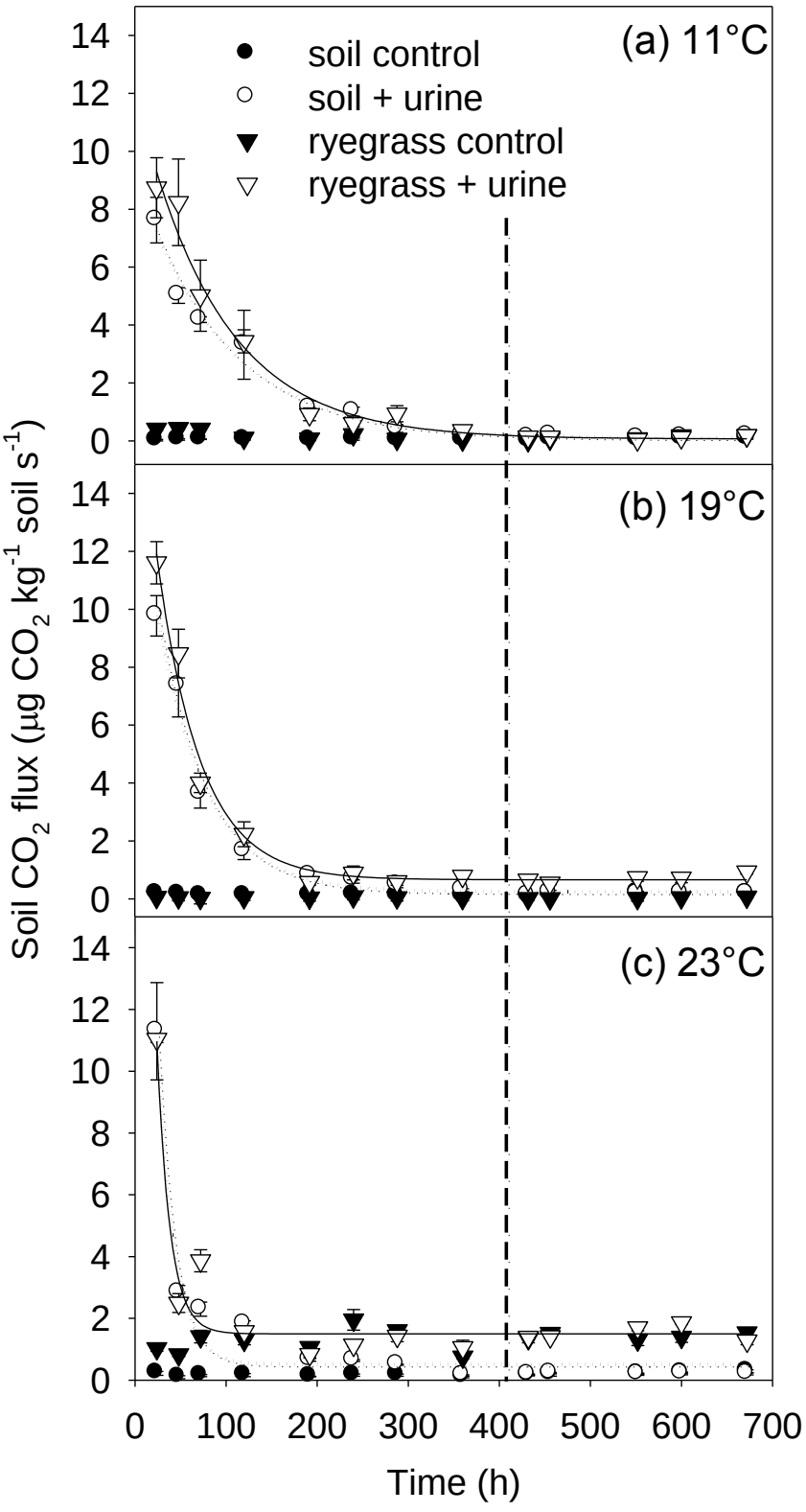

Figure 5.4 The effect of urine and ryegrass treatments on soil CO_2 fluxes (µg CO_2 kg^{-1} s^{-1}) at (a) 11°C, (b) 19°C and (c) 23°C. Values are means (*n* = 3) and error bars represent SEM. The fitted solid and dotted lines represent the exponential decay model (eqn. 5.5) fitted to the data for the *ryegrass + urine* and *soil + urine* treatments, respectively. The parameters for the models were described in Table 5.5. The vertical dashed line shows the timing of the increase of the soil moisture from 50% to 70% WFPS (at 408 h following the urine application).

Table 5.5 The estimated parameters and their standard errors for the time response model (eqn. 5.5) for F_{CO_2} following urine treatment. The errors are the standard errors of the parameter estimation.

	11°C			
	soil + urine		*Ryegrass + urine*	
Parameter	Estimate	s.e.	Estimate	s.e.
R	0.990	± 0.0017	0.989	± 0.0014
B	9.18	± 0.77	12.07	± 0.81
A	0.10	± 0.23	0.07	± 0.22

	19°C			
	soil + urine		*Ryegrass + urine*	
Parameter	Estimate	s.e.	Estimate	s.e.
R	0.981	± 0.0022	0.979	± 0.0022
B	15.36	± 1.33	18.64	± 1.51
A	0.25	± 0.18	0.66	± 0.18

	23°C			
	soil + urine		*Ryegrass + urine*	
Parameter	Estimate	s.e.	Estimate	s.e.
R	0.948	± 0.0067	0.932	± 0.0116
B	38.68	± 7.09	51.30	± 15.90
A	0.51	± 0.16	1.50	± 0.16

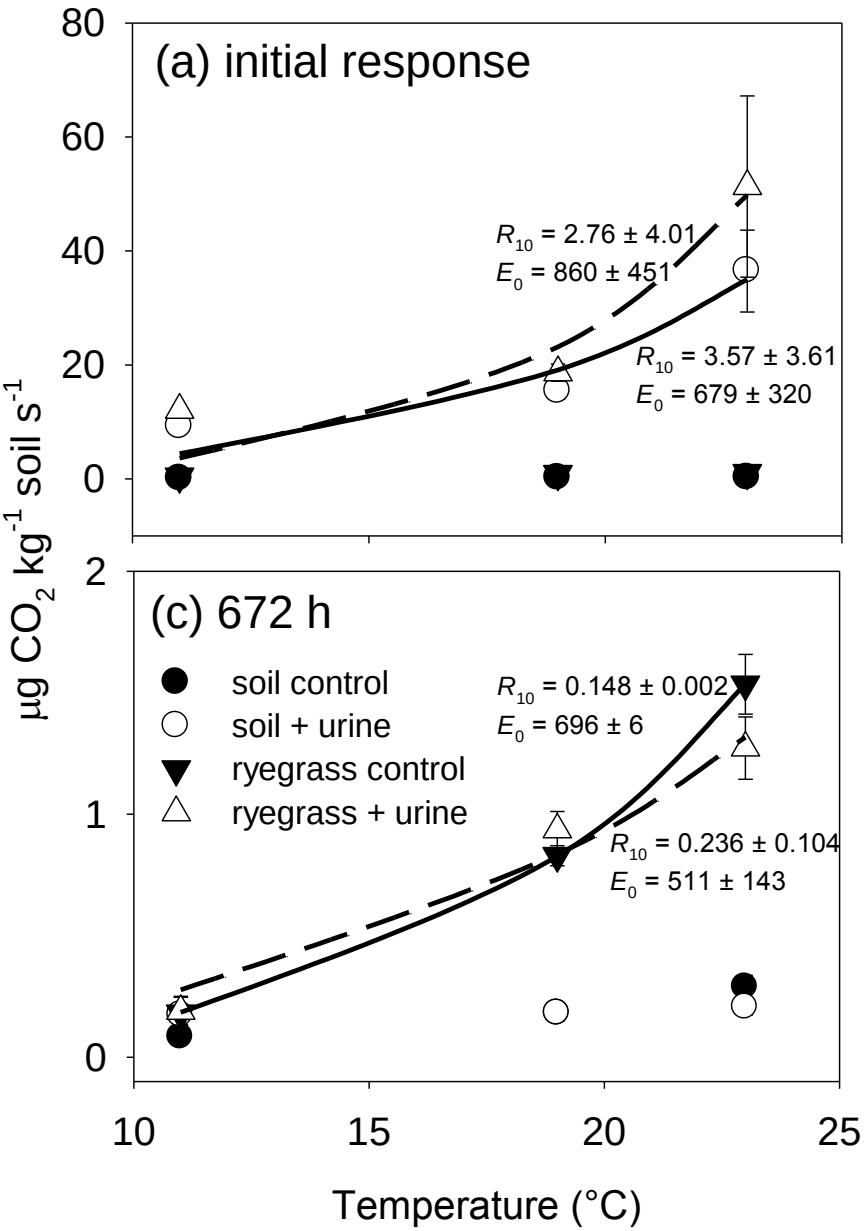

Figure 5.5 Soil CO_2 fluxes (F_{CO_2}) as a function of temperature (a) at the initial peak immediately following the urine treatment and (b) at 672 h following the urine treatment. Bars represent SEM (*n* = 3). Solid and dashed lines represent the best Lloyd and Taylor (1994) model fitted to *soil + urine* and *ryegrass + urine* treatments (a), and *ryegrass control* and *ryegrass + urine* treatments (b). The parameters (E_0 and R_{10}) are next to the corresponding curves, with the standard errors of the parameters.

5.4.8 Cumulative CO_2 production from soil respiration and abiotic processes

Urine treatment markedly increased cumulative CO_2 production from R_S and abiotic processes (e.g. urea hydrolysis) (Table 5.6). With ryegrass, cumulative CO_2 production increased, when compared to cumulative CO_2 production without ryegrass ($p < 0.001$). Increasing T_S also resulted in increases ($p < 0.001$) in cumulative CO_2 production. An interaction ($p < 0.001$) occurred between temperature and ryegrass treatments with cumulative CO_2 production increasing 2.2-fold when T_S increased from 11° to 23°C with plants, whereas it was only 1.3-fold without ryegrass, when averaged over the urine treatment.

After subtracting the maximum potential urine-derived CO_2 flux (see above) from the cumulative CO_2 flux it was observed that the cumulative R_S ($p < 0.001$) was higher under the urine application when compared to the *control* treatments. Without plants, the urine treatment effect was 40% greater at 23°C than at 11°C (Table 5.6(d)). With plants, the urine treatment effect (Table 5.6(d)) was not affected by T_S but urine treatment increased R_S 1.48 g CO_2 kg^{-1} over the course of study, when averaged across temperature treatments. This increase in cumulative R_S, due to the urine application, was interpreted as urine-induced priming. In Table 5.6(e), the magnitude of the urine-induced priming is expressed as a percentage of the cumulative R_S from the + *urine* treatment divided by the cumulative R_S from the *control* treatment in the comparable ryegrass and temperature treatments. Decreasing T_S increased ($p < 0.05$) the magnitude of the urine-induced priming effect but urine-induced priming decreased ($p < 0.05$) with plants present at 19° and 23°C, but not at 11°C. When the urine-induced priming effect based on soil weight (Table 5.6) was normalized using leaf biomass (Table 5.8), the urine-induced priming effect, with plants, was 1134 ± 363, 141 ± 16, and 109 ± 11% at 11°, 19°, and 23°C. Thus, when compared to the calculated urine-induced priming effect based on soil weight (Table 5.6), the magnitude of the urine-induced priming effect based on leaf biomass was smaller. However, the urine-induced priming effect was still clearly observed at 11° and 19°C when plants were present.

Table 5.6 (a) Total cumulative CO_2 flux (R_S plus urine derived abiotic CO_2 production) and (b) CO_2 production due to R_S (cumulative CO_2 flux minus urine derived CO_2 flux) over the course of study (672 h). In the + *urine* treatments, the amount of CO_2 emitted from (a) total cumulative CO_2 flux was calculated first and then the amount of CO_2 emitted from urea hydrolysis (2.09 g CO_2 kg^{-1} soil) was deducted to obtain (b) the flux due to R_S. The urine application effect (b – c) is shown in (d). The relative increase in R_S due to a urine application when compared to (c) cumulative CO_2 flux in the *control* treatments i.e. the urine induced priming is shown as a percentage (e). The errors indicate SEM (n = 3).

Temperature	Ryegrass treatment	(a) Total cumulative CO_2 flux in + *urine* pots (R_S + abiotic)	(b) Cumulative CO_2 flux from R_S in + *urine* pots (a – 2.09 g CO_2 kg^{-1})	(c) Cumulative CO_2 flux from R_S in *control* pots	(d) Urine application effect (b – c)	(e) Magnitude of urine-induced priming (b/c, %)
		g CO_2 kg^{-1} soil				
11°C	Soil	3.45 ± 0.32	1.36 ± 0.32	0.11 ± 0.04	1.25 ± 0.32	1236 ± 535%
	Ryegrass	4.10 ± 0.47	2.01 ± 0.47	0.35 ± 0.07	1.66 ± 0.48	574 ± 177%
19°C	Soil	3.63 ± 0.22	1.54 ± 0.22	0.34 ± 0.06	1.20 ± 0.23	453 ± 103%
	Ryegrass	4.88 ± 0.12	2.79 ± 0.12	1.56 ± 0.14	1.23 ± 0.18	179 ± 18%
23°C	Soil	4.20 ± 0.17	2.11 ± 0.17	0.42 ± 0.03	1.69 ± 0.17	502 ± 54%
	Ryegrass	6.81 ± 0.11	4.72 ± 0.11	3.18 ± 0.03	1.54 ± 0.11	148 ± 4%

5.4.9 The $\delta^{13}C$ values of soil-respired C and the sources of soil respiration

The $\delta^{13}C$ values of CO_2 respired from the *soil control* treatment (C_4 soil, $\delta^{13}C_{OM}$) and of root-respired CO_2 (C_3 plant, $\delta^{13}C_{RD}$) are listed in Table 5.7. Temperature treatment had no effect on the $\delta^{13}C$ values of either $\delta^{13}C_{OM}$ or $\delta^{13}C_{RDU}$. The mixing model (eqn. 5.7) was applied to the *soil + urine*, *ryegrass control*, and *ryegrass + urine* treatments to determine the relative fractions of the C pool sources in the CO_2 fluxes.

Table 5.7 The $\delta^{13}C$ signatures obtained from the end members. The values for $\delta^{13}C_{RDU}$ and $\delta^{13}C_{OM}$ originated with CO_2 produced from plant roots and the *soil control* pots, respectively. The errors are SEM (*n* = 3).

Temperature	$\delta^{13}C_{OM}$	$\delta^{13}C_{RDU}$
11°C	-16.74 ± 0.72	-28.80 ± 0.51
19°C	-18.36 ± 0.37	-29.77 ± 0.72
23°C	-18.82 ± 0.41	-31.23 ± 0.23
significance	ns	ns

Significance refers to the comparison between the temperature treatments. $^{**}p < 0.01$; $^{*}p < 0.05$; ^{ns} (not significant).

At 11°C, there were no significant effects of urine or ryegrass treatments on the $\delta^{13}C$ values of soil-respired C at any sampling time (Fig. 5.6a). At 19°C, the $\delta^{13}C$ values in the *ryegrass + urine* treatments were more enriched ($p < 0.05$) than the $\delta^{13}C$ values of the *ryegrass control* treatments at 360 and 552 h, respectively (Fig. 5.6b). The $\delta^{13}C$ values of the *soil + urine* treatment at 19°C were more enriched ($p < 0.05$) than the *ryegrass + urine* treatment at 360 h. At 23°C, there was no significant difference between treatments at 24 h, but at 360 and 552 h, the $\delta^{13}C$ values of the soil-respired C in the *soil + urine* treatment were more enriched ($p < 0.01$) than in the *ryegrass control* and *ryegrass + urine* treatments (Fig. 5.6c). At 23°C, there was no significant difference between the $\delta^{13}C$ values of soil-respired C in the *ryegrass control* and *ryegrass + urine* treatments at any time.

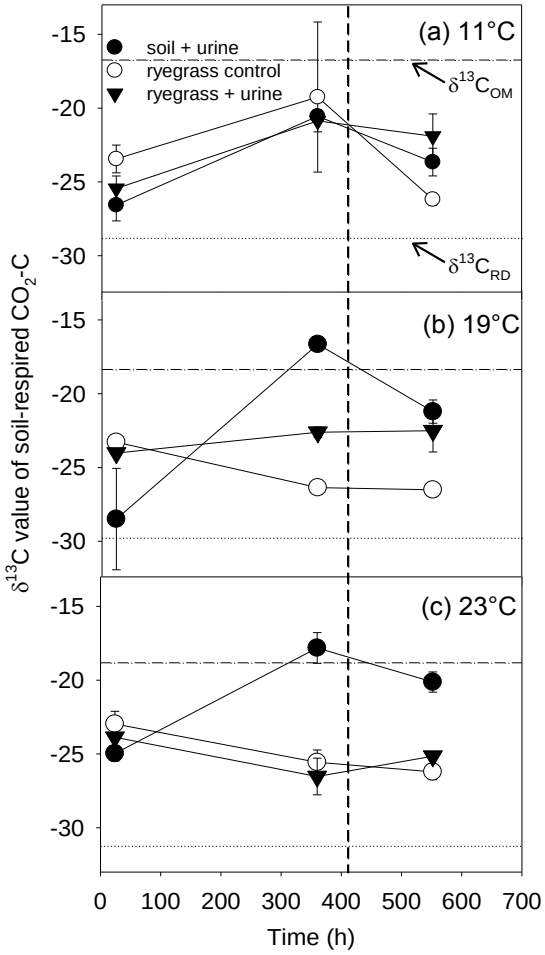

Figure 5.6 The $\delta^{13}C$ values of soil-respired CO_2 over time. Error bars represent SEM (n = 3). The horizontal dashed and dotted lines represent the $\delta^{13}C$ values of soil organic matter C ($\delta^{13}C_{OM}$) and of root-respired CO_2 ($\delta^{13}C_{RDU}$), respectively (see Table 5.7). The lines were shown on the figures to indicate the $\delta^{13}C$ values of the two potential contributing sources of the CO_2 flux. The vertical dashed line shows the timing of the increase of the soil moisture from 50% to 70% WFPS (at 408 h following the urine application).

The $\delta^{13}C$ values used to calculate the contribution of soil organic matter decomposition to F_{CO_2} (f_{OM}) are shown in Table 5.7. Overall, there were significant effects of sampling time ($p < 0.001$), temperature ($p < 0.01$), ryegrass ($p < 0.001$), and urine treatments ($p < 0.001$) on the f_{OM} values (Fig. 5.7). At 11°C, the f_{OM} values changed over time, increasing ($p < 0.05$) from 24 h to 360 h prior to decreasing again at 552 h (Fig. 5.7a). Ryegrass and urine treatments had no statistically significant effect on the f_{OM} values at 11°C. At 19°C, the f_{OM} values were low at 24 h before increasing to dominate the CO_2 flux by 360 h ($p < 0.05$), before they too declined at 552 h, in the *soil + urine* treatment (Fig. 5.7b). In the *ryegrass control* treatment at 19°C the f_{OM} values decreased ($p < 0.05$) from a value of approximately 0.6 at 24 h to a value of approximately 0.3 at 360 and 552 h (Fig. 5.7b). The f_{OM} values were relatively constant over time for the *ryegrass + urine* treatment at 19°C (Fig. 5.7b). At 23°C in the *soil + urine* treatments the f_{OM} values increased between 24 and 360 h and followed the trend seen at 19°C (Fig. 5.7c). The *ryegrass + urine* and *ryegrass control* treatments also showed the same trends, with comparable values, as at 19°C, with the f_{OM} values decreasing over time in the *ryegrass control* and remaining relatively constant in the *ryegrass + urine* treatment (Fig. 5.7c).

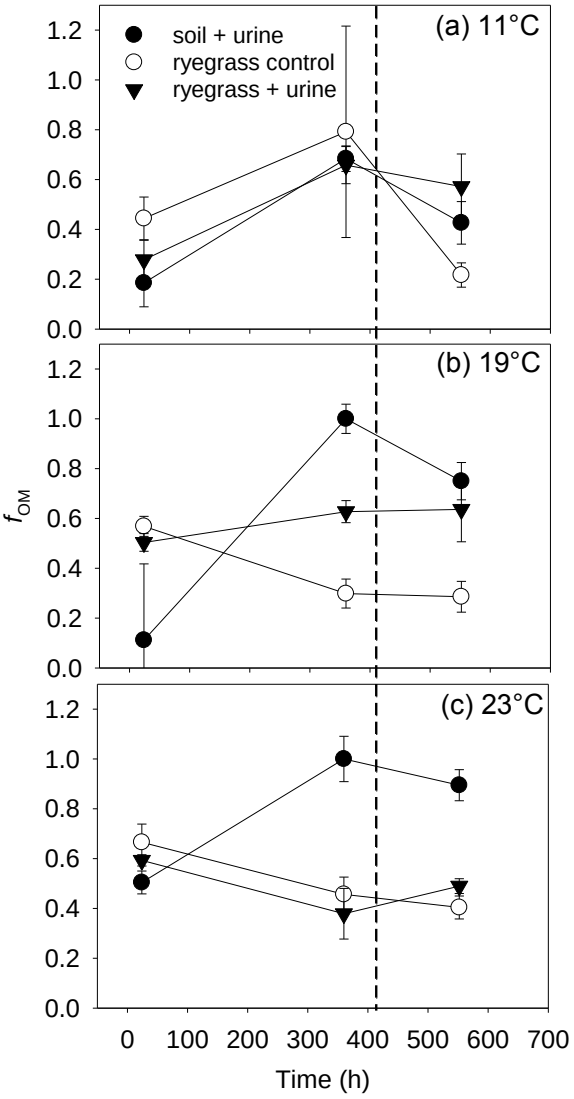

Figure 5.7 The effect of urine and ryegrass treatments on f_{OM} over time (h) at (a) 11°C, (b) 19°C and (c) 23°C. The error bars represent SEM (n = 3). The vertical dashed line shows the timing of the increase of the soil moisture from 50% to 70% WFPS (at 408 h following the urine application).

The R_{OM} rates with plants (R_S minus R_{RDU}), calculated based on the f_{OM} (Fig. 5.7) and R_S values (Fig. 5.4), showed that at 360 and 552 h, R_{OM} in the *ryegrass + urine* treatment was consistently higher than R_{OM} in the *ryegrass control* treatment at all the temperature treatments (Table 5.8). However, the magnitude of the priming effect on R_{OM} in the *ryegrass + urine* treatment was significantly higher than in the *ryegrass control* treatment only at 11°C 360 h and 23°C 552 h (Table 5.8).

Table 5.6 presents the estimation of R_S without urine derived abiotic CO_2 production. However, with plants present, R_S can further be separated into R_{OM} and root-derived respiration (R_{RD}), hence the comparison of R_S without plants (R_{OM} only) and R_S with plants (R_{OM} and R_{RD}) would be difficult if only the data in Table 5.6 was available. Hence, Table 5.8 shows the estimated R_{OM} values with plants present (R_S minus R_{RD} and urine derived abiotic CO_2 production) and it makes possible the direct comparison of 'R_{OM} with plants' and 'R_{OM} without plants'. However, the values in Table 5.6 are based on the cumulative CO_2 flux throughout the experimental period (0 – 672 h), whereas the values in Table 5.8 are based on the CO_2 fluxes at particular times during the experiment (360 and 552 h).

Table 5.8 The magnitude of priming effect on R_{OM} induced by (d) ryegrass presence and (e) ryegrass + urine presence at 360 and 552 h following urine application calculated based on R_{OM} in the (a) *soil control*, (b) *ryegrass control*, and (c) *ryegrass + urine* treatments. The R_{OM} rates were calculated from the fraction of R_{OM} contributing to R_S (f_{OM}, Fig. 5.7), and the measured R_S rates (Fig. 5.4). The error bars represent SEM (n = 3).

		R_{OM} (µg CO_2 kg^{-1} s^{-1})			Magnitude of priming effect (%)	
		(a) soil control	(b) ryegrass control	(c) ryegrass + urine	(d) Priming induced with ryegrass present, (b)/(a)	(e) Priming induced with ryegrass and urine present (c)/(a)
11°C	360 h	0.03 ± 0.01	0.03 ± 0.02	0.24 ± 0.08	99 ± 58%	724 ± 303% *
	552 h	0.03 ± 0.02	0.02 ± 0.00	0.03 ± 0.01	54 ± 31%	86 ± 61% ns
19°C	360 h	0.10 ± 0.04	0.10 ± 0.03	0.49 ± 0.07	100 ± 51%	502 ± 228% ns
	552 h	0.22 ± 0.04	0.23 ± 0.03	0.47 ± 0.08	104 ± 22%	212 ± 51% ns
23°C	360 h	0.10 ± 0.02	0.34 ± 0.03	0.40 ± 0.09	350 ± 91%	407 ± 136% ns
	552 h	0.21 ± 0.02	0.53 ± 0.07	0.83 ± 0.05	257 ± 45%	405 ± 49% *

Significance refers to the differences between (d) and (e). **$p < 0.01$; *$p < 0.05$; ns (not significant)

At 24 h, increasing T_S significantly ($p < 0.001$) increased the f_{OM} values, when averaged across ryegrass and urine treatments (Fig. 5.8a). The f_{OM} values for the *ryegrass control* treatment were higher ($p < 0.01$) than the values for the *ryegrass + urine* and *soil + urine* treatments at 24 h (Fig. 5.8a). At 360 h, there was no significant effect of temperature treatment when values were averaged across treatments (Fig. 5.8b). However, the *soil + urine* treatment had higher ($p < 0.01$) f_{OM} values when compared to the values for the *ryegrass + urine* and *ryegrass control* treatments at 360 h (Fig. 5.9b). At 552 h, there were significant ($p < 0.05$) effects of temperature, ryegrass and urine treatments. Increasing temperature increased the f_{OM} values, at 552 h, for the *soil + urine* and *ryegrass control* treatments. The *soil + urine* treatment again had a significantly ($p < 0.01$) higher f_{OM} value when compared to the values for the *ryegrass control* treatment at 552 h but the difference was smaller than 360 h (Fig. 5.8c). The *soil + urine* treatment also had a significantly ($p < 0.01$) higher f_{OM} value when compared to the values for the *ryegrass + urine* treatment at 552 h (Fig. 5.8c).

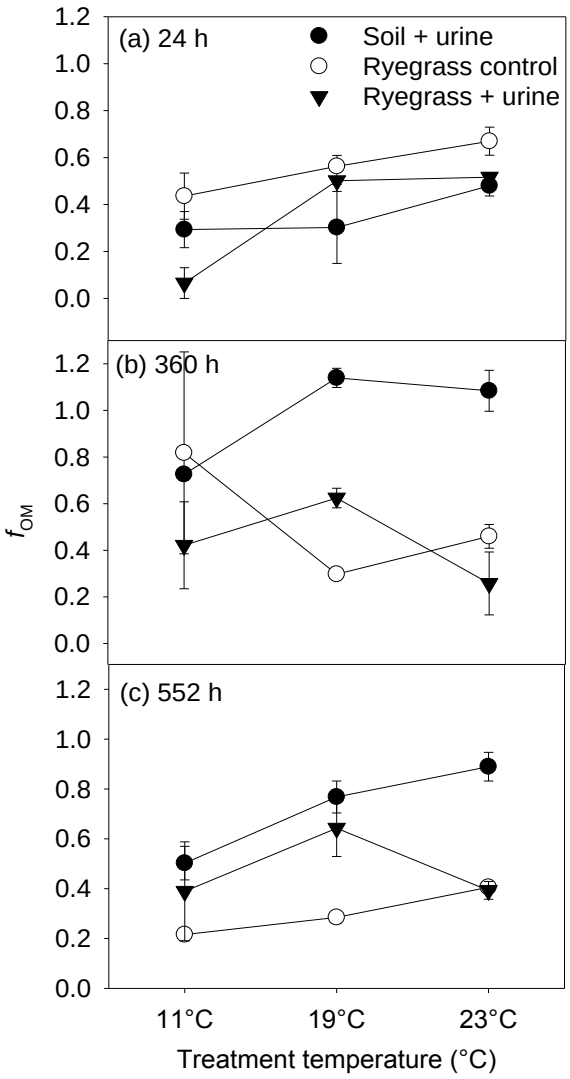

Figure 5.8 The effect of temperature, ryegrass and urine treatments on the contribution of f_{OM} to the CO_2 flux, (a) 24 h, (b) 360 h, and (c) 552 h following a urine application. The error bars represent SEM (n = 3).

5.4.10 Photosynthesis rates and plant growth

Increasing temperature significantly ($p < 0.001$) increased photosynthesis rates (A) when averaged across the urine treatments. Urine application decreased ($p < 0.05$) A at 240 h at 11° and 23°C (Fig. 5.9a and c) but not at 19°C (Fig. 5.9b). At 552 h, A in the + *urine* treatments was higher ($p < 0.05$) than in the *control* treatment at 23°C, but at 11°C A in the + *urine* treatment remained lower ($p < 0.01$) than in the *control* treatment at this time.

Increasing temperatures had a positive effect ($p < 0.01$) on leaf and root biomass (Table 5.9). Urine treatment had a negative effect ($p < 0.01$) on the root biomass at all of the treatment temperatures whereas urine treatment had no effect on the leaf biomass (Table 5.9).

Using the plant biomass data in Table 5.9 and the photosynthesis data at 552 h, the photosynthesis rates were also expressed as per unit of plant biomass (leaf + root) to compare with previously measured values in Chapter 4 (Fig. 4.3). With urine, A increased with temperature whereas without urine, A remained constant across treatment temperatures. This was because, without urine, leaf: root ratio was markedly decreased with increasing temperature (Table 5.9).

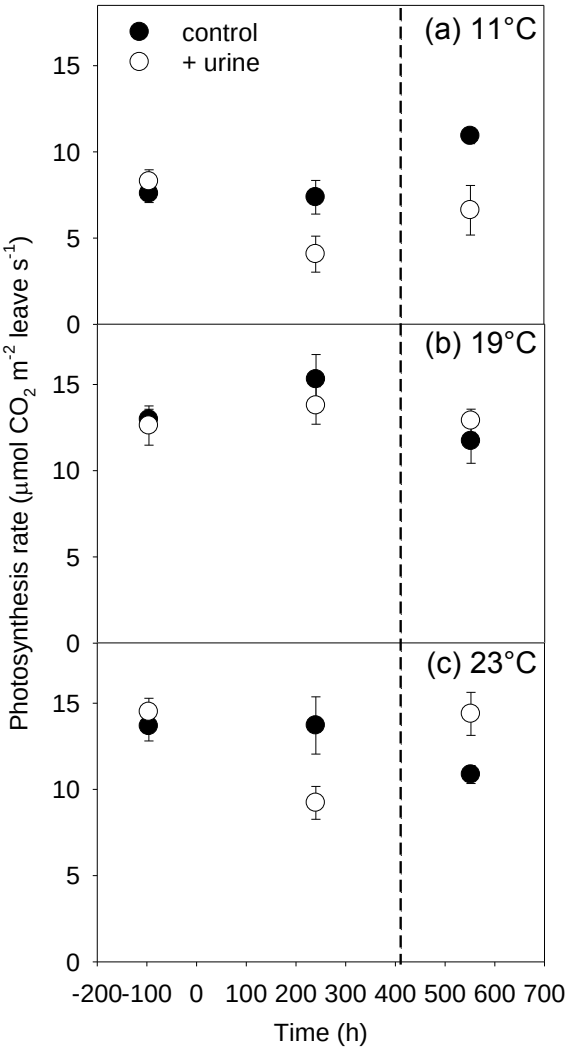

Figure 5.9 Photosynthesis rates (µmol CO_2 m^{-2} s^{-1}) of ryegrass plants in the *ryegrass+urine* and *ryegrass control* treatments at (a) 11°C, (b) 19°C and (c) 23°C over time (h). The measurements were taken 96 h before the urine application (-96 h), and 240 h and 552 h following the urine application. Values are means of 6 measurements taken from three different pots and the error bars represent SEM (n = 6). The vertical dashed line shows the timing of the increase in soil moisture from 50% to 70% WFPS (at 408 h following the urine application).

Table 5.9 Leaf and root dry weight biomass (g pot^{-1}) at the end of the experiment (672 h following the urine treatment). The data is the average of three replicates and the error bars represent SEM (*n* = 3).

Temperature	Vegetation	Leaf	Root
		g pot^{-1}	
11°C	ryegrass control	4.64 ± 0.53	4.36 ± 0.91
	ryegrass + urine	2.52 ± 0.56	0.59 ± 0.19
19°C	ryegrass control	6.72 ± 0.26	8.45 ± 1.74
	ryegrass + urine	8.65 ± 0.44	3.29 ± 0.69
23°C	ryegrass control	7.75 ± 0.80	10.89 ± 1.98
	ryegrass + urine	10.35 ± 0.09	5.34 ± 0.78

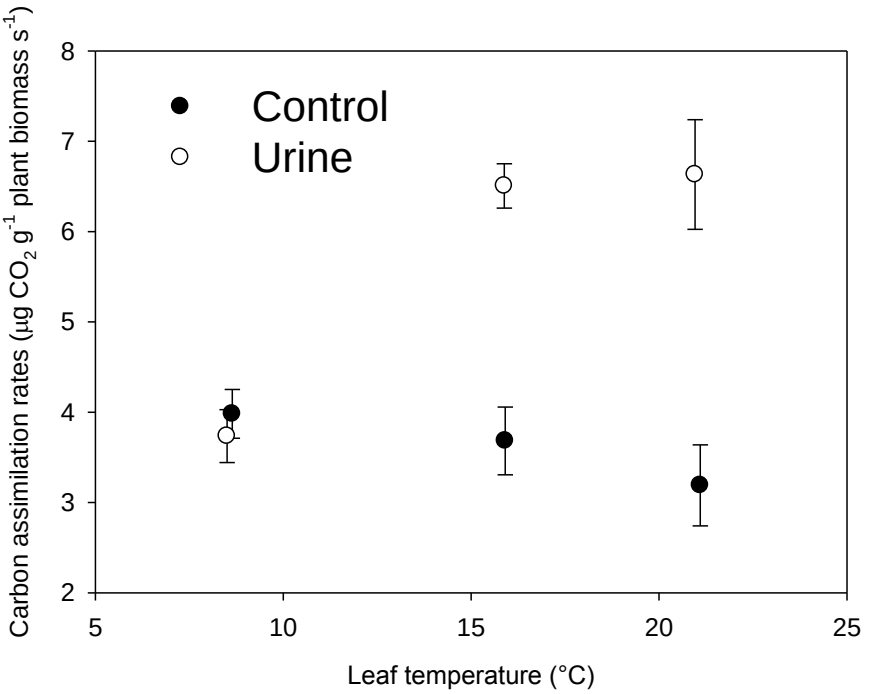

Figure 5.10 Temperature response of the carbon assimilation rates, at 552 h, of ryegrass with (○) and without (●) urine treatment. Error bars represent SEM (*n* = 4).

For the photosynthesis data measured at 552 h, the percentage of CO_2 respired via R_{RDU} per unit weight of net CO_2 fixed by photosynthesis (A) was calculated (Table 5.10). This calculation was performed based on the assumption that abiotic CO_2 fluxes from urine ceased by 552 h following the urine application and root-derived C was the only source of R_{RDU}. Increasing T_S ($p < 0.001$) significantly increased the percentage of R_{RDU} per unit of A. There was a significant interaction ($p < 0.05$) between temperature and urine treatments in terms of the percentage of R_{RDU} per A. At 11°C, urine application increased the percentage of soil-respired CO_2 per A whereas at 19° and 23°C, the effect of urine application was the opposite (Table 5.10).

Table 5.10 The estimated fractions of urine and root-derived C utilisation (R_{RDU}) per unit weight of net CO_2 fixed by photosynthesis at 552 h following urine application. The errors represent SEM (n = 3).

Soil Temperature	Treatment	R_{RDU} / A (%)
11°C	ryegrass control	0.40 ± 0.09
	ryegrass + urine	0.48 ± 0.27
19°C	ryegrass control	2.32 ± 0.25
	ryegrass + urine	0.82 ± 0.40
23°C	ryegrass control	2.89 ± 0.03
	ryegrass + urine	2.19 ± 0.14

5.5 Discussion

5.5.1 Effects of temperature, soil moisture, ryegrass and urine on soil N_2O flux

Increased soil N_2O fluxes both with and without ryegrass present, following urine application, can be attributed to the enhanced supply of N made available following the hydrolysis of the urea-N contained in the urine. This fact is supported by the high soil inorganic-N concentrations measured at the end of the experiment (Table 5.2).

At 11°C, without plants, urine application to the soil induced a greater cumulative N_2O flux than that with plants (Table 5.1). At 11°C, plants corresponded with a halving of the EF. This may have reflected the soil's low N availability due to its low fertility, and consequent urine-N uptake by plants. Any plant uptake of N may have also reduced N_2O via nitrification since nitrifying bacteria and plants compete for NH_4^+-N (Verhagen et al., 1995). Urine-derived N, as NO_3^--N, would still be coming 'on stream' within 48 h of urine application and it is likely that substrates for denitrification would have been limited to antecedent inorganic-N at this time. The hydrolysis of urea was more relatively prolonged at 11°C, based on soil CO_2 flux data (discussed below), and this supports the assumption that substrates for denitrification was limited at this time.

Another reason for lower N_2O fluxes occurred with plants, at 11°C, could have been due to soil structure changes resulting from ryegrass root growth, since roots can reduce the anaerobic environments in soils that are preferred by denitrifying bacteria (Cornish, 1993). Although countering this would be the effect of oxygen consumption by ryegrass roots and exudation of C by roots which could both have enhanced N_2O reduction via denitrification. Even in the presence of urine, the measurements of photosynthesis showed that C was being fixed by ryegrass, despite the possible occurrence of ammonia toxicity and root-scorching due to high soil pH. Some of this C would have been rapidly released via root exudation (Day and Detling, 1990). However, the C exudation by roots at 11°C could have been too small to supply the demand by denitrifiers because photosynthesis rates were approximately a half of those at 19° and 23°C (Fig. 5.9). Without plant-derived readily available C, soil N_2O emissions have been reported to be low under urine patches (Carter et al., 2006). The low photosynthesis rates at 11°C may have been because of higher salt concentrations, a result of reduced N uptake and slower nitrification rates but also most likely because the 11°C temperature is approaching the minimum temperature (6 – 7°C) at which perennial ryegrass grows (Peacock, 1975). The ryegrass root biomass at 11°C, as a proportion of leaf biomass,

was markedly lower (Table 5.9) and this would also have reduced the potential for distributing root C exudates.

At 19° and 23°C, urine application to the soil induced a significant increase of the N_2O fluxes. The emission factors at 19° and 23°C were greater than that at 11°C, with and without plants. At 19° and 23°C, without plants, soil N_2O fluxes following urine application were smaller than those with plants.

The reason that ryegrass increased soil N_2O fluxes at 19° and 23°C in the presence of urine was probably due to enhanced plant activity at these T_S and the associated increase in root exudates. As the T_S increased the leaf and root dry masses also increased along with photosynthetic C fixation, resulting in greater C supply to the soil microbial community. Soil NO_3^--N would also have been prevalent, based upon previous urine studies (e.g. Clough et al., 2009) and in conjunction with the enhanced C exudation and elevated WFPS denitrification could have occurred. A previous field study has also reported a positive influence of plant roots on soil N_2O fluxes using maize plants (Song and Zhang, 2009).

The occurrence of higher N_2O fluxes immediately post urine application, during the moderate moisture phase, agrees with previous studies performed in urine patches at similar soil moisture contents (~50% WFPS) (Carter, 2007; Yamulki et al., 1998). The reason that the soil N_2O fluxes initially peaked within 48 h following the urine application, during the moderate moisture phase, was most likely due to the increase in WFPS that occurred as a result of the urine application, which would have made the soil more anaerobic and potentially enhanced denitrification of antecedent inorganic-N. Bouwman (1998) reported that denitrification rates increased with increasing soil moisture and highest denitrification rates were found at a WFPS above 60%.

Soil N_2O flux rates peaked at 225 µg N_2O-N kg^{-1} h^{-1} (equivalent to 13000 µg N_2O-N m^{-2} h^{-1}) in the current study (19 d following the urine application at 23°C with plants). The N_2O flux rates are comparable to other studies, for example, Luo et al. (2008) measured soil N_2O fluxes following the addition of cow urine (applied at 1000 kg N ha^{-1}) on a New Zealand pasture, and the recorded values for the fluxes were up to 6000 µg N_2O-N m^{-2} h^{-1} under comparable soil NO_3^- and moisture conditions. Uchida et al. (2008) also recorded peak soil N_2O fluxes of 8000 µg N_2O-N m^{-2} h^{-1} 900 h after the application of fresh cow urine (at 15°C, equivalent to 340 kg N ha^{-1}) when a pasture soil was repacked and heavily compacted. Thus the N_2O flux results are well within what would be expected under normal pasture conditions.

Following the increase in soil moisture at 408 h the observed increase in the N_2O flux was again most likely a consequence of the increased WFPS enhancing denitrification. This is also consistent with previous work that has shown a marked increase in N_2O emissions when WFPS exceeds approximately 60% (Clough et al., 2004; Linn and Doran, 1984). Pihlatie et al. (2004) also reported that the dominant N_2O production process in soils changed from nitrification to denitrification when soil moisture content was increased from 40% to 100% WFPS. At a similar soil moisture content to the moderate moisture phase in the current study (WFPS = 50%), nitrification and denitrification were found to contribute equally to the N_2O production under a urine patch (Carter, 2007), but denitrification was reported to be the dominant source of N_2O under the high moisture condition (WFPS = 70%) (Monaghan and Barraclough, 1993). Russow et al. (2009) has shown that denitrification increases significantly when the oxygen percentage in the soil falls below 2%, and as WFPS increases microsites for denitrification become more numerous as oxygen content in the soil decreases. The response to elevated WFPS at 408 h was further influenced by T_S and this is discussed below.

The availability of substrate N strongly controls soil N_2O fluxes (Tiemann and Billings, 2008). During the moderate moisture phase, the lack of any effect of T_S on soil N_2O fluxes, in the absence of urine indicates that the response to T_S depended on N substrate availability, and that there was insufficient N to be lost via nitrification or denitrification pathways. Thus it follows that the presence of ryegrass also had no influence on N_2O fluxes in the absence of urine during the moderate moisture phase, since N substrate would presumably have been even less available for soil microbes when plant competition for N was also present.

Hence the changes in N_2O fluxes to T_S following the soil moisture increase, observed in the current study, were probably due to the responses of both denitrifiers and ryegrass to T_S. Temperature optima for denitrification have been reported to be 60° to 75°C (Keeney et al., 1979) while the optima for ryegrass is 18° to 21°C (Mitchell, 1956). However, N_2O production via nitrifiers cannot be ruled out and NH_4^+-N was still present in all urine treatments at the end of the experiment. Temperature optima for nitrifiers have been reported as approximately 35°C (Stark, 1996) thus the N_2O fluxes may have been partially formed via nitrification.

The results agree with a previous review article, focusing on grassland systems, which concluded that soil N_2O fluxes are the most sensitive to temperature at a soil moisture content range of 60 to 80% WFPS (Flechard et al., 2007). However, the novelty of these current

results is the demonstration of the interaction between pasture plants, temperature and ruminant urine and their essential effects on N_2O fluxes.

5.5.2 ^{15}N enrichment and recovery as N_2O

During the first 120 h at ≥ 19°C, the contribution of urine-N to soil N_2O fluxes was higher with plants presence, when compared to the N_2O fluxes from soils without plants, as shown by the N_2O-^{15}N enrichment (Fig. 5.2). The fact that the same effect was later observed between 120 and 240 h at 11°C indicates that the effect was temperature driven, in addition to any effect the presence of ryegrass had. Temperature affects biological processes, such as microbial activity and plant growth, and abiotic processes such as urea hydrolysis. As described above (section 5.2) the hydrolysis of urea results in elevated CO_2 fluxes and a return to the antecedent pH level, which of course may change further due to nitrification. This elevated CO_2 flux was observed to be of much shorter duration as T_S increased (section 5.4.7), indicating that urea hydrolysis was faster at higher T_S. This means the soil NH_4^+-N pool would have formed more rapidly at higher T_S leading to progressively earlier microbial utilisation of this soil NH_4^+-N pool as T_S increased. During this period, higher ^{15}N enrichment was observed in the N_2O from the *ryegrass + urine* treatment when the soil was at the moderate moisture phase. This would have favoured nitrification and denitrification as opposed to predominantly denitrification after 408 h (the high moisture phase). Thus N_2O could have derived from either mechanism. However, for plants to influence the N_2O-^{15}N enrichment there would have needed to be an increased C exudate supply at the higher T_S influencing denitrification due to enhanced ryegrass growth at these temperatures (after NH_4^+-N subsequent nitrification would have enhanced the nitrite pool and this may have been denitrified), or due to the microbial environment being enhanced by presence of the plant rhizosphere. The exact mechanism cannot be determined from the results obtained so far and warrant further investigation.

It was expected that as T_S increased, the contribution of urine-N to the N_2O flux would decline since soil-N mineralisation is considered to increase with T_S (Kladivko and Keeney, 1987; Stanford et al., 1973). However, the ^{15}N enrichment of the N_2O flux did not differ at 552 h due to T_S indicating that urine-N presence and not T_S determined the N_2O-N source. At the end of the experiment (672 h), ≤ 15% of the N_2O-N originated from soil-N. More soil-N would be available for soil microbes to produce N_2O at higher T_S.

This may have been because the amount of mineralised soil-N was relatively small compared to the applied urine-N. The C:N ratio of the soil used in the current study was 11.7 and the

estimated mineralised C (based on the cumulative R_S during the experiment at 23°C without plants) was 2.11 g CO_2 kg^{-1} (0.58 g CO_2-C kg^{-1}). Given these values, the amount of N mineralised might have been relatively small (0.58 / 11.7 = 0.05 g N kg^{-1}) when compared to the amount of N applied as urine-N (1.02 g N kg^{-1}). Soil respiration data from the *control* treatments in the current study (section 5.5.3) indicates that the soil microbial activity (R_S) was relatively low when compared to similar previous studies using a more fertile pastoral soil with a history of highly productive pasture species i.e. ryegrass and white clover (e.g. Clough and Kelliher, 2005). Thus, soil microbes in the soil may have not been actively mineralising labile organic N even at high T_S in the current study. In support of this hypothesis are the results of Di and Cameron (2008) who reported that approximately 40% of the N_2O flux was derived from soil-N following urine application (equivalent to 1000 kg N ha^{-1}), over a 2 month period, in a high fertility pasture soil with a ryegrass and white clover sward. Soil N mineralisation can also be higher in a pasture with legume species, used in the study by Di and Cameron (2008), compared to mineralisation in a monoculture non-legume sward, used in the current study, because of the N fixation performed by clovers (Elgersma and Hassink, 1997). Also, a previous study reported that N immobilisation rates were positively related to T_S but were also dependent on the nature of the C substrates (Nicolardot et al., 1994). Hence, in the current study, mineralised soil-N might have been immobilised into the microbial-N pool instead of being used for N_2O production.

Nitrous oxide emissions were not measured in previous experiments (Chapter 3 and 4). However, in a fertile pasture soil without plants, I observed a positive relationship between microbial ability to access more recalcitrant organic matter and T_S (Uchida et al., 2010). Similarly, in Chapter 4, I showed that the fraction of R_{OM} to R_S increased with T_S in a fertile soil with plants. However, in a less fertile soil, used in the current experiment, the phenomena described above were not observed and R_{OM} remained constant across T_S ranging from 13° – 27°C (Fig. 4.2). Thus, the increase in soil microbial activity and consequent soil-N mineralisation with increasing T_S might not have been occurring in the low fertility soil used in the current experiment due to low microbial activity. These may explain the relatively low contribution from soil-N to the N_2O fluxes in the current study. It is likely that more soil-N would have been available for soil microbes to produce N_2O if a more fertile soil was used in the current study because in a fertile soil, the mineralization of soil-C and soil-N is more likely to occur and the relatively lower C:N ratio in fertile soils suggest that more N is mineralized per a unit of soil organic matter mineralization.

Although urine-induced soil-N mineralisation did not increase with T_S in the current study, urine-induced priming increased R_S (section 5.5.3). Similarly, Gao et al. (2009) reported that, in soils with high C:N ratio (> 13), R_S positively responded to T_S while N mineralisation rates were below zero and were constant when T_S ranged from 5° – 15°C. However, many models predict a positive relationship between R_S and soil-N mineralisation (Rodrigo et al., 1997). Further study is required to investigate how background soil characteristics, for example, soil fertility, labile organic C and N contents, and general soil fertility, influence the contribution of soil-N to N_2O fluxes under urine patches and the response of the pasture N cycle to soil warming.

The increased recovery of ^{15}N as N_2O-N during the high moisture phase, particularly in the presence of ryegrass, and elevated T_S (Fig. 5.3), occurred for reasons outlined above with respect to N_2O fluxes, i.e. the probable increase in denitrification during this phase due to enhanced anaerobic and warmer conditions with increased root exudate C supply. Such moisture induced effects have also been observed previously, for example, Clough et al. (2004) reported increases in ^{15}N recovery from < 0.1 – 0.4% to 0.4 – 1.7% when soil moisture contents were increased from field capacity to saturated conditions over an 85 day period.

At 11° and 19°C, with and without plants, the EF, estimated from the cumulative N_2O fluxes (Table 5.1), was larger than the percentage recovery of ^{15}N. At 11°C, similar to the EF, plant corresponded with a halving of the percentage recovery of ^{15}N (Fig. 5.3). At 19° and 23°C, plants increased the EF (Table 5.1) and the percentage recovery of ^{15}N (Fig. 5.3). The EF was remarkably similar to the percentage recovery of ^{15}N, at 23°C. This comparison suggests that virtually all of the emitted N_2O derived from the applied urine-N at 23°C and N mineralisation did not contribute to the emitted N_2O.

The range in the percentage recovery of ^{15}N, as N_2O-N, in this study (Fig. 5.3) is comparable to previous studies listed below. In a field study conducted at a T_S of 10°C on a ryegrass dominated pasture, the percentage of ^{15}N recovered as N_2O-N was 0.05% after 720 h following urine application (1030 kg N ha^{-1}) (Wachendorf et al., 2008). Clough et al. (1996) conducted a lysimeter study with a silt loam mineral soil and recovered 3% of ^{15}N applied as N_2O-N 100 d after urine application (500 kg N ha^{-1}). The percentage of ^{15}N recovered as N_2O-N will vary according to soil type (Clough et al., 1998) and experimental duration.

5.5.3 Effects of temperature, soil moisture, ryegrass and urine on soil CO_2 flux

Urine application greatly increased R_S (cumulative F_{CO_2} minus the total amounts of CO_2 produced due to urea hydrolysis and from other urine-C) and this was interpreted as a urine-

induced priming effect (Table 5.6). Clough et al. (2003) observed a similar phenomenon when 500 kg N ha^{-1} of synthetic urine was applied to a silt loam pasture soil. Clough et al. (2003) showed that during a 60 d incubation at 20°C, at field capacity, the control soil and urine treated soil produced 3.4 and 4.0 g CO_2 kg^{-1}, respectively, solely due to R_S in the absence of plants. Another study by Clough and Kelliher (2005) reported R_S from the control and urine treated soils (over 10 d) to be 1.6 and 1.1 g CO_2 kg^{-1}, hence in their study, R_S decreased under urine patch when compared to R_S in the control soil (negative urine-derived priming effect). Another study, performed on a grass-clover pasture, also showed that there was no increase in R_S following a 400 kg N ha^{-1} urine application (Bol et al., 2004). In the current study, at 19°C and without plants, the control soil and urine treated soil produced 0.3 ± 0.1 and 1.5 ± 0.2 g CO_2 kg^{-1} from R_S (Table 5.6). Hence the magnitude of the priming effect (R_S in the urine treated soil / R_S in the control soil) was markedly larger for the current study when compared to the previous studies above (Clough and Kelliher, 2005; Clough et al., 2003). This may have been because of the competition for nutrients between soil microbes under low fertility conditions (discussed below).

The $\delta^{13}C$ values of F_{CO_2} also support the fact that a significant proportion of the F_{CO_2} originated from soil-C. When averaged across temperature treatments, approximately 40 and 70% of F_{CO_2} originated from the soil-C at 24 and 552 h, respectively, in the *soil + urine* treatment (Fig. 5.8). A direct comparison of the amount of respired soil-C estimated by cumulative F_{CO_2} and by the $\delta^{13}C$ values was constrained because the $\delta^{13}C$ values were measured only three times in the current study. However, the trends are the same and of the same magnitude when comparing the two methods with regards to the magnitude of priming effect. A significant amount of soil-C was almost certainly solubilised, due to an increase in soil pH, and respired following urine treatment application.

There is a significant lack of published information with which to ascertain the factor(s) that control the urine-derived priming effect. However, when comparing the current study with the previous studies above (Clough and Kelliher, 2005; Clough et al., 2003) it can be seen that at low rates of R_S the magnitude of the urine-derived priming effect is relatively higher. However, this comparison is confounded by differing urine-N rates between the current study and the previous studies. The control soil in the study of Clough and Kelliher (2005) showed the highest R_S rate (1.6 g CO_2 kg^{-1} in 10 d) but showed the lowest magnitude of urine-derived priming effect, whereas the control soil used in the current study showed the lowest R_S rate (0.3 g CO_2 kg^{-1} in 28 d) but showed the highest magnitude of urine-derived priming effect.

Soils used in the previously referenced studies (see above) were from ryegrass and white clover pastures and they were relatively more fertile when compared to the soil used in the current study (C_4 grass pasture). In a previous experiment (Chapter 4), with plants and without urine treatment, the fraction of R_{OM} in R_S was smaller in a low fertility soil, used in the current study (C_4 soil), when compared to a high fertility soil (Fig. 4.2), suggesting that mineralisation was limited in the low fertility soil when urine was not present. A previous study reported that in forest soils, soil organic C mineralisation in the N-poor soils was limited by N availability and thus increased strongly after the addition of an N containing compound (Hamer and Marschner, 2005), hence similar mechanisms may control the magnitude of urine-induced priming effects in pasture soils. Also, Fontaine et al. (2003) reported that the priming effect results from the competition for nutrient between the microbes. Similarly, Phillips and Fahey (2008) reported that the magnitude of a priming effect was stronger in nutrient-poor soils when compared to nutrient rich soils. Hence, the priming effect is more likely to occur in low fertility soils with relatively less available nutrients, when compared to fertile soils. Therefore, factors such as soil fertility and microbial availability of soil nutrients will almost certainly influence the magnitude of urine-derived priming effect. Further study is required to assess the influence of soil fertility on R_S and possible priming effects.

In the current study, the urine-induced priming effect on R_S was greater at lower T_S (Table 5.6(e)) possibly because of slower rates of chemical and biological reactions in the soil at lower T_S. As noted above the hydrolysis of urea was more relatively prolonged at 11°C thus the soil pH possibly remained higher for longer at this temperature and therefore the solubilisation of soil organic matter due to elevated soil pH was also likely to have occurred over a longer period. This may have made soil C available to the microbial pool for a longer period.

However, the urine treatment effect (increase in the actual amount of CO_2 emissions from R_S when urine was applied, Table 5.6(d)) was not strongly affected by T_S. Soil respiration is expected to increase 120% when T_S increases from 11° to 23°C but in the current study, urine treatment effect (Table 5.6(d)) increased only 40% without plants and with urine when T_S increases from 11° to 23°C, suggesting that the urine treatment effect was limited by insufficient C substrate availability. This may have reflected the soil's low C quality.

The estimated fraction of root- and urine-derived CO_2 (R_{RDU}) per unit weight of net CO_2 fixed by photosynthesis (R_{RDU}/A, Table 5.10) increased with increasing T_S, when averaged across urine treatment. A previous study (Chapter 4) showed that > 70% of soil-respired CO_2

originated from root-derived C and the values for root-derived C/A increased with increasing T_S (Table 4.3). Hence the similar trend was observed in Chapter 4 and 5. Given these facts, increasing R_{RDU}/A with increasing T_S may suggest that plants used assimilated C more efficiently at lower T_S. Urine treatment markedly reduced R_{RDU}/A at 19°C. The reason for this is difficult to fathom but this may be related to the fact that the optimum temperature for the ryegrass growth is approximately 19°C (Mitchell, 1956). Hence, this change in plant physiology with T_S changes might have influenced the magnitude of priming effects. In addition, changes in soil microbial community might have had an influence on the magnitude of priming effects. However, the mechanisms related to this hypothesis are unknown and further research is needed in this area.

The reason why plant presence would reduce the urine-induced priming effect (Table 5.6) is harder to fathom but following on from the logic above it may be that the soil microbial community, in the presence of plants, was larger or growing more rapidly following urine application and some of the primed soil C may have been incorporated into the microbial C pool. It was reported that without plants, the addition of inorganic-N (NH_4NO_3) to a soil induced a short-term (< 30 d) increase in R_S when the inorganic-N was added together with various plant residues, because the addition of the inorganic-N reduced the C:N ratio and this favoured the microbial decomposition of plant residues (Lueken et al., 1962). Thus, the reduction of the C:N ratio due to the urine-N addition may have been a factor controlling the urine-induced priming effect, and this reduction of the C:N ratio may have occurred more efficiently in the absence of plants, due to the lack of plant N uptake, in the current study, driving the urine-induced priming effect.

The time course of F_{CO_2} (R_S plus CO_2 derived from abiotic processes) following the urine treatment in this study was similar to the data obtained from a previous study using a grassland soil (Kelliher et al., 2007) where the experiment was conducted at 19.2 ± 1.2°C and the parameters *b* and *r* obtained by fitting the same model were not significantly different from the parameters obtained in the current study at 19°C (Table 5.5). The range in the parameter *r* in this study (Table 5.5) is comparable to previously reported *r* values in this thesis, using a fertile soil without urine (Table 3.1). In the current study, the decline of F_{CO_2} was faster at higher T_S but this trend was not clear in the fertile soil without urine at T_S ranging from 3° to 24°C (Chapter 3). Based on the differentiation of eqn. 5.5, which may be written as $\mathrm{d}F_{CO_2} / \mathrm{d}t = \{[F_{CO_2}(t) - a]\ln(r)\}$, the natural logarithm (ln) of *r* expresses the magnitude of the decline in F_{CO_2} and this method was previously used to examine the changes

in F_{CO_2} over time following urine application on pasture soils (Kelliher et al., 2005b). The ln(r) values suggest that after urea application, the magnitude of the decay in F_{CO_2} was controlled by T_S and not by the plants present (Table 5.11). This was because of the abiotic release of CO_2 due to urea hydrolysis, as noted above, which increased as T_S increased.

Table 5.11 Natural logarithm of the parameter *r*, determined from eqn. 5.5 (Table 5.5). For each ryegrass treatment (*ryegrass + urine* and *soil + urine*), dissimilar letters represent significant differences between treatment temperatures.

	ryegrass + urine		soil + urine	
11°C	-0.011	± 0.001[a]	-0.010	± 0.002[a]
19°C	-0.021	± 0.002[b]	-0.019	± 0.002[b]
23°C	-0.070	± 0.012[c]	-0.053	± 0.007[c]

The values of *a*, the asymptote, only increased significantly at higher T_S in the presence of ryegrass and urine, when compared to the *a* in the *soil + urine* treatment, because of the increasing contribution of root-derived C to R_S at higher T_S as time progressed. This theory is supported by the measured photosynthesis rates. At 11° and 19°C, photosynthesis rates were reduced or unchanged by the urine treatment (Fig. 5.9a and b), whereas at 23°C, photosynthesis rates had increased 552 h after urine application (Fig. 5.9c). Root exudation has also been shown to be temperature dependent over the range 7° – 30°C in a western wheatgrass (*Agropyron smithii* Rydb.) (Bokhari and Singh, 1974). Also, leaf biomass in the *ryegrass + urine* treatment increased at higher T_S but decreased at lower T_S, when compared to the *ryegrass control* treatment (Table 5.8). This suggests that, at lower T_S, urine application negatively affected plant growth, possibly because of root-scorching. Thus, the decreased plant growth at lower T_S and subsequent decrease in root-derived C input in the *ryegrass + urine* treatment is probably the reason for the unchanged value of *a* with/without ryegrass at lower T_S.

In the first experiment (Chapter 3) the values of *a* increased with increasing T_S in the absence of ryegrass and urine (Table 3.1). The soil used in Chapter 3 was more fertile, compared to the soil used in the current study, and did not receive urine thus direct comparisons are not possible. However, it is possible that increased replication in the current study may have seen the *a* values increasing with T_S in the absence of ryegrass because there was a trend for a positive relationship between T_S and the *a* values but with large errors (Table 5.5).

The fact that the increase in soil moisture content had no significant effect on the F_{CO_2} data may have been because WFPS was not limiting even at the moderate moisture regime. Doran (1984) reported a close relationship between R_S and soil moisture content, with peak activity at 60% WFPS hence the two WFPS phases used in this experiment, 50% and 70%, might also have been close to the optimum in terms of F_{CO_2}.

5.5.4 The $\delta^{13}C$ values of soil CO_2 fluxes

The $\delta^{13}C$ values from soil organic matter decomposition ($\delta^{13}C_{OM}$) and root-derived C respiration ($\delta^{13}C_{RDU}$), measured in this study (Table 5.7), were typical of the $\delta^{13}C$ values from C_4 and C_3 plants previously reported (O'Leary, 1988) and comparable to those in Chapter 4 (Table 4.2). Using the natural ^{13}C abundance, R_{OM} could be separately quantified but urine-derived CO_2 and root-derived CO_2 could not be separated as shown above because both originated from C derived from C_3 sources, hence the sum of urine-derived and root-derived CO_2 was termed R_{RDU}.

The significant increase in f_{OM} in the *ryegrass + urine* treatment, when compared to the *ryegrass control* treatment, was observed at 19°C (360 and 552 h) (Fig. 5.7b). Based on the $\delta^{13}C$ values, this was also able to be interpreted as a urine-induced priming effect on R_{OM} with plants present. A direct comparison between the urine-induced priming effect indicated by f_{OM} and the priming effect previously discussed (Table 5.6) is not appropriate since the previous priming effect discussed (Table 5.6) was based on the cumulative R_S and this did not distinguish between R_{OM} and R_{RDU}. Nevertheless both approaches demonstrate the positive urine-induced priming effect, in the current study.

Fontaine et al. (2003) have reported that a positive priming effect occurred when there was a competition among soil microbes for the readily available C. Hence, in this study, there might have been a competition for the readily available C between plants and soil microbial community only at 19°C while at 11° and 23°C, the priming effect was probably limited due to low soil microbial activity at a low T_S (11°C) or due to the fact that plants were supplying enough readily C substrates hence there was no competition for the readily available C (23°C). The optimal temperature for ryegrass growth is 18° to 21°C (Mitchell, 1956), hence in the presence of ryegrass, urine treatment may have increased f_{OM} only at 19°C because 19°C was the ryegrass' optimum temperature. Also, f_{OM} is controlled by the amount (Blagodatskaya and Kuzyakov, 2008) and types (Hamer and Marschner, 2005) of added C substrates. Blagodatskaya and Kuzyakov (2008) showed that the magnitude of priming effects linearly increased until the additions of easily available organic C became 15% of microbial biomass

C. I did not measure the microbial biomass C in this experiment hence further study is needed in this area. Hamer and Marschner (2005) reported that the combined addition of fructose and alanine induced a higher positive priming effect than the single substrate additions. Again, I did not measure the components of root-derived C in this experiment hence this could be studied in future. In the current study, the combination of urine, ryegrass and the optimum T_S for plant growth possibly created the optimum conditions for the increase in f_{OM}, when compared to the conditions without ryegrass or urine.

However, the significant increase in the proportion of R_{OM} in R_S (f_{OM}) in the *ryegrass + urine* treatment, when compared to the f_{OM} in the *ryegrass control* treatment, observed at 19°C did not result in a significant increase of actual C loss from the soil via R_{OM}. This finding was possibly because of the relatively large errors associated in the actual soil CO_2 fluxes (R_S). However, there was a trend for the magnitude of the urine-induced priming effect to be higher at 360 h and 552 h in the *ryegrass + urine* treatment when compared with the *ryegrass control* treatment (Table 5.8).

With plants and without urine, sources of R_S in the current study were either root-derived C or soil organic matter C. In Chapter 4, using the same plant and soil used in the current study, increasing T_S decreased f_{OM} without plants (Table 4.2). However, this was not clearly observed in the current study (Fig. 5.8). The two experiments were performed under different light levels due to the availability of growth cabinets (700 and 400 µmol m^{-2} s^{-1} PAR in Chapter 4 and 5, respectively), thus the light levels could have influenced the plant activity and consequent C supply to soil, resulting in the different relationship between T_S and f_{OM}. Further study is required in this area.

Without plants, there were only two sources of R_S in the current study, R_{OM} and the abiotic CO_2 production (e.g. urea hydrolysis) because there were no C inputs from plants. Based on the $\delta^{13}C$ values of soil-respired CO_2 from the soils without plants, at 24 h following urine application, approximately 50 to 70% of R_S originated from urine-C and there was no effect of T_S (Fig. 5.8a). However, at 360 and 552 h, only 0 to 20% of R_S originated from urine-C at 19° and 23°C whereas approximately 50% of R_S still originated from urine-C at 11°C (Fig. 5.8b and c). This was because urea hydrolysis was reduced at the lower T_S as shown by the model (eqn. 5.5) fitted to the F_{CO_2} data. Based on the comparison of the *r* values, it was estimated that urea hydrolysis occurred at a slower rate at lower T_S.

5.6 Conclusion

This study showed that increasing plant-derived C, at higher temperatures, increased N_2O fluxes under high moisture conditions. At a lower temperature (11°C), the positive effect on plant-derived C on N_2O fluxes was not observed. At the highest temperature treatment (23°C), plants increased the EF and percentage recovery of ^{15}N by about 3-fold

Urine application induced a priming effect and enhanced C mineralisation of the soil organic matter accounted for the increased R_S. Urine application increased F_{CO_2} but the fluxes decayed faster as T_S increased because urea hydrolysis rates also increased with T_S. The urine-induced priming effect was relatively larger at lower T_S, and was larger without ryegrass present based on cumulative R_S. This was probably because at lower T_S, chemical and biological reactions occurred at slower rates, soil pH remained higher for a longer period and there was a longer time for soil organic matter solubilisation. The relatively large magnitude of the urine-induced priming effect observed in the current study, when compared to previous work, may have been because of the lower fertility and lower nutrient status of the soil. In the presence of ryegrass, urine application increased the contribution of R_{OM} to R_S particularly at 19°C. This was reasoned to be due to the optimum temperature for ryegrass growth (18° – 21°C) enhancing the competition of nutrients between plants and due to microbes increasing the magnitude of priming effect. Further research is necessary to investigate the mechanisms controlling the urine-induced priming effect under pasture soils, in particular the response of the soil microbial pool and also the relationship between urine-induce priming of C and soil N_2O emissions.

Chapter 6

Synthesis and recommendations for further research

This chapter synthesises my research and identifies emergent opportunities for advancement by further research.

6.1 Overall summary and future work

6.1.1 Soil microbial respiration responses to changing temperature and substrate availability in fertile grassland

This study used highly fertile soil had been sampled from beneath pasture grazed by dairy cattle with the shoots and roots removed. The objective of this study was to quantify the interaction between soil temperature and soil substrate availability on soil microbial activity in the absence of plant substrate inputs. The soil was incubated in the laboratory under constant moisture status. Thus without plants present the quantity of total C substrate was fixed. At 3°C, the soil microbial respiration rate (R_M) was relatively constant at 1.05 ± 0.03 μg CO_2 kg^{-1} s^{-1} for 14 d and C substrate had evidently not been depleted, so R_M was constant. Increasing the incubation temperature from 3° to 24°C corresponded with a doubling of R_M over the 14 d. However, at 24°C, R_M declined by one time constant (by 63%) within 3 d, indicating a strong and rapid effect of the depletion of relatively labile C substrate in the absence of plants. The lower, asymptotic level of R_M maintained over the incubation's final 11 d, at 24°C, was considered to have represented utilisation of more recalcitrant C in the soil and it was significantly larger than the constant level of R_M at 3°C. This suggested the microbial community was utilised C substrate at 24°C that was unavailable at 3°C. The methodology employed in this experiment was not able to detect changes in water soluble C with cold (20°C) and hot (80°C) water soluble C contents were found to be ineffective as indicators of the changes in substrate availability that influenced R_M. Clearly a more subtle approach is required if such changes in microbially available C are to be detected. I would very much like to repeat this study but explore the possibility of using nuclear magnetic resonance (NMR) in order to try and detect shifts in the chemical composition of the soil to see if they correspond with temperature changes applied. The following questions need to be answered: (1) did increasing the incubation temperature to 24°C make more C available due to a simple abiotic mechanism or did the temperature change allow the existing microbial pool to more easily assess?; and (2) what is the relationship between the amount of available C substrates and their recalcitrance at different temperatures?

There is another possible scenario to be covered in future research. Again the same type of experiment would be performed but both the microbial composition (e.g. fungi versus bacteria) and the microbial pool size would be examined. The reason for doing this would be to see if dynamic changes in microbial composition or pool size coincided with changes in R_M and maybe NMR data, as T_S were shifted. Bradford et al. (2008) noted that thermal adaptation of microbes can occur when soil temperature is changed for a longer period of time and that this thermal adaptation can result in changes in the apparent temperature response of R_S. Short term laboratory incubations may not of course run for sufficient periods for such effects to occur.

The soil used here was the high fertility (*HF*) soil. In future work it would also be interesting to rerun a similar experiment but include the low fertility soil (*LF*) especially given the lack of temperature sensitivity that the *LF* soil (old C that was not plant derived) displayed in Chapter 4. Again it would be advantageous to include more subtle measures of the microbial community and soil chemistry as discussed above.

6.1.2 Fertility and plants affect the temperature sensitivity of carbon dioxide production in soils

The objective of this study was to determine the temperature response of respiration sourced from root-derived C (R_{RD}) and soil organic matter decomposition (R_{OM}) in two pasture soils of contrasting nutrient status, with and without plants. Chapter 3 showed that without plants present, R_M markedly decreased within a few days due to the depletion of readily available C substrates. Many previous studies indicate that live roots significantly influence R_M sourced from the recalcitrant C substrates via priming effects (section 2.2.2.4). Soil respiration sourced from the recalcitrant C substrates was termed R_{OM} in the second experiment. In the first experiment, R_M measurement was started immediately following the soil sampling and R_M included the respiration of root-derived C. However, the R_{OM} measurement, measured in Chapter 4, was started 8 weeks following the soil sampling hence R_{OM} was purely derived from the soil-C. When live roots are present, the priming effect on R_{OM} can be either positive or negative (section 2.2.2.4) and may influence the effect of T_S on R_{OM} (Bader and Cheng, 2007). Hence, the second experiment focused on R_{OM} when plants were present.

The response of R_{OM} to T_S may also vary due to the soil's nutrient status, e.g. C and N, and with plant C inputs (Kuzyakov et al., 2000). Thus, in addition to the nutrient rich dairy farm soil (*HF* soil), used in Chapter 3, an extensively managed low fertility dairy farm soil (*LF* soil) was used in the second experiment.

When plants are present, soil CO_2 efflux (R_S) includes R_{OM} and respiration sourced from root-derived C (R_{RD}). In Chapter 4, R_S was separated into R_{OM} and R_{RD} using a natural ^{13}C abundance method.

It was concluded from this experiment that without plants, R_{OM} was equally sensitive to T_S in the nutritionally contrasting soils, with increasing T_S increasing R_{OM}. This is in agreement with the results from Chapter 3 with respect to the *HF* soil. However, when plants were present, R_{OM} was markedly less sensitive to T_S in the *LF* soil, but R_{OM} in the *HF* soil remained temperature sensitive. Further research needs to examine this interesting result to find out why the temperature sensitivity of R_{OM} was influenced by plant presence and soil fertility. Factors such as the size of soil microbial biomass, the microbial community, soil nutrient availability, and the type of root-derived C substrates might have influenced the temperature sensitivity of R_{OM}. It will be interesting to study changes in the relative microbial communities in terms of size and composition at different T_S, with/without plants in different types of soils. Both soil types had basal urea fertiliser dressings applied prior to treatment applications to ensure plant growth but clearly this did not have a unifying effect on soil microbes responsible for respiration. In the *HF* soil the old C basically behaved in a similar manner with and without plants. This may have been due to the history of management. In contrast, the quality of the soil C substrate may have been so poor in the *LF* soil that microbes were struggling to utilise it. Fig. 4.1 showed that the answer is no, as judged by the relative rates of respiration without plants present. In which case it also seems likely the soil microbial pool was of differing size and composition. As outlined above for Chapter 3, this component must be examined in future studies. It seems incongruous that in the *LF* soil that root exudate C could make such increasing contributions to the total respiration pool as T_S increased (Fig. 4.2c). It may be that root growth was covering increasing volumes of the pot as T_S increased, but it would also be interesting to know if fungal respiration increased and if it was due to hyphae being able to respond and switch rapidly to more readily available 'new C' from roots.

A suitable technique to explore the soil microbial response to plant added 'new C' would be the use of ^{13}C labelled phospholipid fatty acid signatures (PLFAs). Using the ^{13}C-PLFA analysis, changes in soil microbial communities, due to added readily available C substrates, can be monitored and the data from this analysis can be used to discuss the changes in R_{OM} response to temperature when plants were present. To follow the temporal and spatial (distance from roots) response of soil microbes to the introduction of a plant a C_3 plant could be added to a C_4 soil and vice versa with the incorporation of the newly added plant C highlighted in the microbial biomass ^{13}C signature.

Given the results of Chapter 3 and 4 it would be interesting to enhance our understanding of C availability by trying to apply a treatment that would make the recalcitrant C more available. Soil fertility presents as an influencing factor here and it would be worth investigating how a small change in soil pH of the *LF* soil, or addition of nutrients such as phosphorus might alter the soil's temperature sensitivity and the microbial community response. Especially given what appeared to be an effect of pH on increasing the priming effect observed at lower temperature in Chapter 5, where prolonged elevated pH at lower temperatures enhanced priming.

The observed changes in the temperature sensitivity of R_{OM} caused by plant presence and soil type potentially have some global significance. Ecosystem models predict that global temperature increases tend to reduce soil C storage due to a positive relationship between R_{OM} and temperature, resulting in an increase in the atmospheric concentration of CO_2 and an acceleration of climate change (e.g. Cox et al., 2000). In contrast to the *LF* soil, a relatively more rapid loss of soil C occurred as T_S increased in the *HF* soil, when plants were present. Hence, the results suggested that the warming of high productivity and high fertility soils could increase the rate of soil C loss to the atmosphere more than that from less fertile soils. However, several uncertainties make this far from a simple extrapolation. For example, high fertility soils have higher plant productivity and this compensate for the increased respiration losses. Another factor not considered in this thesis is the potential effect of elevated atmospheric CO_2 concentrations that would accompany predicted increases in global mean temperatures, and exert feed back over time.

6.1.3 Effects of bovine urine, plants and temperature on nitrous oxide and carbon dioxide emissions from a low fertility soil

In addition to soil and plant related factors, animal grazing has a major impact on R_S and N_2O emissions in pastoral ecosystems as outlined above (section 2.2.3.5). In this study, the effect of urine application, soil temperature (T_S = 11°, 19° and 23°C), and plants on soil N_2O fluxes and R_S was investigated.

Nitrous oxide emissions were higher when the WFPS was increased which indicated that denitrification was the driving mechanism. Despite the WFPS being at 70% the N_2O flux was relatively short lived after increasing the WFPS. If plants were driving denitrification by providing heterotrophs with C substrates one might have expected a more prolonged N_2O flux. The data obtained previously in Chapter 4 from the *LF* soil (Figure 4.2a) showed that the fraction of R_S derived from root-C increased substantially as T_S increased. Future experiments need to also measure the N_2 flux and a higher ^{15}N enrichment on the urine-N would enable

this and could help explain the reduction in N_2O at relatively high WFPS. Repetition of such an experiment would warrant an examination of the soil microbial pool for atom % ^{15}N and $\delta^{13}C$ in order to establish where it was resourcing its substrate supply and the relative contributions of the urine-N and plant-derived C.

A previous pilot study (data not presented here) performed as an honours project showed decreasing atom%^{15}N enrichment of the N_2O flux as T_S were increased following a urea application. This was thought to be due to mineralized soil N increasing its contribution to the N_2O flux as soil warmed. I have expected to see a similar result in the current study but in retrospect the lack of such a result may be due to soil type. The pilot study used a more fertile soil where as this study used a low fertility soil. I would like to repeat this type of study with different soil types, varying in fertility (e.g. the *HF* soil in Chapter 3 and 4) to see if the contribution of soil-N to N_2O is in fact soil specific (fertility related) and how it changes with temperature. If soil C is mineralized so too must be the soil-N it's a question of whether or not it goes into the soil microbial pool or the N_2O flux. Again though if we note the earlier result (Figure 4.2a), where R_{OM} in the *LF* soil was relatively insensitive to T_S increase, it is perhaps not surprising we saw no increase in soil-N contributions to the N_2O flux as T_S increased.

With respect to the CO_2 fluxes there was a progressive shift from an abiotic hydrolysis induced mechanism to heterotrophic mechanisms, in the urine treatments, with the shift occurring more rapidly as T_S increased. This temperature effect was strong and the use of T_S to predict this effect could be useful in future studies that try to predict the fate of C in pasture systems, given that the soil priming effect was greater at the lower T_S, which was assumed to be a function of the prolonged elevation of soil pH, which is a direct result of prolonged hydrolysis.

The isotopic $\delta^{13}C$ data clearly supported this shift in CO_2 source, in the urine treatments. In future studies however, more sampling events need be included in the programme. The use of C_3 and C_4 soils in conjunction with C_3 and C_4 pasture species provides an exciting system for research. However, it is not without possible artefacts. The grasses used here both are of similar biochemical composition compared to a ryegrass versus maize comparison, where maize has more recalcitrant C. Thus, so long as the sites have had a long history of paspalum or kikuyu (C_4 pasture species) and ryegrass (C_3 pasture species) use then the soil organic matter loadings should be similar in terms of quality. However, because the soils used here (*LF* and *HF*) were of different parent material we cannot rule out that factors other than general nutritional fertility influenced soil microbial responses to treatments applied. In an

ideal situation if the experimental 'set-up' was repeated a common parent material would be included in the soil selection criteria.

Hence Chapter 4 was performed based on the assumption that soil parent material and plant type did not affect the temperature sensitivity of R_S and I only focused on the soil nutrient status. To test the validity of this assumption, further work needs to be performed using two soils with contrasting nutrient status but from the same location and of the same soil type.

6.2 General comments

- The experiments conducted in this thesis were also constrained by the availability of growth cabinets hence, the number of treatment temperatures were 3 or 4 for each experiment. Because of the limited number of treatment temperatures, uncertainties around mean values are higher than I would have liked. For example, in Chapter 5, the highest magnitude of urine-induced priming of soil C was observed at an intermediate temperature (19°C). However, because this experiment had only three treatment temperatures, the relationship between the urine-induced priming of soil C and temperature could not be clearly described. A model was used to estimate the effect of T_S on soil processes but the uncertainties with regards to the model fitting could be reduced if future studies are performed using a higher number of treatment temperatures.

- In Chapter 3 and 4, soils were pre-conditioned for 7 days at their appropriate treatment temperature before the measurements of R_S commenced. It was reported that soil microbes could adapt to their temperature condition during a pre-conditioning period, and that this phenomena could change the apparent temperature sensitivity of R_S. Hence, further study is needed to investigate the microbial adaptation to different temperatures. However, the current studies were performed using soils with live plants hence plant growth was another factor to consider when determining the pre-conditioning period. Plants grew faster at higher temperature hence the pre-conditioning period had to be kept short to minimize the difference in plant biomass among treatment temperatures. Future studies need to consider these issues.

- The experiment in Chapter 4 was performed using two soils; the low (*LF*) and high fertility (*HF*) soils. However, the first experiment (Chapter 3) was conducted only using the *HF* soil and the third experiment (Chapter 5) was performed only using the *LF* soil. This was because the first experiment had to be started within 4 h of soil sampling and this was impossible to perform the experiment in this way using the *LF* soil, which was

obtained from 700 km north of Lincoln University, where I performed the experiment. Also, for the first experiment, because locally available cow urine was only C_3 labelled, I only had the option of using the *LF* soil, which was a C_4 soil to investigate the contribution of urine-C to soil respiration. Thus, further work can be performed, by developing the third experiment, to investigate the magnitude of urine-induced priming effect in a high fertility soil.

References

Abdalla, M., M. Jones, P. Smith, and M. Williams. 2009. Nitrous oxide fluxes and denitrification sensitivity to temperature in Irish pasture soils. Soil Use and Management 25:376-388.

Allard, V., C. Robin, P.C.D. Newton, M. Lieffering, and J.F. Soussana. 2006. Short and long-term effects of elevated CO_2 on Lolium perenne rhizodeposition and its consequences on soil organic matter turnover and plant N yield. Soil Biology & Biochemistry 38:1178-1187.

Allard, V., P.C.D. Newton, M. Lieffering, J.F. Soussana, R.A. Carran, and C. Matthew. 2005. Increased quantity and quality of coarse soil organic matter fraction at elevated CO_2 in a grazed grassland are a consequence of enhanced root growth rate and turnover. Plant and Soil 276:49-60.

Amador, J.A., and R.D. Jones. 1993. Nutrient limitations on microbial respiration in peat soils with different total phosphorus content. Soil Biology & Biochemistry 25:793-801.

Ambus, P., S.O. Peterson, and J.F. Soussana. 2007. Short-term carbon and nitrogen cycling in urine patches assessed by combined carbon-13 and nitrogen-15 labelling. Agriculture, Ecosystems & Environment 121:84-92.

Amundson, R. 2001. The carbon budget in soils. Annual Review of Earth and Planetary Sciences 29:535-561.

Anderson, I.C., and J.S. Levine. 1986. Relative rates of nitric oxide and nitrous oxide production by nitrifiers, denitrifiers, and nitrate respirers. Applied Environmental Microbiology 51:938-945.

Anderson, T.W., and D.A. Darling. 1952. Asymptotic theory of certain "goodness of fit" criteria based on stochastic processes. The Annals of Mathematical Statistics 23:193-212.

Arrhenius, S. 1898. The effect of constant influences upon physiological relationships. Scandinavian Archives of Physiology 8:367-415.

Atkin, O.K., D. Bruhn, V.M. Hurry, and M.G. Tjoelker. 2005. The hot and the cold: unravelling the variable response of plant respiration to temperature. Functional Plant Biology 32:87-105.

Avalakki, U.K., W.M. Strong, and P.G. Saffigna. 1995. Measurements of gaseous emissions from denitrification of applied nitrogen-15. II. Effects of temperature and added straw. Australian Journal of Soil Research 33:89-99.

Avnimelech, Y., and M. Laher. 1977. Ammonia Volatilization From Soils: Equilibrium Considerations. Soil Science Society of America Journal 41:1080-1084.

Azam, F., C. Müller, A. Weiske, G. Benckiser, and J.C.G. Ottow. 2002. Nitrification and denitrification as sources of atmospheric nitrous oxide – role of oxidizable carbon and applied nitrogen. Biology and Fertility of Soils 35:54-61.

Bader, N.E., and W. Cheng. 2007. Rhizosphere priming effect of Populus fremontii obscures the temperature sensitivity of soil organic carbon respiration. Soil Biology & Biochemistry 39:600-606.

Bailey, L.D. 1976. Effects of temperature and root on denitrification in a soil. Canadian Journal of Soil Science 56:79-87.

Balesdent, J., A. Mariotti, and B. Guillet. 1987. Natural ^{13}C abundance as a tracer for studies of soil organic matter dynamics. Soil Biology & Biochemistry 19:25-30.

Bardgett, R.D., and D.A. Wardle. 2003. Herbivore-Mediated Linkages between Aboveground and Belowground Communities. Ecology 84:2258-2268.

Bardgett, R.D., C. Freeman, and N.J. Ostle. 2008. Microbial contributions to climate change through carbon cycle feedbacks. Multidisciplinary Journal of Microbial Ecology 2:805-814.

Bardgett, R.D., R.D. Lovell, P.J. Hobbs, and S.C. Jarvis. 1999. Seasonal changes in soil microbial communities along a fertility gradient of temperate grasslands. Soil Biology & Biochemistry 31:1021-1030.
Bekku, Y.S., T. Nakatsubo, A. Kume, M. Adachi, and H. Koizumi. 2003. Effect of warming on the temperature dependence of soil respiration rate in arctic, temperate and tropical soils. Applied Soil Ecology 22:205-210.
Bender, M.M. 1968. Mass spectrometric studies of carbon-13 variations in corn and other grasses. Radiocarbon 10:468-472.
Berg, B., B. Wessén, and G. Ekbohm. 1982. Nitrogen Level and Decomposition in Scots Pine Needle Litter. Oikos 38:291-296.
Bingeman, C.W., J.E. Varner, and W.P. Martin. 1953. The effect of the addition of organic materials on the decomposition of an organic soil. Soil Science Society of America Proceedings 29:692-696.
Blackmer, A.M., and J.M. Bremner. 1977. Nitrification of fertilizer nitrogen in soils as a source of atmospheric N_2O. Agronomy Abstract:146.
Blagodatskaya, E., and Y. Kuzyakov. 2008. Mechanisms of real and apparent priming effects and their dependence on soil microbial biomass and community structure: critical review. Biology and Fertility of Soils 45:115-131.
Bokhari, U.G., and J.S. Singh. 1974. Effects of Temperature and Clipping on Growth, Carbohydrate Reserves, and Root Exudation of Western Wheatgrass in Hydroponic Culture. Crop Science 14:790-794.
Bol, R., T. Bolger, and T. Cully. 2003. Recalcitrant soil organic materials mineralize more efficiently at higher temperatures. Journal of Plant Nutrition and Soil Science 166:300-307.
Bol, R., S.O. Petersen, C. Christofides, K. Dittert, and M.N. Hansen. 2004. Short-term N_2O, CO_2, NH_3 fluxes, and N/C transfers in a Danish grass-clover pasture after simulated urine deposition in autumn. Journal of Plant Nutrition and Soil Science 167:568-576.
Bond-Lamberty, B., C. Wang, and S.T. Gower. 2004. A global relationship between the heterotrophic and autotrophic components of soil respiration? Global Change Biology 10:1756-1766.
Bosatta, E., and G.I. Ågren. 1999. Soil organic matter quality interpreted thermodynamically. Soil Biology & Biochemistry 31:1889-1891.
Boutton, T.W., S.R. Archer, and A.J. Midwood. 1999. Stable isotopes in ecosystem science: structure, function and dynamics of a subtropical savanna. Rapid Communications in Mass Spectrometry 13:1263-1277.
Bouwman, A.F. 1998. Environmental science - Nitrogen oxides and tropical agriculture. Nature 392:866-867.
Bowling, D.R., S.D. Sargent, B.D. Tanner, and J.R. Ehleringer. 2003. Tunable diode laser absorption spectroscopy for stable isotope studies of ecosystem-atmosphere CO_2 exchange. Agricultural and Forest Meteorology 118:1-19.
Boyer, J.N., and P.M. Groffman. 1996. Bioavailability of water extractable organic carbon fractions in forest and agricultural soil profiles. Soil Biology & Biochemistry 28:783-790.
Bradford, M.A., C.A. Davies, S.D. Frey, T.R. Maddox, J.M. Melillo, J.E. Mohan, J.F. Reynolds, K.K. Treseder, and M.D. Wallenstein. 2008. Thermal adaptation of soil microbial respiration to elevated temperature. Ecology Letters 11:1316-1327.
Bremer, E., and P. Kuikman. 1997. Influence of competition for nitrogen in soil on net mineralization of nitrogen. Plant and Soil 190:119-126.
Bremner, J.M., and K. Shaw. 1958. Denitrification in soil. II. Factors affecting denitrification. Journal of Agricultural Science 51:40-52.

Bronson, K.F., G.P. Sparling, and I.R.P. Fillery. 1999. Short-term N dynamics following application of ^{15}N-labeled urine to a sandy soil in summer. Soil Biology & Biochemistry 31:1049-1057.

Brown, M., D. Whitehead, J.E. Hunt, T.J. Clough, G.C. Arnold, W.T. Baisden, and R.R. Sherlock. 2009. Regulation of soil surface respiration in a grazed pasture in New Zealand. Agricultural and Forest Meteorology 149:205-213.

Burford, J.R., and J.M. Bremner. 1975. Relationships between the denitrification capacities of soils and total, water-soluble and readily decomposable soil organic matter. Soil Biology & Biochemistry 7:389-394.

Burton, A.J., K.S. Pregitzer, and R.L. Hendrick. 2000. Relationships between fine root dynamics and nitrogen availability in Michigan northern hardwood forests. Oecologia 125:389-399.

Bussink, D.W., and O. Oenema. 1998. Ammonia volatilization from dairy farming systems in temperate areas: a review. Nutrient Cycling in Agroecosystem 51:19-33.

Butler, J.L., P.J. Bottomley, S.M. Griffis, and D.D. Myrold. 2004. Distribution and turnover of recently fixed photosynthate in ryegrass rhizospheres. Soil Biology & Biochemistry 36:371-382.

Buyer, J.S., D.P. Roberts, and E. Russek-Cohen. 2002. Soil and plant effects on microbial community structure. Canadian Journal of Microbiology 48:955-964.

Bååth, E., and H. Wallander. 2003. Soil and rhizosphere microorganisms have the same Q_{10} for respiration in a model system. Global Change Biology 9:1788-1791.

Cabrera, M.L., and D.E. Kissel 1989. Review and simplification of calculations in ^{15}N tracer studies. Fertilizer Research 20:11-15.

Carter, M., K. Klumpp, and X. Le Roux. 2006. Lack of increased availability of root-derived C may explain the low N_2O emission from low N-urine patches. Nutrient Cycling in Agroecosystems 75:91-100.

Carter, M.S. 2007. Contribution of nitrification and denitrification to N_2O emissions from urine patches. Soil Biology & Biochemistry 39:2091-2102.

Chalamat, A. 1985. Effects of environmental factors on denitrification, p. 7-29, *In* H. L. Golterman, ed. Denitrification in the Nitrogen Cycle. Plenum Press, New York.

Cheng, W., D.W. Johnson, and S. Fu. 2003. Rhizosphere effects on decomposition: controls of plant species, phenology, and fertilization. Soil Science Society of America Journal 67:1418-1427.

Cheng, W.X. 1996. Measurement of rhizosphere respiration and organic matter decomposition using natural C-13. Plant and Soil 183:263-268.

Christensen, J.H., B. Hewitson, A. Busuioc, A. Chen, X. Gao, I. Held, R. Jones, R.K. Kolli, W.-T. Kwon, R. Laprise, V. Magaña Rueda, L. Mearns, C.G. Menéndez, J. Räisänen, A. Rinke, A. Sarr and P. Whetton. 2007. Regional Climate Projections *In* S. Solomon, D. Qin, M. Manning, Z. Chen, M. Marquis, K.B. Averyt, M. Tignor and H.L. Miller, ed. Climate Change 2007: The Physical Science Basis. Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA.

Christensen, T.R., S. Jonasson, T.V. Callaghan, and M. Havström. 1999. On the potential CO_2 release from tundra soils in a changing climate. Applied Soil Ecology 11:127-134.

Clough, T.J., and F.M. Kelliher. 2005. Dairy farm effluent effects on urine patch nitrous oxide and carbon dioxide emissions. Journal of Environmental Quality 34:979-986.

Clough, T.J., R.R. Sherlock, and F.M. Kelliher. 2003. Can liming mitigate N_2O fluxes from a urine-amended soil? Australian Journal of Soil Research 41:439-457.
Clough, T.J., R.R. Sherlock, K.C. Cameron, and S.F. Ledgard. 1996. Fate of urine nitrogen on mineral and peat soils in New Zealand. Plant and Soil 178:141-152.
Clough, T.J., S.F. Ledgard, M.S. Sprosen, and M.J. Kear. 1998. Fate of ^{15}N labelled urine on four soil types. Plant and Soil 199:195-203.
Clough, T.J., F.M. Kelliher, R.R. Sherlock, and C.D. Ford. 2004. Lime and soil moisture effects on nitrous oxide emissions from a urine patch. Soil Science Society of America Journal 68:1600-1609.
Clough, T.J., J.L. Ray, L.E. Buckthought, J. Calder, D. Baird, M. O'Callaghan, R.R. Sherlock, and L.M. Condron. 2009. The mitigation potential of hippuric acid on N_2O emissions from urine patches: An in situ determination of its effect. Soil Biology & Biochemistry 41:2222-2229.
Conen, F., J. Leifeld, B. Seth, and C. Alewell. 2006. Warming mineralises young and old soil carbon equally. Biogeosciences 3:515-519.
Conrad, R. 1996. Soil microorganisms as controllers of atmospheric trace gases (H_2, CO, CH_4, OCS, N_2O, and NO). Microbiology and Molecular Biology Reviews 60:609-640.
Cornish, P.S. 1993. Soil macrostructure and root growth of establishing seedlings. Plant and Soil 151:119-126.
Cox, P.M., R.A. Betts, C.D. Jones, S.A. Spall, and I.J. Totterdell. 2000. Acceleration of global warming due to carbon-cycle feedbacks in a coupled climate model. Nature 408:184-187.
Craine, J.M., D.A. Wedin, and F.S. Chapin. 1999. Predominance of ecophysiological controls on soil CO_2 flux in a Minnesota grassland. Plant and Soil 207:77-86.
Crutzen, P.J. 1970. The influence of nitrogen oxides on the atmospheric ozone content. Quarterly Journal of Royal Meteorological Society 96:320-325.
Davidson, E.A., and A.R. Mosier. 2004. Controlling losses to air, p. 251-259, *In* D. J. Hatch, et al., eds. Controlling nitrogen flows and losses. Wageningen Academic Publishers, Wageningen.
Davidson, E.A., and I.A. Janssens. 2006. Temperature sensitivity of soil carbon decomposition and feedbacks to climate change. Nature 440:165-173.
Davidson, E.A., L.F. Galloway, and M.K. Strand. 1987. Assessing available carbon: Comparison of techniques across selected forest soils. Communications in Soil Science and Plant Analysis 18:45-64.
Davidson, E.A., I.A. Janssens, and Y. Luo. 2006. On the variability of respiration in terrestrial ecosystems: moving beyond Q_{10}. Global Change Biology 12:154-164.
Day, T.A., and J.K. Detling. 1990. Grassland Patch Dynamics and Herbivore Grazing Preference Following Urine Deposition. Ecology 71:180-188.
de Graaff, M.A., K.J. van Groenigen, J. Six, B. Hungatte, and C. van Kessel. 2006. Interactions between plant growth and soil nutrient cycling under elevated CO_2: a meta-analysis. Global Change Biology 12:2077-2091.
de Klein, C.A.M., and R.S.P. Van Logtestijn. 1996. Denitrification in grassland soils in the netherlands in relation to irrigation, N-application rate, soil water content and soil temperature. Soil Biology & Biochemistry 28:231-237.
de Klein, C.A.M., R.R. Sherlock, K.C. Cameron, and T.J. van der Weerden. 2001. Nitrous oxide emissions from agricultural soils in New Zealand - a review of current knowledge and directions for future research. Journal of The Royal Society of New Zealand 31:543-574.

Delwiche, C.C., and B.A. Bryan. 1976. Denitrification. Annual Review of Microbiology 30:241-262.

Denman, K.L., G. Brasseur, A. Childthaisong, P. Ciais, P.M. Cox, R.E. Dickinson, D. Hauglustaine, C. Heinze, E. Holland, D. Jacob, U. Lohmann, S. Ramachandran, P.L. da Silva Dias, S.C. Wofsy, and X. Zhang. 2007. Couplings Between Changes in the Climate System and Biogeochemistry., *In* S. Solomon, et al., eds. Climate Change 2007: The Physical Science Basis. Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA.

Dijkstra, F.A., W. Cheng, and D.W. Johnson. 2006. Plant biomass influences rhizosphere priming effects on soil organic matter decomposition in two differently managed soils. Soil Biology & Biochemistry 38:2519-2526.

Doak, B.W. 1952. Some chemical changes in the nitrogenous constituents of urine when voided on pasture. Journal of Agricultural Science 42:162-171.

Domanski, G., Y. Kuzyakov, S.V. Siniakina, and K. Stahr. 2001. Carbon flows in the rhizosphere of ryegrass (*Lolium perenne*). Journal of Plant Nutrition and Soil Science 164:381-387.

Dormaar, J.F. 1990. Effects of active roots on the decomposition of soil organic materials. Biological Fertility of Soils 10:121-126.

During, C., and K.J. McNaught. 1961. Effects of cow urine on growth of pasture and uptake of nutrients. New Zealand Journal of Agricultural Research 4:591-605.

Ekblad, A., and P. Högberg. 2001. Natural abundance of ^{13}C in CO_2 respired from forest soils reveals speed of link between tree photosynthesis and root respiration. Oecologia 127:305-308.

Elgersma, A., and J. Hassink. 1997. Effects of white clover (*Trifolium repens* L.) on plant and soil nitrogen and soil organic matter in mixtures with perennial ryegrass (*Lolium perenne* L.). Plant and Soil 197:177-186.

Fang, C., and J.B. Moncrieff. 2001. The dependence of soil CO_2 efflux on temperature. Soil Biology & Biochemistry 33:155-165.

Feng, X., and M.J. Simpson. 2009. Temperature and substrate controls on microbial phospholipid fatty acid composition during incubation of grassland soils contrasting in organic matter quality. Soil Biology & Biochemistry 41:804-812.

Firestone, M.K. 1982. Biological denitrification. Agronomy Monograph 22:289-326.

Fitter, A.H., J.D. Graves, G.K. Self, T.K. Brown, D.S. Bogie, and K. Taylor. 1998. Root production, turnover and respiration under two grassland types along an altitudinal gradient: influence of temperature and solar radiation. Oecologia 114:20-30.

Flanagan, L.B., and B.G. Johnson. 2005. Interacting effects of temperature, soil moisture and plant biomass production on ecosystem respiration in a northern temperate grassland. Agricultural and Forest Meteorology 130:237-253.

Flechard, C.R., P. Ambus, U. Skiba, R.M. Rees, A. Hensen, A. van Amstel, A. van den Pol-van Dassellar, J.F. Soussana, M. Jones, J. Clifton-Brown, A. Raschi, L. Horvath, A. Neftel, M. Jocher, C. Ammann, J. Leifeld, J. Furhrer, P. Calance, E. Thalman, K. Pilegaard, C. Di Marco, C. Campbell, E. Nemitz, K.J. Hargreaves, P.E. Levy, B.C. Ball, S.K. Jones, W.C.M. van de Bulk, T. Groot, M. Blom, R. Domingues, G. Kasper, V. Allard, E. Ceschia, P. Cellier, P. Laville, C. Henault, F. Bizouard, M. Abdalla, M. Williams, S. Baronti, F. Berretti, and B. Grosz. 2007. Effects of climate and management intensity on nitrous oxide emissions in grassland systems across Europe. Agriculture, Ecosystems & Environment 121:135-152.

Flessa, H., R. Ruser, P. Dörsch, T. Kamp, M.A. Jimenez, J.C. Munch, and F. Beese. 2002. Integrated evaluation of greenhouse gas emissions (CO_2, CH_4, N_2O) from two farming systems in southern Germany. Agriculture, Ecosystems & Environment 91:175-189.

Focht, D.D., and W. Verstraete. 1977. Biochemical ecology of nitrification and denitrification. Advanced Microbial Ecology 1:135-214.

Follett, R.F., J.M. Kimble, and R. Lal. 2001. The potential of U.S. grazing lands to sequester soil carbon, p. 401-432, *In* R. F. Follett, et al., eds. The Potential of U.S. Grazing Lands to Sequester Carbon and Mitigate the Greenhouse Effect. CRC Press, United States of America.

Fontaine, S., A. Mariotti, and L. Abbadie. 2003. The priming effect of organic matter: a question of microbial competition? Soil Biology & Biochemistry 35:837-843.

Forster, P., V. Ramaswamy, P. Artaxo, T. Berntsen, R. Betts, D.W. Fahey, J. Haywood, J. Lean, D.C. Lowe, G. Myhre, J. Nganga, R. Prinn, G. Raga, S. Mand, and R. Van Dorland. 2007. Changes in Atmospheric Constituents and in Radiative Forcing, *In* S. Solomon, et al., eds. Climate Change 2007: The Physical Science Basis. Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA.

Fowler, D., U. Skiba, and K.J. Hargreaves. 1997. Emissions of nitrous oxide from grasslands., p. 147-164, *In* S. C. Jarvis and B. F. Pain, eds. Gaseous Nitrogen Emissions from Grasslands. Cab International, Wallingford, UK.

Gao, J.-Q., H. Ouyang, X.-L. Xu, C.-P. Zhou, and F. Zhang. 2009. Effects of Temperature and Water Saturation on CO_2 Production and Nitrogen Mineralization in Alpine Wetland Soils. Pedosphere 19:71-77.

Garwood, E.A. 1967. Studies on the roots of grasses. Annual Report 1966, Grassland Research Institute, Hurley:72-9.

Gaudinski, J.B., S.E. Trumbore, E.A. Davidson, and S. Zheng. 2000. Soil carbon cycling in a temperate forest: radiocarbon-based estimates of residence times, sequestration rates and partitioning of fluxes. Biogechemistry 51:33-69.

Ghani, A., M. Dextor, and K.W. Perrott. 2003. Hot-water extractable carbon in soils: a sensitive measurement for determining impacts of fertilisation, grazing and cultivation. Soil Biology & Biochemistry 35:1231-1243.

Grable, A.R. 1971. Effects of compaction on content and transmission of air in soils, p. 154-164, *In* K. K. Barnes, et al., eds. Compaction of Agricultural Soils. the American Society of Agricultural Engineers, St. Joseph.

Gu, L., W.M. Post, and A.W. King. 2004. Fast labile carbon turnover obscures sensitivity of heterotrophic respiration from soil to temperature: a model analysis. Global Biogeochemical Cycles 18:1022-1032.

Gödde, M., and R. Conrad. 1999. Immediate and adaptational temperature effects on nitric oxide production and nitrous oxide release from nitrification and denitrification in two soils. Biology and Fertility of Soils 30:33-40.

Hadas, A., S. Feigenbaum, A. Feigin, and R. Portnoy. 1986. Distribution of nitrogen forms and availability indices in profiles of differently managed soil types. Soil Science Society of America Journal 50:308-313.

Hamer, U., and B. Marschner. 2005. Priming effects in different soil types induced by fructose, alanine, oxalic acid and catechol additions. Soil Biology & Biochemistry 37:445-454.

Hanson, P.J., N.T. Edwards, C.T. Garten, and J.A. Andrews. 2000. Separating root and soil microbial contributions to soil respiration: a review of methods and observations. Biogechemistry 48:115-146.

Hantschel, R.E., T. Kamp, and F. Beese. 1995. Increasing the soil temperature to study global warming effects on the soil nitrogen cycle in agroecosystems. Journal of Biogeography 22:375-380.
Hartley, I.P., and P. Ineson. 2008. Substrate quality and the temperature sensitivity of soil organic matter decomposition. Soil Biology & Biochemistry 40:1567-1574.
Hartley, I.P., D.W. Hopkins, M.H. Garnett, M. Sommerkorn, and P.A. Wookey. 2008. Soil microbial respiration in arctic soil does not acclimate to temperature. Ecology Letters 11:1092-1100.
Hauck, R.D., and S.W. Melstead. 1956. Some aspects of the problem of evaluating denitrification in soils. Soil Science Society of America Proceedings 20:361-364.
Haynes, R.J. 2000. Labile organic matter as an indicator of organic matter quality in arable and pastoral soils in New Zealand. Soil Biology & Biochemistry 32:211-219.
Haynes, R.J., and P.H. Williams. 1993. Nutrient cycling and soil fertility in the grazed pasture ecosystem. Advances in Agronomy 49:119-199.
Heilmeier, H., M. Erhard, and E.D. Schulze. 1997. Biomass allocation and water use under arid conditions., p. 93-112, *In* F. A. Bazzaz and J. Grace, eds. Plant Resource Allocation. Academic Press, Sandiego, CA.
Hewitt, A.E. 1993. New Zealand Soil Classification. Landcare Research Science Series No. 1., Lincoln, New Zealand.
Hobbie, S.E., and P.M. Vitousek. 2000. Nutrient limitation of decomposition in Hawaiian forests. Ecology 81:1867-1877.
Hoschstein, L.T., and G.A. Tomlinson. 1988. The enzymes associated with denitrification. Annual Review of Microbiology 42:231-261.
Houghton, R.A., and G.M. Woodwell. 1989. Global climate change. Scientific American 260:36-44.
Howard, D.M., and P.J.A. Howard. 1993. Relationships between CO_2 evolution, moisture content and temperature for a range of soil types. Soil Biology & Biochemistry 25:1537-1546.
Humphreys, L.R. 1997. The Evolving Science of Grassland Improvement Cambridge University Press, U.K.
Hütsch, B.W., J. Augustin, and W. Merbach. 2002. Plant rhizodeposition - an important source for carbon turnover in soils. Journal of Plant Nutrition and Soil Science 165:397-407.
IPCC. 2007a. Climate Change 2007: Synthesis Report. Contribution of Wroking Groups I, II and III to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change [Core Writing Team, Pachauri, R.K and Reisinger, A. (eds)]. IPCC, Geneva, Switzerland.
IPCC. 2007b. Climate Change 2007: The Physical Science Basis. Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change, *In* S. Solomon, et al., eds. Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA.
Islam, A., D. Chen, and R.E. White. 2007. Heterotrophic and autotrophic nitrification in two acid pasture soils. Soil Biology & Biochemistry 39:972-975.
Jacobson, S.N., and M. Alexander. 1980. Nitrate loss from soil in relation to temperature, carbon source and denitrifier populations. Soil Biology & Biochemistry 12:501-505.
Jandl, R., and P. Sollins. 1997. Water-extractable soil carbon in relation to the belowground carbon cycle. Biological Fertility of Soils 25:196-201.

Joergensen, R.G., P.C. Brookes, and D.S. Jenkinson. 1990. Survival of the soil microbial biomass at elevated temperatures. Soil Biology & Biochemistry 22:1129-1136.
John, P. 1977. Aerobic and anaerobic bacterial respiration monitored by electrodes. Journal of General Microbiology 98:231-238.
Johnson, K.A., and D.E. Johnson. 1995. Methane emissions from cattle. Journal of Animal Science 73:2483-2492.
Johnson, M.G., E.E. Levine, and J.S. Kern. 1995. Soil organic matter, distribution, genesis, and management to reduce greenhouse gas emissions. Water, Air, and Soil Pollution 82:593-615.
Keeney, D.R., I.R. Fillery, and G.P. Marx. 1979. Effect of Temperature on the Gaseous Nitrogen Products of Denitrification in a Silt Loam Soil. Soil Science Society of America Journal 43:1124-1128.
Kelliher, F.M., M.M. Barbour, and J.E. Hunt. 2005a. Sucrose application, soil microbial respiration and evolved carbon dioxide isotope enrichment under contrasting land uses. Plant and Soil 268:233-242.
Kelliher, F.M., J.R. Sedcole, I. Emery, and L.M. Condron. 2007. Grassland soil microbial respiration responses to urea and litter applications. New Zealand Journal of Agricultural Research 50:321-326.
Kelliher, F.M., A.R. Reisinger, R.J. Martin, M.J. Harvey, S.J. Price, and R.R. Sherlock. 2002. Measuring nitrous oxide emission rate from grazed pasture using Fourier-transform infrared spectroscopy in the nocturnal boundary layer. Agricultural and Forest Meteorology 111:29-38.
Kelliher, F.M., J.R. Sedcole, R.F. Minchin, Y. Wan, L.M. Condron, T.J. Clough, and R. Bol. 2005b. Soil microbial respiration responses to repeated urea applications in three grasslands. Australian Journal of Soil Research 43:905-913.
Khalil, M.I., and E.M. Baggs. 2005. CH_4 oxidation and N_2O emissions at varied soil water-filled pore spaces and headspace CH_4 concentrations. Soil Biology & Biochemistry 37:1785-1794.
Khdyer, I.I., and C.M. Cho. 1983. Nitrification and denitrification of nitrogen fertilizers in a soil column. . Soil Science Society of America Journal 47:1134-1139.
Kirschbaum, M.U.F. 1995. The temperature dependence of soil organic matter decomposition, and the effect of global warming on soil organic C storage. Soil Biology & Biochemistry 27:753-760.
Kirschbaum, M.U.F. 2004. Soil respiration under prolonged soil warming: are rate reductions caused by acclimation or substrate loss? Global Change Biology 10:1870-1877.
Kirschbaum, M.U.F. 2006. The temperature dependence of organic-matter decomposition - still a topic of debate. Soil Biology & Biochemistry 38:2510-2518.
Kladivko, E.J., and D.R. Keeney. 1987. Soil nitrogen mineralization as affected by water and temperature interactions. Biology and Fertility of Soils 5:248-252.
Klemedtsson, L., B.H. Svensson, and T. Rosswall. 1988. Relationship between soil moisture content and nitrous oxide production during nitrification and denitrification. Biology and Fertility of Soils 6:106-111.
Koch, O., D. Tscherko, and E. Kandeler. 2007. Temperature sensitivity of microbial respiration, nitrogen mineralization, and potential soil enzyme activities in organic alpine soils. Global Biogeochemical Cycles 21:GB4017.
Koepf, H. 1953. Die Temperatur/Zeit - Abhangigkeit der Bodenatmung. Zeitschrift fur Pflanzenernahrung. Dungung und Bodenkunde 61:29-48.

Kononova, M.M. 1966. Soil Organic Matter: Its Nature, Its Role in Soil Formation and in Soil Fertility. Pergamon, Oxford.
Kortschak, H.P., C.E. Hartt, and G.O. Burr. 1965. Carbon dioxide fixation in sugar cane leaves. Plant Physiology 40:209-213.
Kuzyakov, Y. 2002. Review: Factors affecting rhizosphere priming effects. Journal of Plant Nutrition and Soil Science 165:382-396.
Kuzyakov, Y. 2004. Separation of root and rhizomicrobial respiration by natural ^{13}C abundance: theoretical approach, advantages, and difficulties. Eurasian Soil Science 37:S79-S84.
Kuzyakov, Y. 2006. Sources of CO_2 efflux from soil and review of partitioning methods. Soil Biology & Biochemistry 38:425-448.
Kuzyakov, Y., and W. Cheng. 2001a. Photosynthesis controls of rhizosphere respiration and organic matter decomposition. Soil Biology & Biochemistry 33:1915-1925.
Kuzyakov, Y., and W. Cheng. 2001b. Photosynthesis controls of rhizosphere respiration and organic matter decomposition. Soil Biology and Biochemistry 33:1915-1925.
Kuzyakov, Y., and R. Bol. 2006. Sources and mechanisms of priming effect induced in two grassland soils amended with slurry and sugar. Soil Biology & Biochemistry 38:747-758.
Kuzyakov, Y., A. Kretzschmar, and K. Stahr. 1999. Contribution of *Lolium perenne* rhizodeposition to carbon turnover of pasture soil. Plant and Soil 213:127-136.
Kuzyakov, Y., J.K. Friedel, and K. Stahr. 2000. Review of mechanisms and quantification of priming effects. Soil Biology and Biochemistry 32:1485-1498.
Kuzyakov, Y., H. Ehrensberger, and K. Stahr. 2001. Rhizodeposition of *Lolium perenne* on two nitrogen levels and enhance of humus decomposition. Soil Biology & Biochemistry 33:61-75.
Kyveryga, P.M., A.M. Blackmer, J.W. Ellsworth, and R. Isla. 2004. Soil pH effects on nitrification of fall-applied anhydrous ammonia. Soil Science Society of America Journal 68:545-551.
Larionova, A., D. Sapronov, V. Lopez de Gerenyu, L. Kuznetsova, and V. Kudeyarov. 2006. Contribution of plant root respiration to the CO_2 emission from soil. Eurasian Soil Science 39:1127-1135.
Ledgard, S.F., K.W. Steele, and W.H.M. Saunders. 1982. Effects of cow urine and its major constituents on pasture properties. New Zealand Journal of Agricultural Research 25:61-68.
Leininger, S., T. Urich, M. Schloter, L. Schwark, J. Qi, G.W. Nicol, J.I. Prosser, S.C. Schuster, and C. Schleper. 2006. Archaea predominate among ammonia-oxidizing prokaryotes in soils. Nature London 442:806-809.
Letey, J., N. Valoras, A. Hadas, and D.D. Focht. 1980. Effect of air-filled porosity, nitrate concentration, and time on the ratio of N_2O/N_2 evolution during denitrification. Journal of Environmental Quality 9:227-231.
Li, C., S. Frolking, and T.A. Frolking. 1992. A model of nitrous oxide evolution from soil driven by rainfall events: 1. Model structure and sensitivity. Journal of Geophysical Research 97:9759-9776.
Li, C., V. Narayanan, and R.C. Harriss. 1996. Model estimates of nitrous oxide emissions from agricultural lands in the United States. Global Biogeochemical Cycles 10:297-396.
Liljeroth, E., P. Kuikman, and J.A. Van Veen. 1994. Carbon translocation to the rhizosphere of maize and wheat and influence on the turn-over of native soil organic matter at different soil nitrogen levels. Plant and Soil 161:233-240.

Lin, G., J.R. Ehleringer, P.T. Rygiewicz, M.G. Johnson, and D.T. Tingey. 1999. Elevated CO_2 and temperature impacts on different components of soil CO_2 efflux in Douglas-fir terracosms. Global Change Biology 5:157-168.
Linn, D.M., and J.W. Doran. 1984. Effect of water-filled pore space on carbon dioxide and nitrous oxide production in tilled and nontilled soils. Soil Science Society of America Journal 48:1267-1272.
Liu, H.S., H.L. Li, X.G. Han, J.H. Huang, J.X. Sun, and H.Y. Wang. 2006. Respiratory substrate availability plays a crucial role in the response of soil respiration to environmental factors. Applied Soil Ecology 32:284-292.
Lloyd, J., and J.A. Taylor. 1994. On the temperature dependence of soil respiration. Functional Ecology 8:315-323.
Lloyd, J., and G.D. Farquhar. 1996. The CO2 dependence of photosynthesis, plant growth responses to elevated atmospheric CO2 concentrations and their interaction with soil nutrient status. I. general principles and forest ecosystems. Functional Ecology 10:4-32.
Lloyd, J., and G.D. Farquhar. 2008. Effects of rising temperatures and [CO2] on the physiology of tropical forest trees. Philosophical Transactions of the Royal Society B 363:1811-1817.
Long, S.P. 1999. Environmental responses, p. 215-250, *In* R. F. Sage and R. K. Monson, eds. C4 Plant Biology. Academic Press, San Diego.
Lovell, R.D., and S.C. Jarvis. 1996. Effects of urine on soil microbial biomass, methanogenesis, nitrification and denitrification in grassland soils. Plant and Soil 186:265-273.
Lovell, R.D., S.C. Jarvis, and R.D. Bardgett. 1995. Soil microbial biomass and activity in long-term grassland: effects of management changes. Soil Biology & Biochemistry 27:969-975.
Lucht, W., I.C. Prentice, R.B. Myneni, S. Sitch, P. Friedlingstein, W. Cramer, P. Bousquet, W. Buermann, and B. Smith. 2002. Climatic control of the high-latitude vegetation greening trend and pinatubo effect. Science 296:1687-1689.
Lueken, H., W.L. Hutcheon, and E.A. Paul. 1962. The influence of nitrogen on the decomposition of crop residues in the soil. Canadian Journal of Soil Science 42:276-288.
Luo, J., S.B. Lindsey, and S.F. Ledgard. 2008. Nitrous oxide emissions from animal urine application on a New Zealand pasture. Biological Fertility of Soils 44:463-470.
Luo, Y. 2007. Terrestrial carbon-cycle feedback to climate warming. Annual Review of Ecology, Evolution, and Systematics 38:683-712.
Luo, Y., and X. Zhou. 2006. Soil respiration and the environment Academic Press, Oxford, UK.
Luo, Y., S. Wan, D. Hui, and L.L. Wallace. 2001. Acclimatization of soil respiration to warming in a tall grass prairie. Nature 413:622-625.
Lynch, J.M., and J.M. Whipps. 1990. Substrate flow in the rhizosphere. Plant and Soil 129:1-10.
Lükewille, A., and R.F. Wright. 1997. Experimentally increased soil temperature causes release of nitrogen at a boreal forest catchment in southern Norway. Global Change Biology 3:13-21.
Maag, M., and F.P. Vinther. 1996. Nitrous oxide emission by nitrification and denitrification in different soil types and at different soil moisture contents and temperatures. Applied Soil Ecology 4:5-14.
Manning, P., M. Saunders, R.D. Bardgett, M. Bonkowski, M.A. Bradford, R.J. Ellis, E. Kandeler, S. Marhan, and D. Tscherko. 2008. Direct and indirect effects of

nitrogen deposition on litter decomposition. Soil Biology & Biochemistry 40:688-698.
Matson, P.A., and P.M. Vitousek. 1990. Ecosystem approach to a global nitrous oxide budget. BioScience 40:667-672.
Mattew, C., and J.Z. Yang. 1998. Determination of tiller and root appearance in perennial ryegrass (*Lolium perenne*) swards by observation of the tiller axis, and potential application in mechanistic modelling. New Zealand Journal of Agricultural Research 41:1-10.
Mawdsley, J.L., and R.D. Bardgett. 1997. Continuous defoliation of perennial ryegrass (*Lolium perenne*) and white clover (*Trifolium repens*) and associated changes in the microbial population of an upland grassland soil. Biology and Fertility of Soils 24:52-58.
McKenney, D.H., K.F. Shuttleworth, and W.I. Frindlay. 1980. Temperature dependence of nitrous oxide production from brookston clay. Canadian Journal of Soil Science 60:665-674.
McKenzie, B.A., P.D. Kemp, D.J. Moot, C. Matthew, and R.J. Lucas. 1999. Environmental effects on plant growth and development, p. 29-44, *In* J. White and J. Hodgson, eds. New Zealand Pasture and Crop Science. Oxford University Press, New York.
Meehl, G.A., T.F. Stocker, W.D. Collins, P. Friendlingstein, A.T. Gaye, J.M. Gregory, A. Kitoh, R. Knutti, J.M. Murphy, A. Noda, S.C.B. Raper, I.G. Watterson, A.J. Weaver, and Z.C. Zhao. 2007. Global Climate Projections, *In* S. Solomon, et al., eds. Climate Change 2007: The Physical Science Basis. Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA.
Meharg, A.A., and K. Killham. 1990. Carbon distribution within the plant and rhizosphere in laboratory and field grown *Lolium perenne* at different stages of development. Soil Biology & Biochemistry 22:471-477.
Melillo, J.M., J.D. Aber, and J.F. Muratore. 1982. Nitrogen and lignin control of hardwood leaf litter decomposition dynamics. Ecology 63:621-626.
Melillo, J.M., P.A. Steudler, J.D. Aber, K. Newkirk, H. Lux, F.P. Bowles, C. Catricala, A. Magill, T. Ahrens, and S. Morrisseau. 2002. Soil warming and carbon-cycle feedbacks to the climate system. Science 298:2173-2176.
Midwood, A.J., B. Thornton, and P. Millard. 2008. Measuring the ^{13}C content soil-respired CO_2 using a novel open chamber system. Rapid Communications in Mass Spectrometry 22:2073-2081.
Mikan, C.J., J.P. Schimel, and A.P. Doyle. 2002. Temperature controls of microbial respiration in arctic tundra soils above and below freezing. Soil Biology & Biochemistry 34:1785-1795.
Millard, P., A.J. Midwood, J.E. Hunt, D. Whitehead, and T.W. Boutton. 2008. Partitioning soil surface CO_2 efflux into autotrophic and heterotrophic components, using natural gradients in soil $\delta^{13}C$ in an undisturbed savannah soil. Soil Biology & Biochemistry 40:1575-1582.
Ministry for the Environment. 2008. Climate Change Effects and Impacts Assessment: A Guidance Manual for Local Government in New Zealand. Ministry for the Environment, Wellington.
Mitchell, K.J. 1956. Growth of pasture species under controlled environment. 1. Growth at various levels of constant temperature. New Zealand Journal of Science and Technology, Section A 38:203-15.

Monaghan, R.M., and D. Barraclough. 1993. Nitrous oxide and dinitrogen emissions from urine-affected soil under controlled conditions. Plant and Soil 151:127-138.
Mosier, A., and L. Mack. 1980. Gas chromatographic system for precise, rapid analysis of nitrous oxide. Soil Science Society of American Journal 44:1121-1123.
Moyano, F.E., W.L. Kutsch, and C. Rebmann. 2008. Soil respiration fluxes in relation to photosynthetic activity in broad-leaf and needle-leaf forest stands. Agricultural and Forest Meteorology 148:135-143.
Mäkiranta, P., K. Minkkinen, J. Hytönen, and J. Laine. 2008. Factors causing temporal and spatial variation in heterotrophic and rhizospheric components of soil respiration in afforested organic soil croplands in Finland. Soil Biology & Biochemistry 40:1592-1600.
Mäkiranta, P., R. Laiho, H. Fritze, J. Hytönen, J. Laine, and K. Minkkinen. 2009. Indirect regulation of heterotrophic peat soil respiration by water level via microbial community structure and temperature sensitivity. Soil Biology & Biochemistry 41:695-703.
Müller, C., R.R. Sherlock, and P.H. Williams. 1997. Mechanistic model for nitrous oxide emission via nitrification and denitrification. Biology and Fertility of Soils 24:231-238.
Nakadai, T., M. Yokozawa, H. Ikeda, and H. Koizumi. 2002. Diurnal changes of cabon dioxide flux from bare soil in agricultural field in Japan. Applied Soil Ecology 19:161-171.
Nicolardot, B., G. Fauvet, and D. Cheneby. 1994. Carbon and nitrogen cycling through soil microbial biomass at various temperatures. Soil Biology & Biochemistry 26:253-261.
Nielsen, T.H., and N.P. Revsbech. 1998. Nitrification, denitrification, and N-liberation associated with two types of organic hot-spots in soil. Soil Biology & Biochemistry 30:611-619.
Nissenbaum, A., and I.R. Kaplan. 1972. Chemical and Isotopic Evidence for the in Situ Origin of Marine Humic Substances. Limnology and Oceanography 17:570-582.
O'Leary, M.H. 1988. Carbon isotopes in photosynthesis. BioScience 38:328-336.
Oenema, O., G.L. Velthof, S. Yamulki, and S.C. Jarvis. 1997. Nitrous oxide emissions from grazed grassland. Soil Use and Management 13:288-295.
Oenema, O., A. Bannink, S.G. Sommer, and G.L. Velthof. 2001. Geseous nitrogen emissions from livestock farming system, p. 255-289, *In* R. F. Follett and J. L. Hatfield, eds. Nitrogen in the Environment: Sources, Problems, and Management. Elsevier Science.
Oenema, O., N. Wrage, G.L. Velthof, J.W. van Groenigen, J. Dolfing, and P.J. Kuikman. 2005. Trends in global nitrous oxide emissions from animal production systems. Nutrient Cycling in Agroecosystems 72:51-65.
Ohlsson, K.E.A., B. Singh, S. Holm, A. Nordgren, L. Lövdahl, and P. Högberg. 2005. Uncertainties in static closed chamber measurements of the carbon isotopic ratio of soil-respired CO_2. Soil Biology & Biochemistry 37:2273-2276.
Ojima, D.S., B.O.M. Dirks, E.P. Genn, C.E. Owensby, and J.O. Scurlock. 1993. Assessment of C budget for grasslands and drylands of the world. Water Air and Soil Pollution 70:95-109.
Olsson, P., S. Linder, R. Giesler, and P. Högberg. 2005. Fertilization of boreal forest reduces both autotrophic and heterotrophic soil respiration. Global Change Biology 11:1745-1753.

Orchard, V.A., F.J. Cook, and D.M. Corderoy. 1992. Field and laboratory studies on the relationship between respiration and moisture of two soils of contrasting fertility status. Pedobiologia 36:21-33.
Parkin, T.B., and J.M. Tiedje. 1984. Application of a soil core method to investigate the effect of oxygen concentration on denitrification. Soil Biology & Biochemistry 16:331-334.
Parnas, H. 1976. A theoretical explanation of the priming effect based on microbial growth with two limiting substrates. Soil Biology & Biochemistry 8:139-144.
Parton, W.J., D.S. Schimel, C.V. Cole, and D.S. Ojima. 1987. Analysis of Factors Controlling Soil Organic Matter Levels in Great Plains Grasslands. Soil Science Society of America Journal 51:1173-1179.
Paul, E.A., D. Harris, H.P. Collins, U. Schulthess, and G.P. Robertson. 1999. Evolution of CO_2 and soil carbon dynamics in biologically managed, row-crop agroecosystems. Applied Soil Ecology 11:53-65.
Payne, W.J. 1973. Reduction of nitrogenous oxide by microorganisms. Bacteriological Reviews 37:409-452.
Peacock, J.M. 1975. Temperature and Leaf Growth in Lolium perenne. II. The Site of Temperature Perception. Journal of Applied Ecology 12:115-123.
Pendall, E., L. Rustad, and J. Schimel. 2008. Towards a predictive understanding of belowground process responses to climate change: have we moved any closer? Functional Ecology 22:937-940.
Petrie, S., C. Sweeney, C. Cameron, S. Gulliver, K. Tsui, M. Gabriels, D. Austin, K. Cao, A. Pickering, R.R. Palma, W. Hennessy, M. McCurdy, H. Clark, C.A.M. de Klein, G. Rys, S. Wakelin, P. Lane, and A. Whiteford. 2007. New Zealand Greenhouse Gas Inventory 1990-2007, 15 April 2009 ed. Ministry for the Environment, Wellington. http://www.mfe.govt.nz/publications/climate/greenhouse-gas-inventory-2009/html/index.html.
Phillips, D.L., and J.W. Gregg. 2001. Uncertainty in source partitioning using stable isotopes. Oecologia 127:171–179.
Phillips, R.P., and T.J. Fahey. 2008. The Influence of Soil Fertility on Rhizosphere Effects in Northern Hardwood Forest Soils. Soil Science Society of America Journal 72:453-461.
Piao, S., P. Ciais, P. Friedlingstein, P. Peylin, M. Reichstein, S. Luyssaert, H. Margolis, J. Fang, A. Barr, A. Chen, A. Grelle, D.Y. Hollinger, T. Laurila, A. Lindroth, A.D. Richardson, and T. Vesala. 2008. Net carbon dioxide losses of northern ecosystems in response to autumn warming. Nature 451:49-52.
Pihlatie, M., E. Syvasalo, A. Simojoki, M. Esala, and K. Regina. 2004. Contribution of nitrification and denitrification of N_2O production in peat, clay and loamy sand soils under different soil moisture conditions. Nutrient Cycling in Agroecosystems 70:135-141.
Potter, C.S., P.A. Matson, P.M. Vitousek, and E.A. Davidson. 1996. Process modeling of controls on nitrogen trace gas emissions from soils worldwide. Journal of Geophysical Research 101:1361-1377.
Raich, J.W., and K.J. Nadelhoffer. 1989. Belowground carbon allocation in forest ecosystems: global trends. Ecology 70:1346-1354.
Raich, J.W., and W.H. Schlesinger. 1992. The global carbon dioxide flux in soil respiration and its relationship to vegetation and climate. Tellus B 44:81-99.
Raich, J.W., and C.S. Potter. 1995. Global patterns of carbon dioxide emissions from soils. Global Biogeochemical Cycles 9:23-26.
Raich, J.W., and A. Tufekciogul. 2000. Vegetation and soil respiration: Correlations and controls. Biogeochemistry 48:71-90.

Ravishankara, A.R., J.S. Daniel, and R.W. Portmann. 2009. Nitrous Oxide (N_2O): The Dominant Ozone-Depleting Substance Emitted in the 21st Century. Science 326:123-125.

Redmann, R.E. 1992. Primary productivity, p. 75-94, *In* R. T. Coupland, ed. Ecosystems of the world: natural grasslands: introductionand western hemisphere, Vol. 8A. Elsevier, Amsterdam.

Rey, A., C. Petsikos, P.G. Jarvis, and J. Grace. 2005. Effect of temperature and moisture on rates of carbon mineralization in a Mediterranean oak forest soil under controlled and field conditions. European Journal of Soil Science 56:589-599.

Reynolds, C.M., D.C. Wolf, and J.A. Armbruster. 1985. Factors related to urea hydrolysis in soils. Soil Science Society of America Journal 49:104-108.

Richards, B.N. 1987. The microbiology of terrestrial ecosystems. John Wiley & Sons, New York.

Richardson, A.D., and D.Y. Hollinger. 2005. Statistical modeling of ecosystem respiration using eddy covariance data: Maximum likelihood parameter estimation, and Monte Carlo simulation of model and parameter uncertainty, applied to three simple models. Agricultural and Forest Meteorology 131:191-208.

Robertson, G.P., K.M. Klingensmith, M.J. Klug, E.A. Paul, J.R. Crum, and B.G. Ellis. 1997. Soil resources, microbial activity, and primary production across an agricultural ecosystem. Ecological Applications 7:158-170.

Robinson, D., and C.M. Scrimgeour. 1995. The contribution of plant C to soil CO_2 measured using $d^{13}C$. Soil Biology & Biochemistry 27:1653-1656.

Rochette, P., L.B. Flanagan, and E.G. Gregorich. 1999. Separating soil respiration into plant and soil components using analyses of the natural abundance of Carbon-13. Soil Science Society of America Proceedings 63:1207-1213.

Rodrigo, A., S. Recous, C. Neel, and B. Mary. 1997. Modelling temperature and moisture effects on C-N transformations in soils: comparison of nine models. Ecological Modelling 102:325-339.

Rovira, P., and V.R. Vallejo. 2002. Labile and recalcitrant pools of carbon and nitrogen in organic matter decomposing at different depths in soil: an acid hydrolysis approach. Geoderma 107:109-141.

Rudaz, A.O., E.A. Davidson, and M.K. Fireston. 1991. Sources of nitrous oxide production following wetting of dry soil. FEMS Microbiology Letters 85:117-124.

Rudaz, A.O., E. Walti, G. Kyburz, P. Lehmann, and J. Fuhrer. 1999. Temporal variation in N_2O and N_2 fluxes from a permanent pasture in Switzerland in relation to management, soil water content and soil temperature. Agriculture, Ecosystems & Environment 73:83-91.

Ruser, R., H. Flessa, R. Russow, G. Schmidt, F. Buegger, and J.C. Munch. 2006. Emission of N_2O, N_2 and CO_2 from soil fertilized with nitrate: effect of compaction, soil moisture and rewetting. Soil Biology & Biochemistry 38:263-274.

Russow, R., C.F. Stange, and H.-U. Neue. 2009. Role of nitrite and nitric oxide in the processes of nitrification and denitrification in soil: results from ^{15}N tracer experiments. Soil Biology & Biochemistry 41:785-795.

Rustad, L., J. Campbell, G. Marion, R. Norby, M. Mitchell, A. Hartley, J. Cornelissen, and J. Gurevitch. 2001. A meta-analysis of the response of soil respiration, net nitrogen mineralization, and aboveground plant growth to experimental ecosystem warming. Oecologia 126:543-562.

Rustad, L.E., T.G. Huntington, and R.D. Boone. 2000. Controls on soil respiration: Implications for climate change. Biogeochemistry 48:1-6.

Saggar, S., and C.B. Hedley. 2001. Estimating seasonal and annual carbon inputs, and root decomposition rates in a temperate pasture following field ^{14}C pulse-labelling. Plant and Soil 236:91-103.

Sakata, T., S. Ishizuka, and M. Takahashi. 2007. Separation of soil respiration into CO_2 emission sources using ^{13}C natural abundance in a deciduous broad-leaved forest in Japan. Soil Science and Plant Nutrition 53:328-336.

Sauer, T.J., S.R. Compston, C.P. West, G. Hernandez-Ramirez, E.E. Gbur, and T.B. Parkin. 2009. Nitrous oxide emissions from a bermudagrass pasture: interseeded winter rye and poultry litter. Soil Biology & Biochemistry 41:1417-1424.

Schimel, D.S. 1995. Terrestrial ecosystems and the carbon cycle. Global Change Biology 1:77-91.

Schimel, D.S., B.H. Braswell, E.A. Holland, R. McKeown, D.S. Ojima, T.H. Painter, W.J. Parton, and A.R. Townsend. 1994. Climatic, edaphic, and biotic controls over storage and turnover of carbon in Soils. Global Biogeochemical Cycles 8:279-293.

Schindlbacher, A., S. Zechmeister-Boltenstern, and K. Butterbach-Bahl. 2004. Effects of soil moisture and temperature on NO, NO_2, and N_2O emissions from European forest soils. Journal of Geophysical Research 109:D17032.

Schipper, L.A., W.T. Baisden, R.L. Parfitt, C. Ross, J.J. Clayton, and G. Arnold. 2007. Large losses of soil C and N from soil profiles under pasture in New Zealand during the past 20 years. Global Change Biology 13:1138-1144.

Schlesinger, W.H. 1977. Carbon Balance in Terrestrial Detritus. Annual Review of Ecology and Systematics 8:51-81.

Schulze, E.D., F.M. Kelliher, and C.H. Korner. 1994. Relationships among maximum stomatal conductance, ecosystem surface conductance, carbon assimilation rate, and plant nitrogen nutrition: a global scaling exercise. Annual Review of Ecology and Systematics 25:629-660.

Schuman, G.E., J.D. Reeder, J.T. Manley, R.H. Hart, and W.A. Manley. 1999. Impact of grazing management on the carbon and nitrogen balance of a mixed-grass rangeland. Ecological Applications 9:65-71.

Schönwitz, R., W. Stichler, and H. Ziegler. 1986. $\delta^{13}C$ values of CO_2 from soil respiration on sites with crops of C3 and C4 type of photosynthesis. Oecologia 69:305-308.

Scott-Denton, L.E., T. Rosenstiel, and R.K. Monson. 2006. Differential controls by climate and substrate over the heterotrophic and rhizospheric components of soil respiration. Global Change Biology 12:205-216.

Shamroot, S., L. McDonald, and W.V. Bartholomew. 1968. Rhizo-deposition of organic debris in soil. Soil Science Society of America Proceedings 32:817-820.

Shand, C.A., M. Williams, L.A. Dawson, S. Smith, and M.E. Young. 2002. Sheep urine affects soil solution nutrient composition and roots: differences between field and sward box soils and the effects of synthetic and natural sheep urine. Soil Biology & Biochemistry 34:163-171.

Sherlock, R.R., and K.M. Goh. 1984. Dynamics of ammonia volatilization from simulated urine patches and aqueous urea applied to pasture I. Field experiments. Fertilizer Research 5:181-195.

Sikora, L.J., and J.L. McCoy. 1990. Attempts to determine available carbon in soils. Biology and Fertility of Soils 9:19-24.

Singh, J.S., and S.R. Gupta. 1977. Plant decomposition and soil respiration in terrestrial ecosystems. Botanical Review 43:449-528.
Singh, K.P., N. Ghoshal, and S. Singh. 2009. Soil carbon dioxide flux, carbon sequestration and crop productivity in a tropical dryland agroecosystem: influence of organic inputs of varying resource quality. Applied Soil Ecology 42:243-253.
Six, J., R.T. Conant, E.A. Paul, and K. Paustian. 2002. Stabilization mechanisms of soil organic matter: Implications for C-saturation of soils. Plant and Soil 241:155-176.
Skopp, J., M.D. Jawson, and J.W. Doran. 1990. Steady-state aerobic microbial activity as a function of soil water content. Soil Science Society of America Journal 54:1619-1625.
Smith, K.A. 1997. The potential for feedback effects induced by global warming on emissions of nitrous oxide by soil. Global Change Biology 3:327-338.
Smith, K.A., T. Ball, F. Conen, K.E. Dobbie, J. Massheder, and A. Rey. 2003. Exchange of greenhouse gases between soil and atmosphere: interactions of soil physical factors and biological processes. European Journal of Soil Science 54:779-791.
Soil Survey Staff. 1998. 'Keys to Soil Taxonomy.' 8th edn United States Department of Agriculture, Natural Resources Conservation Service, Washington, DC.
Song, C., and J. Zhang. 2009. Effects of soil moisture, temperature, and nitrogen fertilization on soil respiration and nitrous oxide emission during maize growth period in northeast China. Acta Agricultura Scandinavica. Section B, Plant Soil Science 59:97-106.
Sparling, G., M. Vojvodic-Vukovic, and L.A. Schipper. 1998. Hot-water-soluble C as A simple measure of labile soil organic matter: the relationship with microbial biomass C. Soil Biology & Biochemistry 30:1469-1472.
Stanford, G., M.H. Frere, and D.H. Schwaninger. 1973. Temperature coefficient of soil nitrogen mineralization. Soil Science 115:321-323.
Stark, J.M. 1996. Modeling the temperature response of nitrification. Biogeochemistry 35:433-445.
Ste-Marie, C., and D. Paré. 1999. Soil, pH and N availability effects on net nitrification in the forest floors of a range of boreal forest stands. Soil Biology & Biochemistry 31:1579-1589.
Steinweg, J.M., A.F. Plante, R.T. Conant, E.A. Paul, and D.L. Tanaka. 2008. Patterns of substrate utilization during long-term incubations at different temperatures. Soil Biology & Biochemistry 40:2722-2728.
Stevens, R.J., R.J. Laughlin, G.J. Atkins, and S.J. Prosser. 1993. Automated determination of nitrogen-15-labelled dinitrogen and nitrous oxide by mass spectrometry. Soil Science Society of America Journal 57:981-988.
Struwe, S., and A. Kjøller. 1991. Denitrification in wet forest soil systems in situ and in slurry experiments. Forest Ecology and Management 44:41-52.
Tang, J., and D.D. Baldocchi. 2005. Spatial–temporal variation in soil respiration in an oak–grass savanna ecosystem in California and its partitioning into autotrophic and heterotrophic components. Biogeochemistry 73:183-207.
Tans, P. 2009. NOAA/ESRL (www.esrl.noaa.gov/gmd/ccgg/trends/).
Tate, K.R., N.A. Scott, A. Parshotam, L. Brown, R.H. Wilde, D.J. Giltrap, N.A. Trustrum, B. Gomez, and D.J. Ross. 2000. A multi-scale analysis of a terrestrial carbon budget: is New Zealand a source or sink of carbon. Agriculture, Ecosystems & Environment 82:229-246.

Thompson, R.B., and I.R.P. Fillery. 1997. Transformation in soil and turnover to wheat of nitrogen from components of grazed pasture in the south of Western Australia. Australian Journal of Agricultural Research 48:1033-1047.
Thomsen, I.K., B.M. Petersen, S. Bruun, L.S. Jensen, and B.T. Christensen. 2008. Estimating soil C loss potentials from the C to N ratio. Soil Biology & Biochemistry 40:849-852.
Thornley, J.H.M. 1970. Respiration, growth and maintenance in plants. Nature 227:304-305.
Thornley, J.H.M., and M.G.R. Cannell. 1997. Temperate grassland responses to climate change: an analysis using the Hurley pasture model. Annals of Botany 80:205-221.
Tiedje, J.M. 1988. Ecology of denitrification and dissimilatory nitrate reduction to ammonium, p. 179–244, *In* A. J. B. Zehnder, ed. Biology of anaerobic microorganisms. Wiley, New York.
Tiemann, L.K., and S.A. Billings. 2008. Carbon controls on nitrous oxide production with changes in substrate availability in a north american grassland. Soil Science 173:332-341.
Tjoelker, M.G., J. Oleksyn, and P.B. Reich. 2001. Modelling respiration of vegetation: evidence for a temperature-dependent Q_{10}. Global Change Biology 7:223-230.
Townsend, A.R., and P.M. Vitousek. 1995. Soil organic matter dynamics along gradients in temperature and land use on the island of Hawaii. Ecology 76:721-733.
Townsend, A.R., P.M. Vitousek, D.J. Desmarais, and A. Tharpe. 1997. Soil carbon pool structure and temperature sensitivity inferred using CO_2 and $^{13}CO_2$ incubation fluxes from five Hawaiian soils. Biogechemistry 38:1-17.
Trenberth, K.E., P.D. Jones, P. Ambenje, R. Bojariu, D. Easterling, A. Klein Tank, D. Parker, F. Rahimzadeh, J.A. Renwick, M. Rusticucci, B. Soden and P. Zhai. 2007. Observations: Surface and Atmospheric Climate Change, *In* S. Solomon, D. Qin, M. Manning, Z. Chen, M. Marquis, K.B. Averyt, M. Tignor and H.L. Miller, ed. Climate Change 2007: The Physical Science Basis. Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA.
Trumbore, S. 2000. Age of soil organic matter and soil respiration: radio carbon constraints on below ground C dynamics. Ecological Applications 10:399-411.
Uchida, Y., T.J. Clough, F.M. Kelliher, and R.R. Sherlock. 2008. Effects of aggregate size, soil compaction, and bovine urine on N_2O emissions from a pasture soil. Soil Biology & Biochemistry 40:924-931.
Uchida, Y., T.J. Clough, F.M. Kelliher, and R.R. Sherlock. 2010. Soil microbial respiration responses to changing temperature and substrate availability in fertile grassland. Australian Journal of Soil Research In press.
van Cleemput, O., and L. Baert. 1984. Nitrite: a key compound in N loss processes under acid conditions? Plant and Soil 76:197-212.
van Groenigen, J.W., P.J. Kuikman, W.J.M. de Groot, and G.L. Velthof. 2005. Nitrous oxide emission from urine-treated soil as influenced by urine composition and soil physical conditions. Soil Biology & Biochemistry 37:463-473.
van Hees, P.A.W., C.D. Jones, R. Finlay, D.L. Godbold, and U.S. Lundström. 2005. The carbon we do not see—the impact of low molecular weight compounds on carbon dynamics and respiration in forest soils: a review. Soil Biology & Biochemistry 37:1-13.
van't Hoff, J.H. 1884. Ètudes de dynamique chimique (Studies of chemical dynamics) Frederik Muller and Co., Amsterdam, the Netherlands.

Vanhala, P., K. Karhu, M. Tuomi, E. Sonninen, H. Jungner, H. Fritze, and J. Liski. 2007. Old soil carbon is more temperature sensitive than the young in an agricultural field. Soil Biology & Biochemistry 39:2967-2970.
Veen, B.W. 1981. Relation between root respiration and root activity. Plant and Soil 63:73-76.
Verhagen, F.J.M., H.J. Laanbroek, and J.W. Woldendorp. 1995. Competition for ammonium between plant roots and nitrifying and heterotrophic bacteria and the effects of protozoan grazing. Plant and Soil 170:241-250.
Vines, H.M., and R.T. Wedding. 1960. Some effects of ammonia on plant metabolism and a possible mechanism for ammonia toxity. Plant Physiology 35:820-825.
Wachendorf, M., C. Lambe, F. Taube, and K. Dittert. 2008. Nitrous oxide emissions and dynamics of soil nitrogen under ^{15}N-labeled cow urine and dung patches on a sandy grassland soil. Journal of Plant Nutrition and Soil Science 171:171-180.
Waldrop, M.P., and M.K. Firestone. 2004a. Microbial community utilization of recalcitrant and simple carbon compounds: impact of oak-woodland plant communities. Oecologia 138:275-284.
Waldrop, M.P., and M.K. Firestone. 2004b. Altered utilization patterns of young and old soil C by microorganisms caused by temperature shifts and N additions. Biogeochemistry 67:235-248.
Wan, S., and Y. Luo. 2003. Substrate regulation of soil respiration in a tallgrass prairie: Results of a clipping and shading experiment. Global Biogeochemical Cycles 17:1054.
Wang, W., and J. Fang. 2009. Soil respiration and human effects on global grasslands. Global and Planetary Change 67:20-28.
Wang, W.J., R.C. Dalal, P.W. Moody, and C.J. Smith. 2003. Relationships of soil respiration to microbial biomass, substrate availability and clay content. Soil Biology & Biochemistry 35:273-284.
Wardle, D.A., R.D. Bardgett, J.N. Klironomos, H. Setäl ä, W.H. van der Putten, and D.H. Wall. 2004. Ecological linkages between aboveground and belowground biota. Science 304:1634-1637.
Warembourg, F.R., and H.D. Esterlich. 2001. Plant phenology and soil fertility effects on below-ground carbon allocation for an annual (*Bromus madritensis*) and a perennial (*Bromus erectus*) grass species. Soil Biology & Biochemistry 33:1291-1303.
Webster, F.A., and D.W. Hopkins. 1996. Contributions from different microbial processes to N_2O emission from soil under different moisture regimes. Biology and Fertility of Soils 22:331-335.
Weitz, A.M., E. Linder, S. Frolking, P.M. Crill, and M. Keller. 2001. N_2O emissions from humid tropical agricultural soils: effects of soil moisture, texture and nitrogen availability. Soil Biology & Biochemistry 33:1077-1093.
Weixin, C., D.C. Coleman, C.R. Carroll, and C.A. Hoffman. 1993. In situ measurement of root respiration and soluble C concentrations in the rhizosphere. Soil Biology & Biochemistry 25:1189-1196.
Wezermal, C.T., and J.J. Gannon. 1967. Oxygen-nitrogen relationships in autotrophic nitrification. Applied Microbiology 15:1211-1215.
White, J.G.H. 2000. The farmlands of New Zealand, p. 1-10, *In* J. D. H. White and J. Hodgson, eds. New Zealand Pasture and Crop Science. Oxford University Press, Oxford.
White, R., S. Murray, and M. Rohweder. 2000. Pilot analysis of global ecosystems: grassland ecosystems technical report World Resources Institute, Washington DC.

Williams, B.L., S.J. Grayston, and E.J. Reid. 2000. Influence of synthetic sheep urine on the microbial biomass, activity and community structure in two pastures in the Scottish uplands. Plant and Soil 225:175-185.
Winkler, J.P., R.S. Cherry, and W.H. Schlesinger. 1996. The Q_{10} relationship of microbial respiration in a temperate forest soil. Soil Biology & Biochemistry 28:1067-1072.
Wrage, N., G.L. Velthof, M.L. van Beusichem, and O. Oenema. 2001. Role of nitrifier denitrification in the production of nitrous oxide. Soil Biology & Biochemistry 33:1723-1732.
Wrage, N., J.W.v. Groenigen, O. Oenema, and E.M. Baggs. 2005. A novel dual-isotope labelling method for distinguishing between soil sources of N_2O. Rapid Communications in Mass Spectrometry 19:3298-3306.
Yamulki, S., S.C. Jarvis, and P. Owen. 1998. Nitrous oxide emissions from excreta applied in a simulated grazing pattern. Soil Biology & Biochemistry 30:491-500.
Yoshida, T., and M. Alexander. 1970. Nitrous oxide formation by *Nitrosomonas europaea* and heterotrophic microorganisms. Soil Science Society of America Proceedings 34:880-882.
Zaman, M., H.J. Di, and K.C. Cameron. 1999. A field study of gross rates of N mineralization and nitrification and their relationships to microbial biomass and enzyme activities in soils treated with dairy effluent and ammonium fertilizer. Soil Use and Management 15:188-194.
Zobitz, J., D. Moore, W. Sacks, R. Monson, D. Bowling, and D. Schimel. 2008. Integration of Process-based Soil Respiration Models with Whole-Ecosystem CO_2 Measurements. Ecosystems 11:250-269.
Šimek, M., L. Jíšová, and D.W. Hopkins. 2002. What is the so-called optimum pH for denitrification in soil? Soil Biology & Biochemistry 34:1227-1234.