COMPONENTS OF ECOSYSTEM CARBON DIOXIDE EXCHANGE IN A NEW ZEALAND TUSSOCK GRASSLAND UNDER SOIL WARMING AND NITROGEN ADDITION

THESIS

Submitted in partial fulfilment of the requirements
for the degree of Doctor of Philosophy in Ecology
at the University of Canterbury
by
Scott Graham
2012

Dedicated to my grandfather, James H. Graham, MD,
a life-long advocate of education and an academic role model.

ACKNOWLEDGEMENTS

I would like to thank my advisors, Dr. David Whitehead of Landcare Research and Dr. Jason Tylianakis of the University of Canterbury, for inviting me to New Zealand and offering their time in order to guide me through the development and execution of this research. I have benefited greatly from having worked with both of them.

I would also like to thank Landcare Research for hosting me over the last three years. In particular, I am grateful to Peter Millard for offering his expertise in stable isotopes and general advice on research, writing and careers in science. John Hunt, Graeme Rogers and Tony McSeveny generously offered their technical expertise, as well as good humour and a wealth of information on New Zealand natural history and culture. I thank Kev Drew for help with the tedious task of sorting vegetation samples.

Guy Forrester, Elena Moltchonova and Ellen Cieraad all provided essential guidance with statistical analyses. Claudio de Sassi was instrumental in setting up the Cass Soil Warming site and I thank the University of Canterbury Field Stations for use of the Cass Field Station.

I am very grateful to the Miss E.L. Hellaby Indigenous Grassland Research Trust for their generous funding of my time and research.

I thank my parents, Jim and Susan Graham, and grandparents, Jim and Gloria Graham, for their on-going support and for urging me to continue my studies over the years.

Most importantly, I thank my partner, Jennifer Peters, who helped in the lab and field, proofread drafts and offered her support throughout the entire process. None of this would have been possible if not for her.

SLG

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α	quantum yield
$\delta^{13}C_{RS}$	$\delta^{13}C$ signature of soil respired CO_2
$\delta^{13}C_{RA}$	$\delta^{13}C$ signature of root-respired CO_2
$\delta^{13}C_{RH}$	$\delta^{13}C$ signature of heterotrophic respiration
E_0	activation energy
F_N	net ecosystem exchange
fR_H	proportion of soil respiration contributed by heterotrophic respiration
fR_S	proportion of ecosystem respiration contributed by soil respiration
θ	soil volumetric water content
θ_N	non-limiting soil volumetric water content
P_G	gross primary productivity
P_{max}	maximum photosynthetic rate
Q	irradiance
R_A	autotrophic respiration
R_E	ecosystem respiration
$R_{E,a}$	above-ground ecosystem respiration
R_S	soil respiration
$R_{S,A}$	autotrophic soil respiration
$R_{S,H}$	heterotrophic soil respiration
$R_{S,HF}$	heterotrophic soil respiration measured in root-free soil microcosms
R_{10}	respiration rate at 10°C
S_I	substrate-induced respiration
SOM	soil organic matter
T_a	air temperature
T_S	soil temperature

ABSTRACT

Global temperatures are expected to increase by 1.1 to 6.4°C over the next century and over the same period, nitrogen inputs to terrestrial ecosystems are expected to increase as a result of increased crop fertilisation and atmospheric nitrogen deposition. Both of these global change drivers are expected to affect net carbon balance by increasing both gross primary production and ecosystem respiration, yet the balance between these processes, and the potential interactive effects of the drivers, require quantification. The ability to accurately predict the effects of warming and nitrogen addition on all components of terrestrial carbon balance will be critical in determining the likely positive feedback to rising atmospheric CO_2 from terrestrial ecosystems. Tussock grasslands are a widespread and important carbon store within New Zealand and are representative of temperate grasslands worldwide. This thesis addresses the question: *Will tussock grasslands act as a positive feedback to rising atmospheric CO_2 concentration* in response to soil warming and nitrogen addition?

Using a combination of controlled-environment and field-scale studies of tussock grassland, net ecosystem carbon exchange was partitioned into gross primary production, ecosystem respiration and the autotrophic and heterotrophic components of soil respiration. Soil respiration in the field increased by 41% in response to a 3°C soil warming treatment and by 12% in response to a 50 kg N ha^{-1} y^{-1} nitrogen addition treatment. Only warming resulted in enhanced heterotrophic decomposition of soil organic matter (37% increase). However, a controlled-environment study indicated that caution must be used when interpreting temperature responses of heterotrophic respiration from root-free soils, as priming effects were shown to decrease the sensitivity of heterotrophic respiration to temperature. Measurements of net ecosystem exchange in the field showed that warming-enhanced heterotrophic respiration lead to a significant 49 g m^{-2} reduction in net ecosystem carbon uptake. Neither nitrogen addition nor combined warming and nitrogen addition treatment resulted in significant changes in net ecosystem carbon balance.

These results suggest that tussock grasslands will act as a positive feedback to rising atmospheric CO_2 concentration. However, increased nitrogen deposition will serve as a potential mitigating factor for climate driven feedbacks.

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Chapter 2 (90% contribution)
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SLG co-designed the field experiments with DW, obtained funding, collected the data, carried out analyses and wrote the manuscript. JMT and DW reviewed all drafts and provided comments on analyses.

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The undersigned certifies that:

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- In cases where the PhD candidate was the lead author of the co-authored work he or she wrote the text

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CHAPTER 1: INTRODUCTION

1.1 Terrestrial Feedbacks to Global Change

Atmospheric CO_2 concentration is rising at a rate of 1.8 ppm y^{-1} due to anthropogenic CO_2 emissions (IPCC, 2007). However, this rate of increase reflects the accumulation of only 43% of the estimated 9.9 Pg C y^{-1} added to the atmosphere by human activities, including fossil-fuel burning and land-use change (Le Quere et al., 2009). At present, the terrestrial biosphere represents a sink for 4.7 Pg C y^{-1}, which, along with the oceanic carbon sink, is partially mitigating anthropogenic emissions.

The terrestrial carbon sink is determined by the global balance of gross primary production and ecosystem respiration, which can be represented as:

$$F_N = R_A + R_{S,H} - P_G \qquad (1.1)$$

where R_A and $R_{S,H}$ are the autotrophic and heterotrophic components of ecosystem respiration, P_G is gross primary production and F_N is the net impact on the atmosphere (a negative value indicates a terrestrial sink). Approximately 120 Pg C y^{-1} are removed from the atmosphere by gross primary production globally (Schlessinger, 1997). About half of this is then returned to the atmosphere via plant respiration (R_A) and the remaining carbon accumulates as biomass (net primary production = P_G - R_A). This accumulation of carbon by net primary production is largely offset by heterotrophic decomposition processes, particularly within the soil ($R_{S,H}$) (Fig. 1.1).

Soil respiration (R_S) is an important component of terrestrial carbon balance and comprises approximately two thirds of ecosystem respiration globally (Schlesinger and Andrews, 2000). At the global scale, patterns of soil respiration are related closely to net primary production and its drivers, temperature and precipitation (Raich and Schlesinger, 1992). Likewise, at the field-scale, temperature and soil water content are important drivers of soil respiration (Lloyd and Taylor, 1994, Savage and Davidson, 2001). As well, photosynthesis is an important source of respiratory substrate and has been acknowledged to be a driver of soil respiration across a range of ecosystems (Högberg et al., 2001, Tang et al., 2005, Bahn et al., 2009, Vargas et al., 2009).

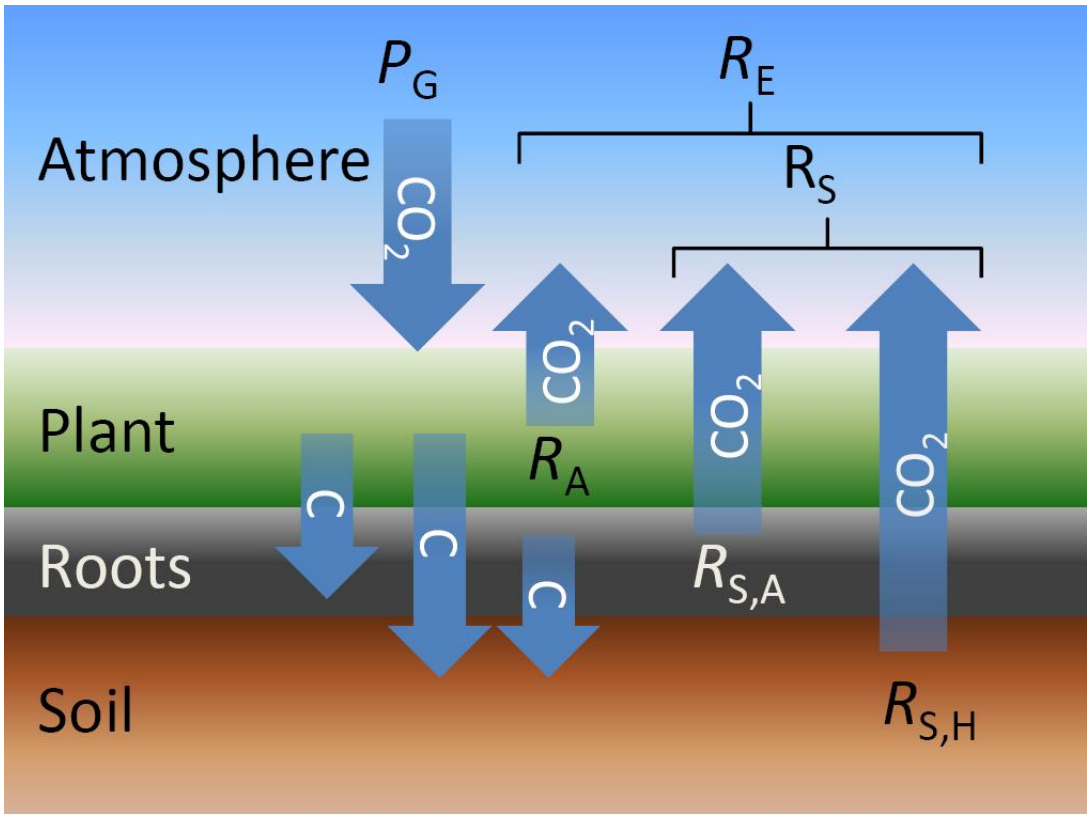

Figure 1.1: Conceptual diagram of plant-soil-atmosphere carbon exchange depicting carbon flows from plants to their roots and soil, as well as exchanges of CO_2.

Soil respiration is the sum of CO_2 fluxes from multiple, distinct carbon sources, primarily respiration of roots and associated rhizosphere microbes, termed autotrophic soil respiration ($R_{S,A}$), and the heterotrophic decomposition of soil organic matter ($R_{S,H}$) (Hanson et al., 2000). These components of soil respiration are likely to exhibit divergent responses to environmental drivers, and to have different impacts on net ecosystem carbon balance. As autotrophic soil respiration is a component of plant respiration, it is regulated largely by substrate supply from photosynthesis (Craine et al., 1999, Högberg et al., 2001, Wan and Luo, 2003). As such, carbon losses due to autotrophic soil respiration have consequences for the carbon balance of plants. However, the extent to which autotrophic respiration can affect the atmosphere is limited by net primary production. Heterotrophic soil respiration involves turnover of soil carbon, the largest terrestrial carbon pool of approximately 1500 Pg C (Schlesinger and Andrews, 2000, Amundson, 2001). As well as being large, the soil carbon pool represents long-term

storage of carbon, a portion of which may have turnover times of up to millennia (Trumbore, 2000). Persistent increases in respiration of this 'old carbon' could greatly affect net ecosystem carbon balance.

All components of terrestrial carbon balance are sensitive to environmental variables such as temperature, nitrogen availability and water availability. Therefore, the persistence of the terrestrial biosphere as a net sink for carbon under future climatic conditions will be related strongly to the relative responses of gross primary productivity and the components of ecosystem respiration (Grace and Rayment, 2000). Alarmingly, coupled-climate models suggest that the terrestrial biosphere will become a positive feedback to rising atmospheric CO_2 concentration under future climate scenarios (Cox et al., 2000, Friedlingstein et al., 2006, Sitch et al., 2008). This predicted weakening of the terrestrial carbon sink is driven largely by enhanced heterotrophic respiration in response to climate warming. However, large uncertainties in these predictions still exist because the exact response of heterotrophic respiration to temperature, and thus any potential effects on soil carbon storage, remain unclear (Davidson and Janssens, 2006, von Lützow and Kögel-Knabner, 2009, Conant et al., 2011). Additionally, modelling efforts have yet to fully integrate interactions between climate and other important global change drivers, such as nitrogen deposition.

This thesis sets out to investigate the impact that climate warming and nitrogen deposition, two prominent drivers of global environmental change (Sala et al., 2000), will have on net ecosystem carbon balance of New Zealand tussock grasslands, an ecosystem of importance to the national carbon balance that is representative of temperature grasslands globally. Emphasis will be placed on potential impact that these to global change drivers will have on soil respiration as a measure of potential losses to stored soil carbon.

1.2 Temperature as a Global Change Driver

Global temperature is expected to increase by between 1.1 and 6.4°C over the next century in response to rising atmospheric CO_2 concentrations (IPCC, 2007). Rising temperature is predicted to result in wide-ranging consequences for ecosystem structure and function through changes in species ranges

(Malcolm et al., 2002, Harsch et al., 2009), phenology (Price and Waser, 1998), physiology (Saxe et al., 2001, Bradford et al., 2008), biodiversity (Thomas et al., 2004) and species interactions (Petchey et al., 1999) among others.

With regard to ecosystem carbon balance, temperature affects gross primary production and ecosystem respiration both directly, through temperature effects on enzymatic processes involved in photosynthesis and respiration, and indirectly, through changes in phenology, biomass allocation, altered nitrogen cycling and community functional shifts. In order to assess these potential implications of climate warming, numerous studies of the impacts of temperature on physiological and ecological processes have been initiated utilising long-term climate records (Oechel et al., 2000, Bond-Lamberty and Thomson, 2010), climactic gradients (Raich and Schlesinger, 1992, Giardina and Ryan, 2000, Valentini et al., 2000), elevation gradients (Rodeghiero and Cescatti, 2005), passive warming experiments including greenhouses (Hobbie and Chapin, 1998) and open-top chambers (Arft et al., 1999), and active warming experiments including infrared heaters to increase air temperature (Saleska et al., 1999, Luo et al., 2001) and heating cables to warm the soil (Melillo et al., 2002).

In general, warming experiments have shown that a temperature increase of 0.3 to 6° C increases net primary productivity by 19% on average (Rustad, 2001). Gross primary production may increase as a result of the temperature dependence of photosynthesis (Farquhar et al., 1980), although decreases in photosynthesis may result if temperature optima are exceeded due to warming. Increased aboveground biomass allocation and leaf area as a result of warming can also increase gross primary production (Hobbie and Chapin, 1998). Such increases in gross primary production may also be facilitated by increased nitrogen availability, as warming has been shown to increase nitrogen mineralization in a variety of ecosystems by 43% on average (Rustad, 2001, Melillo et al., 2011). As well, changes in plant phenology may contribute to warming-enhanced carbon uptake. Temperate and boreal forests have experienced increased growing season length in response to warming (Menzel and Fabian, 1999, Tucker et al., 2001). Such changes in growing season length have been related to increased carbon uptake by forests (White et al., 1999). Conversely, in grasslands, early plant senescence has also been observed in

response to warming, thereby decreasing growing season length (Zavaleta et al., 2003). Similarly, shifts in carbon allocation from growth to reproduction have been observed in tundra ecosystems (Arft et al., 1999), with likely consequences for net primary production.

While carbon uptake by plants may increase with warmer temperatures, plant respiration is also expected to increase in response to global warming due to an increased cost of maintenance respiration (Ryan, 1991, King et al., 2006). Such increases in plant respiration would present a cost to the carbon economy of plants, reducing net primary production. However, such increases appear to be outweighed by increased gross primary production, as net primary production has been shown to increase as a result of warming (Rustad, 2001). Plant respiration depends on photosynthesis as a source of respiratory substrate and has been observed to acclimate to a constant growing temperature, which may limit the contribution of warming enhanced plant respiration as a potential feedback to rising atmospheric CO_2 concentration (Atkin and Tjoelker, 2003, Loveys et al., 2003).

Soil respiration is widely predicted to increase in response to warming. The global soil respiration record indicates an increase in soil respiration of 0.1 Pg C y^{-1} over the last four decades (Bond-Lamberty and Thomson, 2010). This increase is hypothesised to be a result of the present climate warming trend. Likewise, a meta-analysis of artificial warming experiments has shown that soil respiration increased by 20% on average as a result of temperature increases of 0.3 to 6°C (Rustad, 2001). In particular, the heterotrophic component of soil respiration has been implicated as a likely positive feedback to rising atmospheric CO_2 concentration, as the temperature sensitivity of heterotrophic respiration has been shown to be greater than that of net primary production (Kirschbaum, 1995). Such an imbalance would lead to chronic losses of stored soil carbon to the atmosphere. However, some studies suggest that heterotrophic respiration will be largely insensitive to long-term changes in temperature, with only a small, rapid-turnover component of soil responding to warming while the majority of soil carbon remains inert (Liski et al., 1999, Giardina and Ryan, 2000).

The latter results are supported by several long-running artificial warming experiments, which have shown the effect of warming on soil respiration to be a transient response (Luo et al., 2001, Melillo

et al., 2002). This effect, termed ‘acclimation’, has been explained by the depletion of labile carbon substrates from the soil (Melillo et al., 2002, Hartley et al., 2007), changes in the microbial community (Luo et al., 2001), and physiological acclimation of soil microbes (Bradford et al., 2008). While acclimation is not universal among soil warming experiments (Reth et al., 2009), these experiments do highlight the fact that the response of soil respiration to climate change will not be a simple function of temperature.

The above research on acclimation highlights that substrate availability plays an important role in regulating the response of heterotrophic soil respiration to temperature (Kirschbaum, 2004, Davidson et al., 2006, Larionova et al., 2007). As such, the response of heterotrophic respiration to warming will be determined, in part, by plant activity. Plant roots, in particular, exert a strong influence on the physical and chemical environment within the soil through exudation and turnover, which subsequently affect the rate of heterotrophic soil respiration. The effect that living roots have on the rate of heterotrophic soil respiration is termed the ‘rhizosphere priming effect’. Priming has been shown to result in changes in both the rate of heterotrophic soil respiration, as well as its response to temperature (Uchida et al., 2010, Zhu and Cheng, 2011b).

The net effect of warming on ecosystem carbon balance has been shown to vary greatly. One temperate forest experiment showed ecosystem carbon losses in response to soil warming, although this effect was shown to decrease over a period of 11 y as a result of acclimation of soil respiration and increased net primary productivity (Melillo et al., 2011). Similarly, tundra ecosystems have been shown to acclimate to warming over a period of 10 y, limiting warming-enhanced carbon losses (Oechel et al., 2000). Other temperate grassland sites have shown full compensation of enhanced carbon losses to respiration through increased gross primary production (Wan et al., 2009). Warming increased carbon losses in another temperate grassland site (Saleska et al., 1999), although comparison of experimental warming with a climate gradient study suggested that, over time, potential feedbacks from a changing plant community would limit these losses (Saleska et al., 2002).

1.3 Nitrogen Deposition as a Global Change Driver

Nitrogen deposition as a result of fossil fuel combustion and agricultural fertiliser application currently totals 160 Tg N y^{-1}, exceeding biological nitrogen fixation (Gruber and Galloway, 2008). Due to the global prevalence of nitrogen limitation to plant productivity, anthropogenic nitrogen deposition is expected to affect terrestrial carbon cycling and act as negative feedback to rising atmospheric CO_2 through increased net primary production (LeBauer and Treseder, 2008). Current increases in forest carbon storage have already been attributed to enhanced net primary production as a result of nitrogen deposition (Magnani et al., 2007). However, the sustainability of this enhanced forest carbon sink has been called into question, as nitrogen saturation may occur (Aber et al., 1998).

In addition to enhancing net primary production, nitrogen addition has been shown to have important below-ground effects. In general, increased availability of mineral nutrients lead to decreased carbon allocation to roots (Tilman and Wedin, 1991). Likewise, meta-analysis has shown that microbial biomass decreases by 15% on average following nitrogen addition (Treseder, 2008). As a result of this decreased allocation to roots and smaller microbial biomass, soil respiration has been shown to decrease as a result of nitrogen fertilisation. Although, in tundra, nitrogen addition has increased soil carbon losses, possibly due to rhizosphere priming effects (Mack et al., 2004).

The net effect of nitrogen deposition on ecosystem carbon balance is widely predicted to be a negative feedback to rising atmospheric CO_2 which would result from increased plant uptake and reduced soil carbon losses. As such, nitrogen deposition will act as a potential counterbalance to the expected positive feedback response from warming.

1.4 Study Site

The study site selected for investigating the likely feedbacks of climate warming and nitrogen deposition to rising atmospheric CO_2 concentration is a New Zealand tussock grassland. While forests have received the majority of the research attention, grasslands are an important component of the global terrestrial carbon cycle and may contribute substantially to the terrestrial carbon sink (Scurlock and Hall,

1998, Conant et al., 2001). Temperate grasslands, while representing less than 5% of global net primary production, store 10% of soil organic matter due to high below-ground allocation (Schlessinger, 1997). Large-scale models such as CENTURY suggest that grasslands will be vulnerable to climate change, losing up to 0.1 Pg C y^{-1} globally as a result of warming-enhanced heterotrophic respiration (Parton et al., 1995). However, models also suggest that increased nitrogen deposition will potentially mitigate this effect (Thornley et al., 1991)

Within New Zealand, tussock grasslands are an important land use, totalling 4.3 Mha or 16% of the total land area (Trotter et al., 2004). Though extensive, national-scale models show tussock grasslands to have a near-neutral impact on national carbon balance (Tate et al., 2000). Measurements of net ecosystem exchange in tussock grassland have confirmed the tendency for the ecosystem to shift between a small source and small sink from year to year, dependent on rainfall, which is indicative of a system in steady state (Hunt et al., 2004). However, temperature has been shown to be an important driver of soil carbon turnover in tussock grasslands, indicating a potential for increased CO_2 emissions from soil respiration under climate warming scenarios (Tate, 1992, Tate et al., 1995). As a result, tussock grasslands may become a net source and positive feedback to rising atmospheric CO_2 concentration if such increases in R_S are not countered by net primary production. Additionally, nitrogen fertiliser inputs to New Zealand's ecosystems, currently equivalent to 12.8 kg N ha^{-1} y^{-1} are increasing with agricultural intensification (Parfitt et al., 2006). The individual, as well as combined impacts of these global change drivers on New Zealand's tussock grasslands will be of critical importance to the national carbon inventory.

The Cass Soil Warming Experiment (Fig. 1.2) was constructed at the University of Canterbury Cass Field Station (43.03 S, 171.75 E, 590 m a.s.l.) in an area of montane tussock grassland. In preparation of the experimental site, the vegetation and topsoil were cleared to 200 mm soil depth. The soil was homogenised and redistributed and the native New Zealand tussock species *Chionochloa rigida, Chionochloa flavescens, Poa cita* and *Festuca novae-zelandiae* were planted in consistent composition in each of 20, 12.25 m^2 plots. Subsequent to site construction (completed in January 2009), inter-tussock

areas were allowed to regenerate naturally. Inter-tussock growth was primarily represented by the pasture grass species *Agrostis capillaris*.

Figure 1.2: The Cass Soil Warming Experiment, Cass, New Zealand.

A 3° C soil warming treatment was implemented using buried heating cables. Following the initial clearing of the site, and prior to re-establishment of soil and vegetation, heating cables were installed at a depth of 200 mm in 10 plots following Peterjohn et al. (1993) (Fig. 1.3). Similarly, in control plots, unheated cables were installed. The soil warming treatment was initiated in July 2009 and controlled to maintain a continuous 3° C temperature difference at 100 mm soil depth between control and warming plots.

A 50 kg N ha^{-1} y^{-1} nitrogen addition treatment began in February 2009. Nitrogen was added to 10 plots as calcium ammonium nitrate dissolved in water. Nitrogen addition and warming treatments were applied in a factorial design, resulting in five control plots, five soil warming plots, five nitrogen addition plots, and five combined warming and nitrogen addition plots.

Figure 1.3: Layout of heating cables prior to re-establishment of soil and vegetation in November 2008. Photo by Claudio de Sassi.

The small spatial scale, relative homogeneity of the soil and vegetation, as well as the short stature of the plants made the Cass Soil Warming Experiment an optimal site to study the impacts of warming and nitrogen addition on ecosystem CO_2 exchange using a variety of chamber-based approaches. As well, the factorial design allowed for investigation of possible interactive effects of temperature and nitrogen. As global environmental change will certainly involve changes in a number of drivers in concert, such interactive effects will be important in determining the net effect of global change on ecosystem processes (Norby and Luo, 2004, Leuzinger et al., 2011).

1.5 Thesis Objectives

Given the national- and global-scale implications of increasing global temperatures and nitrogen deposition, this thesis set out to investigate the question: *Will New Zealand tussock grasslands act as a positive feedback to rising atmospheric CO_2 concentration?* The answer to this question is related

strongly to the relative changes in carbon uptake through gross primary production and carbon losses through ecosystem respiration, particularly heterotrophic respiration.

The following objectives were formulated in order to assess the likely feedbacks to rising CO_2 concentration as a result of soil warming and nitrogen addition:

1. Determine the role of autotrophic and heterotrophic respiration in regulating the short-term response of soil respiration to temperature.
2. Determine the drivers of soil respiration and heterotrophic respiration at the seasonal-scale. Further, investigate the impact of long-term warming and nitrogen addition on the response of soil respiration to seasonal temperature.
3. Determine the net effect of soil warming and nitrogen addition on ecosystem carbon balance at the annual time-scale by:
    a. Measuring net carbon exchange and partitioning these exchanges into gross primary production and autotrophic and heterotrophic components of respiration; and
    b. Modelling the responses of each of these processes to seasonal climate drivers in order to integrate point measurements to the annual time-scale.

1.6 Thesis Outline

This thesis addresses the question of how ecosystem CO_2 exchange will be affected by climate warming and nitrogen deposition in three experimental chapters. In Chapter 2, I present the results of a controlled-environment study on temperature responses of soil respiration, measured in microcosms of tussock grassland soil planted with the tussock grass species *Poa cita*. Soil respiration was partitioned into its autotrophic and heterotrophic components at three different temperatures using 1) root-free microcosms as an estimate of heterotrophic respiration and 2) a natural-abundance $\delta^{13}C$ approach for partitioning soil respiration into autotrophic and heterotrophic respiration in the presence of plant roots.

In Chapter 3, I present the results of a long-term field soil warming and nitrogen addition experiment. Soil respiration and its components, autotrophic and heterotrophic respiration, were measured over the course of 27 months at a tussock grassland field site under a 3° C soil warming treatment and a 50 kg N ha^{-1} y^{-1} nitrogen addition treatment. Response curve analyses were used to investigate the effects of seasonal temperature and soil water content on soil respiration and heterotrophic respiration.

In Chapter 4, I present data from one year, over which net ecosystem exchange, ecosystem respiration and soil respiration, and its autotrophic and heterotrophic components were measured under soil warming and nitrogen fertilisation using a chamber-based approach. Analyses of physiological response curves were used to model the response of each component of ecosystem carbon balance to seasonal temperature and soil water content. An empirically-based model of net ecosystem exchange was then used to estimate differences in time-integrated net ecosystem carbon balance as a result of warming and nitrogen addition treatments.

Chapter 5 is a synthesis of the three previous chapters, discussing the integrated effects of soil warming and nitrogen on soil respiration and net carbon balance. I then discuss the implications of these results for national, as well as global carbon balance.

CHAPTER 2: ROOTS AFFECT THE RESPONSE OF HETEROTROPHIC SOIL RESPIRATION TO TEMPERATURE IN TUSSOCK GRASS MICROCOSMS

2.1 Introduction

Soil respiration (R_S) represents an important source of CO_2 from the terrestrial biosphere to the atmosphere, approximately ten times greater in magnitude than anthropogenic CO_2 emissions (Raich et al., 2002, Bond-Lamberty and Thomson, 2010). It is widely accepted that R_S is likely to increase in response to increasing soil temperature, resulting in a positive feedback to rising atmospheric CO_2 concentration and resultant global warming. However, the exact nature of the relationship between R_S and temperature is still poorly understood. Much of this uncertainty arises from confounding effects of the other important drivers of soil respiration, which include soil water content and carbon substrate supply (Davidson et al., 2006). As a result, soil-driven positive feedbacks to climate change remain a critical source of uncertainty in coupled climate models (Sitch et al., 2008).

Soil respiration is a combination of CO_2 fluxes from multiple, distinct carbon sources, primarily autotrophic respiration ($R_{S,A}$), originating from roots and closely associated rhizosphere microbes, and heterotrophic respiration ($R_{S,H}$) from microbial decomposition of soil organic matter (SOM) (Hanson et al., 2000). Separation of these components remains a challenge to evaluating the direct effects of temperature on R_S, as $R_{S,A}$ and $R_{S,H}$ are likely to have different drivers and distinct temperature sensitivities. Much disagreement exists over the relative temperature sensitivities of $R_{S,A}$ and $R_{S,H}$ with some studies have showing that $R_{S,A}$ is more sensitive than $R_{S,H}$ to temperature (Boone et al., 1998, Wan and Luo, 2003), while others show that $R_{S,A}$ and $R_{S,H}$ are similarly sensitive to changes in temperature (Bååth and Wallander, 2003). However, photosynthetic allocation to roots has also been shown as an important driver, particularly of $R_{S,A}$, and potential confounding factor in determining the temperature sensitivity of R_S (Högberg et al., 2001, Bhupinderpal et al., 2003).

While both $R_{S,A}$ and $R_{S,H}$ could potentially increase under climate warming scenarios, it is the breakdown of SOM by soil microbes (i.e., $R_{S,H}$) that is of particular importance, as SOM represents a large pool of stored carbon rather than the carbon recently assimilated by plants. Syntheses of theory and experimental data have led to identification of processes involved in the regulation of SOM dynamics and their likely responses to temperature (Davidson and Janssens, 2006, von Lützow and Kögel-Knabner,

2009, Conant et al., 2011). However uncertainty remains over the net response of decomposition of SOM to temperature. Central to this uncertainty is the existence of multiple pools of carbon in the soil, which have varying turnover times and degrees of recalcitrance (Trumbore, 2000). A small fraction of carbon residing in soils is rapidly turned over by microbial mineralisation, while the majority of SOM remains relatively inert, with turnover times ranging from decades to millennia. Kinetic theory predicts that the more recalcitrant carbon will have a higher temperature sensitivity (Bosatta and Ågren, 1999). However some studies show that only the small, labile carbon component of SOM will increase in turnover under warmer conditions, while the majority of soil carbon will be insensitive to temperature (Liski et al., 1999, Giardina and Ryan, 2000). This is supported by the finding of many soil warming experiments that R_S is enhanced initially by warming, but this effect is reduced over time (Rustad, 2001, Melillo et al., 2002). This apparent acclimation of R_S is hypothesised to be related, in part, to the depletion of a small active carbon pool that is vulnerable to temperature increase, while the majority of soil carbon remains unaffected. However, this observed acclimation is not exclusive of a temperature response of recalcitrant SOM (Kirschbaum, 2004, Larionova et al., 2007).

Another source of uncertainty in evaluating the temperature response of R_S is that the rate of $R_{S,H}$ is known to be influenced by roots, the so called 'rhizosphere priming effect' (Kuzyakov, 2002). The rhizosphere priming effect refers to the influence that living roots exert on SOM turnover due to their impact on the physical and chemical environment within surrounding soil resulting in an increase or decrease in $R_{S,H}$ relative to root-free soil. Priming effects have been shown to range from a 50% decrease in $R_{S,H}$ to a 380% increase in response to the presence of roots (Gärdenäs et al., 2011), varying with plant species, plant phenology and soil fertility (Cheng et al., 2003, Dijkstra et al., 2006, Phillips and Fahey, 2008). Despite the potentially large effect of roots on rates of $R_{S,H}$, the effects of rhizosphere priming on the temperature sensitivity of $R_{S,H}$ have been largely unexplored.

Stable isotope techniques provide a powerful tool for evaluating rates of $R_{S,H}$ in undisturbed systems (Hanson et al., 2000). These techniques utilise distinct $\delta^{13}C$ signatures of CO_2 respired by roots and associated rhizosphere microbes and the respiration of microbes involved in heterotrophic SOM

decomposition to partition R_S into $R_{S,A}$ and $R_{S,H}$. Some recent studies have used such techniques to evaluate temperature effects on $R_{S,H}$ and rhizosphere priming (Bader and Cheng, 2007, Uchida et al., 2010, Zhu and Cheng, 2011b). While these studies have utilised C_3/C_4 shifts and continuous ^{13}C labelling techniques to provide greater contrast in the $\delta^{13}C$ signatures of $R_{S,A}$ and $R_{S,H}$, Millard et al.(2010) have demonstrated successful partitioning of R_S in native C_3 systems. Soil organic matter is typically enriched in $\delta^{13}C$, compared with plant biomass, and microbial biomass is still further enriched compared with the $\delta^{13}C$ signature of bulk soil (Ehleringer et al., 2000, Bowling et al., 2008). This, combined with the fact that $R_{S,A}$ is typically more depleted than root biomass (Zhu and Cheng, 2011a), allows for the possibility of partitioning R_S using a $\delta^{13}C$ approach, without the use of labelling or C_3/C_4 vegetation transitions.

In this study we use a natural abundance $\delta^{13}C$ approach to partition R_S into $R_{S,A}$ and $R_{S,H}$ in microcosms of the C_3 tussock grass *Poa cita* and native tussock grassland soil in order to investigate the short-term responses of $R_{S,A}$, $R_{S,H}$ and rhizosphere priming to changes in soil temperature. This will resolve important sources of uncertainty in temperature effects on R_S by allowing for direct comparison of the temperature sensitivities of root-derived and SOM derived components of R_S in an undisturbed soil-plant system. As a model system, our tussock grass microcosms are representative of grasslands in New Zealand, which allocate a large proportion of their carbon belowground and are an important store of soil carbon nationally (Trotter et al., 2004). As well, globally, grasslands represent an important belowground carbon sink (Scurlock and Hall, 1998). We present an experimental framework which could be applied to other soil and vegetation types in order to constrain temperature responses of SOM and reduce uncertainty in soil-driven feedbacks in coupled-climate models.

2.2 Methods

2.2.1 Soil description

The soil used in this experiment was a silt loam collected from a tussock grassland in central South Island, New Zealand (lat: 43.034°S, long: 171.758°E, elevation: 590 m above sea level). Soils are classified as acidic allophanic brown by the New Zealand Soil Classification System (Hewitt, 2010), with

a total carbon content of 4.2%, total nitrogen content of 0.30% and an average microbial biomass carbon of 592 mg kg soil^{-1} . The top 300 mm of the mineral horizon was excavated, sieved through an 8 mm sieve and well mixed. Field-moist conditions were maintained in order to preserve microbial biomass.

2.2.2 Microcosm design

Twenty-eight polyvinyl chloride (PVC) pots, 200 mm in diameter and 300 mm deep, were each filled with 8.5 kg of field-moist soil. The bottom of each microcosm was covered with 80% shade cloth to ensure adequate drainage. A 100 mm diameter PVC measurement collar was inserted to a depth of 50 mm in the centre of each microcosm. In 20 of the microcosms, three plants of the tussock grass *Poa cita*, 100 mm in height were planted around the periphery of the pot. Remaining microcosms were left unplanted as root-free controls. Microcosms were left for five months in a shade house to allow the soil to settle and roots to proliferate before being moved into two controlled environment chambers set to 15°C with a 14 hour day length and 1000 μmol m^{-2} s^{-1} irradiance (400-700 nm). Microcosms were maintained in growth cabinets for five months prior to measurement. At the time of measurement, average root biomass, as estimated from a soil core taken from the centre of each measurement collar at the conclusion of the experiment, was 12.2 ± 3.9 g pot^{-1} dry weight (approximately 7.8 l soil volume).

Microcosm soil was maintained at approximately 35% volumetric water content by wetting soils to field capacity two days prior to measurement. When measurements were conducted on consecutive days, changes in mass were used to determine water loss. Additional water was added after each day's measurements to maintain constant soil water content over the entire period of measurement.

2.2.3 Temperature control

Soil temperature (T_S) was manipulated by wrapping each microcosm with a 100 W resistance heating cable (Argus Heating Ltd. Christchurch, New Zealand) covered with insulation. Soil temperature was measured by a thermocouple (Type-T, Omega Engineering, Inc., Stamford, Ct USA), placed at 100 mm depth in the centre of each microcosm, and recorded on a datalogger (CR-5000, Campbell Scientific,

Logan, UT). Heating was applied by pulse width modulation with maximum heating times of 40-60 s with off times of 40-120 s in order to achieve a constant rate of warming (2°C h^{-1} and hold a steady soil temperature once measurement temperature was reached. Though some lateral temperature gradient was inevitable, by applying heat in pulses, the temperature difference between the edge of the pot and centre could be maintained at less than 5°C and thermocouple placement was selected to represent the temperature of the soil most relevant to the measured respiration rates, directly beneath the measurement collars. Heating was started 8 hours prior to measurement in order to bring soil temperature to a measurement temperature of 15 (no heating), 20, or 25°C.

2.2.4 Isotope measurements

R_S was partitioned into $R_{S,A}$ and $R_{S,H}$ using a natural-abundance $\delta^{13}C$ approach (Midwood et al., 2008, Millard et al., 2010). Soil surface efflux was collected using a dynamic chamber system described by Midwood and Millard (2011). A PVC chamber was sealed on the measurement ring of a microcosm with a foam gasket. Chamber CO_2 concentration was maintained at 50-100 μmol mol^{-1} above ambient by supplying CO_2-free air at a rate of 20-120 ml min^{-1} depending on the rate of R_S. Soil surface efflux was sampled from the chamber at a rate 5-10 ml min^{-1} lower than the CO_2-free air supply to prevent incursion of atmospheric air. Ambient pressure was maintained by allowing excess air to escape through a vent tube. CO_2-free air flow was maintained over a period of 150 min, the maximum time required to achieve steady-state conditions within the chamber and purge all atmospheric air. CO_2 was then collected into an evacuated Tedlar® bag for 20-30 min and analysed for $\delta^{13}C$ (‰) on a tunable diode laser (TGA-100A, Campbell Scientific Inc., Logan, UT, USA). This method has been tested by Midwood et al. (2008) on columns of sand with CO_2 flow of a known $\delta^{13}C$ signature and shown to provide an accurate sample of soil surface efflux, without fractionation. Immediately following gas collection, chambers were removed and, following a ten minute equilibration period, the rate of R_S was measured with a portable respiration system (SRC-1 and EGM-4, PP Systems, Hitchin, UK). The proportion of R_S contributed by heterotrophic

respiration (fR_H) was determined using the two end member mixing model (Robinson and Scrimgeour, 1995):

$$fR_H = 1 - \frac{\delta^{13}C_{RS} - \delta^{13}C_{RH}}{\delta^{13}C_{RA} - \delta^{13}C_{RH}} \quad (2.1)$$

where $\delta^{13}C_{RS}$ is the $\delta^{13}C$ signature of soil surface efflux and $\delta^{13}C_{RA}$ and $\delta^{13}C_{RH}$ are the $\delta^{13}C$ signatures of the autotrophic and heterotrophic respiration. Soil surface efflux was collected from each microcosm at measurement temperatures of 15, 20 and 25°C. The order of measurements was randomised with the limitation that, on the final day of measurement, half of the microcosms were measured at 15°C and half at 25°C. Likewise, time of measurement was randomised, as half of the measurements were conducted in the morning and the remainder in the afternoon.

Microcosms containing no plants were used as an independent measure of $R_{S,H}$ in the absence of roots, $R_{S,HF}$. Only respiration rate was measured in these microcosms, CO_2 was not collected for isotopic analysis as no partitioning was necessary.

The $\delta^{13}C$ signature of $R_{S,H}$ was obtained by taking a 65 mm diameter soil core from the centre of the measurement collar immediately following the final collection of soil surface efflux. Roots were rapidly removed within 3-5 min and root-free soil was then sealed in a Tedlar® bag. The bag was evacuated using a suction pump and repeatedly flushed with nitrogen. Soil microbes are known to rapidly shift their substrate utilisation following disruption of the soil structure (Crow et al., 2006, Pendall and King, 2007), so nitrogen was used to remove oxygen and slow soil microbial activity while atmospheric air was purged from the soil. Following 2-3 min of flushing with nitrogen, CO_2-free air was added to the bag and newly-respired CO_2 was allowed to accumulate. When CO_2 concentration reached a value within the calibrated range of the TDL (300-500 μmol mol^{-1}), CO_2 was analysed for $\delta^{13}C$. This value, obtained within 10-15 min of taking the core, is the most consistent estimate of the isotopic signature of $R_{S,H}$ and

has been validated in the field through comparison with soil surface efflux collected from root-exclusion plots.

The $\delta^{13}C$ signature of $R_{S,A}$ was obtained by rinsing roots removed from the soil core, sealing them in a Tedlar® bag, evacuating the air with a suction pump and flushing repeatedly with CO_2-free air. Roots were then incubated in CO_2-free air until the concentration of root respired CO_2 was within the calibrated range of the TDL.

2.2.5 Statistical analyses

An Arrhenius-type equation was used to model the effect of temperature on respiration rate (Lloyd and Taylor, 1994):

$$R = R_{10} exp E_0 \left(\frac{1}{56.02} - \frac{1}{T - 227.13}\right) \quad (2.2)$$

where R_{10} is the basal rate of respiration at 10°C, E_0 is related to the energy of activation and T_S is soil temperature (K). Equation (2.2) was fitted to measurements of R_S, $R_{S,A}$, $R_{S,H}$ and $R_{S,HF}$ using non-linear mixed-effects models conducted in the 'nlme' package (Pinheiro and Bates, 2000) for R v.2.12.1 (R Development Core Team 2010). Each sample in the analysis consisted of one measurement of respiration (R_S, $R_{S,A}$, $R_{S,H}$ or $R_{S,HF}$), at a given temperature on a single day. Microcosms were included as random effects to account for repeated measurement of the same microcosm at different temperatures. A model including the presence of roots as a fixed effect on R_{10} and E_0 was compared to a model that did not include the fixed effect of roots using a likelihood ratio test.

The effect of temperature on $\delta^{13}C_{RA}$ was evaluated by conducting a Student's t-test between $\delta^{13}C$ signatures of root-respired CO_2 at 15 and 25°C. This was repeated for $\delta^{13}C_{RH}$. The effect of temperature on $\delta^{13}C_{RH}$ was modelled using least squares regression.

In order to include the error associated with the isotopic partitioning method into data analyses, we used a simulation-based approach to generate a distribution of $R_{S,H}$ values against which to compare our observed values. Mean fR_H and standard error were calculated according to Phillips and Gregg (2001)

at 15 and 25°C using the subset of microcosms for which each $\delta^{13}C_{RS}$, $\delta^{13}C_{RA}$ and $\delta^{13}C_{RH}$ were collected at the same time and at the same temperature (n = 10 at each 15 and 25°C). A population of 1000 samples of fR_H was simulated using means and standard errors calculated as described above. Simulated populations of 1000 samples each were also produced using means and standard errors of R_S calculated from measurements. Simulated values of fR_H were then used to partition simulated values of R_S to produce 1000 samples of $R_{S,H}$, which reflect variation resulting from both measurements of R_S and the partitioning method. This population, reflecting all sources of variation, was then used to produce 95% confidence intervals for $R_{S,H}$ at each 15 and 25°C.

2.3 Results

Average (± SE) $\delta^{13}C_{RA}$ and $\delta^{13}C_{RH}$ were -29.73 ± 0.08 and -22.94 ± 0.17‰ respectively. A Student's t-test determined that $\delta^{13}C_{RA}$ was not significantly different between 15 and 25°C (p = 0.40, Table 2.1), and thus the value of $\delta^{13}C_{RA}$ that was measured for a given microcosm was used for partitioning that microcosm at each of the three measurement temperatures. The $\delta^{13}C$ signature of $R_{S,H}$ was significantly affected by temperature ($p < 0.002$), becoming depleted by 1‰ at 25°C compared to 15°C. As it was only possible to measure $\delta^{13}C_{RA}$ and $\delta^{13}C_{RH}$ at 15 and 25°C, a $\delta^{13}C_{RH}$ (‰) value was modelled from the linear regression equation:

$$\delta^{13}C_{RH} = -0.08 * T_S - 21.26 \quad (2.3)$$

where T_S is soil temperature (°C). This soil end-member agrees well with the $\delta^{13}C_{RS}$ of -22.1 ± 0.2‰ (n = 6) measured for root exclusion plots in the tussock grassland from which the microcosm soil was sampled. Average $\delta^{13}C_{RS}$ was -27.73 ± 0.10‰. Similar to $\delta^{13}C_{RH}$, $\delta^{13}C_{RS}$ became more depleted as temperature increased, though to a greater extent, reflecting both the more depleted signature of $R_{S,H}$ as well as a greater contribution of roots to R_S (Table 2.1). Given the differences between $\delta^{13}C_{RS}$, $\delta^{13}C_{RA}$ and $\delta^{13}C_{RH}$, all microcosms were successfully partitioned at all temperatures.

Table 2.1: Average (± SE) $\delta^{13}C$ signatures of soil surface efflux (R_S), root respiration ($R_{S,A}$) and heterotrophic respiration ($R_{S,H}$) by measurement temperature.

Respiration Component	*n*	Soil temperature (°C)	$\delta^{13}C$ (‰)
R_S	20	14.4 ± 0.1	-26.97 ± 0.13
	20	20.6 ± 0.1	-27.98 ± 0.12
	20	25.6 ±0.2	-28.25 ± 0.14
$R_{S,H}$	10	14.5 ± 0.1	-22.46 ± 0.24
	10	25.8 ± 0.1	-23.42 ± 0.13
$R_{S,A}$	10	14.5 ± 0.1	-29.81 ± 0.11
	10	25.8 ± 0.1	-29.65 ± 0.13

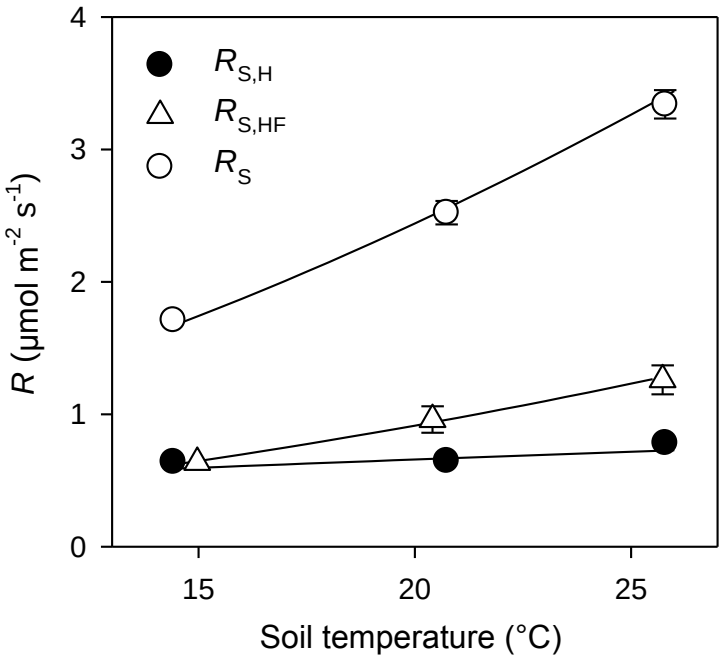

Figure 2.1: Relationship between temperature and rate of total soil respiration (R_S, open circles), heterotrophic respiration in the presence of roots ($R_{S,H}$, filled circles) and heterotrophic respiration in root-free soil ($R_{S,HF}$, triangles) with curves fitted using Equation (2.2).

As well as isotopic signature, the rate of R_S was strongly influenced by the presence of roots, with $R_{S,HF}$ equivalent to approximately 37% of R_S at all temperature levels (Fig. 2.1). Likewise, partitioned $R_{S,H}$ was a small proportion of R_S, with fR_H ranging from 0.38 at 15°C to 0.23 at 25°C (Table 2.2). The temperature sensitivity of $R_{S,H}$ was also affected by the presence of roots (Fig. 2.1). With roots present, $R_{S,H}$ had a much lower temperature sensitivity, i.e. a lower E_0, than R_S, while the temperature sensitivity of $R_{S,HF}$ was very similar to that of R_S (Table 2.3). Likelihood ratio test indicated that a model of $R_{S,H}$ which included the presence of roots as a fixed effect on E_0 provided a significantly better fit than a model which did not include the effect of presence of roots on $R_{S,H}$ ($p = 0.0002$).

Table 2.2: Average (± SE) rate of total soil respiration (R_S) and heterotrophic respiration in the presence ($R_{S,H}$) and absence of roots ($R_{S,HF}$) at three different measurement temperatures and the proportion of soil respiration constituted by heterotrophic respiration (fR_H).

Respiration Component	*n*	Soil temperature (°C)	Respiration rate (μmol CO_2 m^{-2} s^{-1})	fR_H
R_S	20	14.4 ± 0.1	1.71 ± .07	0.38 ±.02
	20	20.6 ± 0.1	2.52 ± .09	0.26 ±.01
	20	25.6 ±0.2	3.34 ± .11	0.23 ±.02
$R_{S,H}$	20	14.4 ± 0.1	0.64 ± .03	-
	20	20.6 ± 0.1	0.65 ± .04	-
	20	25.6 ±0.2	0.78 ± .07	-
$R_{S,HF}$	8	14.4 ± 0.1	0.64 ± .05	-
	8	20.6 ± 0.1	0.96 ± .10	-
	8	25.8 ± 0.1	1.26 ± .11	-

Table 2.3: Average (± SE) parameter values of E_0 (kJ mol^{-1}) and R_{10} (μmol m^{-2} s^{-1}) for total soil respiration (R_S), autotrophic respiration ($R_{S,A}$) and heterotrophic respiration in the presence ($R_{S,H}$) and absence ($R_{S,HF}$) of roots. Parameters were fitted by non-linear mixed-effects model using Equation (2.2).

Respiration Component	*n*	E_0	*p*-value	R_{10}	*p*-value
Soil and roots:					
R_S	60	272 ± 12	<0.0001	1.17 ± 0.06	<0.0001
$R_{S,H}$	60	80 ± 37	<0.0001	0.53 ± 0.05	0.0377
$R_{S,A}$	60	347 ± 19	<0.0001	0.67 ± 0.05	<0.0001
Root-free soil:					
$R_{S,HF}$	64	279 ± 27	<0.0001	0.43 ± 0.03	0.0001

Rhizosphere priming effects, calculated as the difference between $R_{S,H}$ and $R_{S,HF}$, as a percentage of $R_{S,HF}$, were 0, -32 and -38% at 15, 20 and 25°C respectively, indicating no priming effects at 15°C and negative priming effects at 20 and 25°C.

Calculation of errors in fR_H associated with the isotopic partitioning method following Phillips and Gregg (2001) resulted in average values (± SE) for fR_H of 0.40 ± 0.04 at 15°C and 0.25 ± 0.04 at 25°C. These values differ slightly from those in Table 2.2 as they represent a subset of the data for which $\delta^{13}C_{RS}$, $\delta^{13}C_{RA}$ and $\delta^{13}C_{RH}$ were measured in the same microcosm, at the same temperature, while the mixed-effects models utilised the entire dataset by using a single $\delta^{13}C_{RA}$ for each microcosm and a value of $\delta^{13}C_{RH}$ modelled from T_S. The simulation of $R_{S,H}$ resulted in 95% confidence intervals between 0.54 and 0.83 μmol m^{-2} s^{-1} at 15°C, which overlap completely with the confidence intervals of $R_{S,HF}$ which occurred between 0.54 and 0.73 μmol m^{-2} s^{-1}, suggesting no significant priming effects at 15°C. At 25°C, confidence intervals of $R_{S,H}$ occurred between 0.57 and 1.09 μmol m^{-2} s^{-1} and confidence intervals of $R_{S,HF}$ were between 1.02 and 1.49 μmol m^{-2} s^{-1}. The confidence intervals of $R_{S,H}$ and $R_{S,HF}$ overlap slightly,

however, in ananalysis where 1000 values of each $R_{S,H}$ and $R_{S,HF}$, treated as pairs, were simulated, 99.5% of simulations resulted in values of $R_{S,H}$ lower than those of $R_{S,HF}$, supporting the results of the non-linear mixed-effects model which indicated negative priming effects at higher temperatures.

2.4 Discussion

In our system, $R_{S,A}$ is primarily responsible for driving both the rate and temperature sensitivity of R_S. On average, $R_{S,H}$ contributed less than 30% of total R_S and, in the presence of roots, $R_{S,H}$ was insensitive to a temperature increase of 10°C. In the absence of roots, $R_{S,H}$ ($R_{S,HF}$ here) exhibited a similar temperature response to R_S, suggesting that roots play a critical role, not only in contributing to overall rate of R_S, but also in regulating the temperature response of $R_{S,H}$ in this system.

The priming effects documented here, ranging from no effect at 15°C to a 38% decrease in $R_{S,H}$ (negative priming effects) in the presence of roots at 25°C, fall well within the range of published values for priming effects reviewed by Gärdenäs et al. (2011). Mechanistic explanations for negative priming effects include competition for mineral nutrients (particularly nitrogen) between the rhizosphere and heterotrophic soil microbes and preferential substrate utilisation (Kuzyakov, 2002). The 'preferential substrate use' hypothesis states that soil microbes preferentially use labile, root-derived substrates over more recalcitrant SOM resulting in decreased SOM decomposition in the presence of roots.

In our study, low labile substrate availability is a particularly likely scenario due to the fact that harvest and preparation of our microcosm soils constituted a significant soil disturbance. Average total carbon concentration (± SE) measured for undisturbed tussock grassland adjacent to the area where microcosm soils were sampled was 6.2 ± 0.2%, 30% higher than that for microcosm soil. This suggests that soil carbon was lost during the disturbance, likely from the most labile fraction. This supports preferential substrate utilisation as a possible explanation for suppressed rates of $R_{S,H}$ with increasing temperature in rooted soil, as it is likely there was a large difference in the quality of root-derived and soil-derived carbon.

While preferential substrate use serves to explain negative priming effects at a single temperature, it does not provide an explicit mechanism for the observed temperature response of rhizosphere priming. A previous study showed similar dampening of the temperature response of $R_{S,H}$, which was also attributed to preferential use of root-derived carbon at warmer temperatures (Uchida et al., 2010). However, this study involved warming of both plant and soil, and a corresponding measured increase in photosynthesis was hypothesised to fuel the increasing carbon demand by the rhizosphere. In our study, only T_S was manipulated, thus without an increase in leaf temperature, it is unlikely that photosynthetic rates were strongly affected by soil warming. Long term temperature increases have been known to increase carbon exudation by roots (Uselman et al., 2000). However, it is uncertain how root exudation responds to short-term warming, especially in the absence of increased photosynthesis, as in our study. Without further measurements of carbon substrate availability and microbial biomass and activity at the different temperatures, it is impossible to propose a definitive explanation for the observed temperature response of rhizosphere priming. However our results indicate a shift toward microbial use of root-derived carbon with increasing temperature.

Regardless of the mechanism behind the observed decrease in temperature sensitivity of $R_{S,H}$, these results have important implications for the use of temperature responses of $R_{S,H}$ measured using root exclusion methods or incubations of root-free soils. In our study, parameterisation of models of $R_{S,H}$ using data from our root-free soils would result in a substantial overestimate of $R_{S,H}$. Of particular relevance are diurnal temperature responses of R_S. In the tussock grassland field site from which our microcosm soils were sampled, during the five months when plants are most active, diurnal temperature variation at 100 mm soil depth is 6°C on average, but can be as great as 13°C. Our results suggest that temperature related variation in R_S can mainly be attributed to $R_{S,A}$ at this time scale.

While it is certain that these results should not be generalised to other soil and vegetation types, and represent only short-term temperature responses of $R_{S,H}$, it is also clear that temperature responses of $R_{S,H}$ that do not include the effects of roots should be applied with caution, as they may produce erroneous estimates of SOM turnover when applied in models of carbon cycling. We present here an experimental

approach, which could be applied to other soil types, vegetation types and temperature regimes in order to produce more precise temperature responses of $R_{S,H}$ and include the effects of roots in models of soil carbon cycling.

CHAPTER 3: EFFECTS OF SOIL WARMING AND NITROGEN ADDITION ON SOIL RESPIRATION IN A NEW ZEALAND TUSSOCK GRASSLAND

3.1 Introduction

Soils represent a pool of organic carbon approximately double that stored in terrestrial biomass (Schlesinger and Andrews, 2000). At present, soil respiration, the primary pathway for return of soil carbon to the atmosphere, is increasing globally by 0.1 Pg C y^{-1} (Bond-Lamberty and Thomson, 2010). This increase, hypothesised to be a result of global warming, is concerning as temperatures are expected to rise by as much as 6.4°C over the next century (IPCC, 2007). Coupled climate models indicate a likely soil-driven positive feedback to climate change, although uncertainty remains in the magnitude of this feedback (Cox et al., 2000, Sitch et al., 2008)

Global change scenarios also suggest that nitrogen cycling in terrestrial ecosystems will be altered. Nitrogen deposition due to crop fertilisation and fossil fuel combustion currently exceeds terrestrial nitrogen fixation and is expected to increase in the future (Gruber and Galloway, 2008). As warming has also been shown to increase nitrogen mineralization (Rustad, 2001), there lies the possibility for synergistic effects of warming and anthropogenic nitrogen deposition on plant-available nitrogen. Feedbacks between the nitrogen and carbon cycles are well documented (Melillo et al., 2011), and it is likely that nitrogen availability will play a role in determining the magnitude of the terrestrial feedback to rising atmospheric CO_2 concentration.

At short time scales, soil respiration (R_S) is primarily regulated by temperature and soil water availability. At these scales, empirically-based temperature response curves can provide good estimates of R_S (Lloyd and Taylor, 1994, Savage and Davidson, 2001). However, these short-term temperature response curves have limited ability to predict the effects of sustained climate warming. This is due to the likelihood of interactions between temperature and other drivers of R_S including belowground photosynthetic allocation, carbon substrate availability, and nitrogen availability (Davidson et al., 2006, Subke and Bahn, 2010).

Numerous warming experiments have been undertaken across biomes in order to investigate the impacts of long-term climate warming on carbon cycling. Meta-analysis suggests that, on average, warming (0.3-6.0°C) increases R_S by 20% (Rustad, 2001). However, several notable examples have

shown the effect of warming on R_S to be only transient (Luo et al., 2001, Melillo et al., 2002). Mechanisms for this acclimation of R_S to prolonged warming include depletion of labile carbon substrates (Melillo et al., 2002, Kirschbaum, 2004, Hartley et al., 2007), changes to the microbial community structure (Luo et al., 2001, Belay-Tedla et al., 2009), physiological acclimation of soil microbes (Bradford et al., 2008), reduction in root biomass (Zhou et al., 2011) and reduction in specific root respiration rate (Burton et al., 2008). Regardless of the mechanism, acclimation of soil respiration may represent a limit to the amount of carbon lost from soils as a result of climate warming.

While warming-induced increases in R_S represent a likely positive feedback to rising atmospheric CO_2 concentration, nitrogen deposition has been implicated as a possible mitigating factor in temperate forests due to negative impacts of nitrogen addition on R_S (Janssens et al., 2010). Meta-analysis suggests that reduction in R_S may represent a carbon offset equivalent to the nitrogen fertilisation effect on primary production. Similarly, depression of R_S has been observed in grasslands as a result of nitrogen addition (de Jong et al., 1974, Yan et al., 2010).

The net response of R_S to warming and nitrogen depends largely on the combined response of its components, autotrophic soil respiration ($R_{S,A}$) and heterotrophic soil respiration ($R_{S,H}$), which are likely to have differing responses to environmental drivers. Autotrophic respiration refers to respiratory activity of roots and associated rhizosphere microbes, while heterotrophic respiration refers to soil organic matter decomposition by soil microbes (Hanson et al., 2000). The important distinction between the two is that $R_{S,A}$ represents respiration of carbon recently assimilated by plants, whereas $R_{S,H}$ represents release of carbon that may have residence times in the soil reaching millennia (Trumbore, 2000).

The temperature response of $R_{S,H}$ has been well studied and $R_{S,H}$ is widely expected to increase under warming scenarios (Kirschbaum, 1995, Davidson and Janssens, 2006). Such increases in $R_{S,H}$ have been demonstrated in numerous soil warming experiments (Zhou et al., 2007, Schindlbacher et al., 2009). Conversely, nitrogen addition has been shown to decrease microbial biomass (Treseder, 2008). This may be the mechanism for the overall decrease in R_S observed in response to nitrogen addition (Janssens et al., 2010).

Changes in $R_{S,A}$ can result from both changes in specific metabolic activity of roots as well as changes in root biomass, which have consequences for plant carbon balance. Specific root respiration is expected to increase with warmer temperatures (Pregitzer et al., 2000). However, root metabolic activity can acclimate to warming, potentially mitigating respiratory losses in response to warming (Atkin et al., 2000, Loveys et al., 2003). In one temperate forest soil warming experiment, roots were shown to exhibit lower specific respiration rates in response to warming (Burton et al., 2008). The contribution of $R_{S,A}$ to R_S depends not only on specific root respiration rate, but also on roots biomass. Responses of root biomass to warming range from 18% increase in root biomass at a temperate grassland site (Wan et al., 2005), to a 42% decrease in biomass at a temperate forest site (Zhou et al., 2011).

Responses of $R_{S,A}$ to nitrogen are also likely to be variable. In general, there is a good relationship between root nitrogen concentration and specific respiration rate (Pregitzer et al., 1998, Bahn et al., 2006). As such, increases in plant available nitrogen are likely to result in increases in $R_{S,A}$. However, along with root activity, root biomass will also play a role in the net response of $R_{S,A}$ to nitrogen addition. Plants are known to reduce allocation to roots in response to increased nutrient availability (Tilman and Wedin, 1991). Consequently, reductions in root biomass have been observed in response to nitrogen addition in both forests and grassland (Ryan et al., 1996, Bardgett et al., 1999), although increases in root biomass in response to nitrogen addition have also been noted (Yan et al., 2010).

In this study, we investigated the impacts of soil warming and nitrogen addition, as well as their interaction, on R_S and its components, $R_{S,A}$ and $R_{S,H}$. Such multifactor experiments are important to improve the predictive ability of coupled-climate models, as single factor experiments may fail to predict interactive effects of global change drivers (Norby and Luo, 2004, Leuzinger et al., 2011). Likewise, partitioning the components of R_S can lead to greater mechanistic understanding of the response of R_S to its drivers (Chen et al., 2011).

We used a native tussock grassland system as a model system as grasslands are a widespread and important store of carbon nationally (Trotter et al., 2004), as well as globally (Scurlock and Hall, 1998). R_S and $R_{S,H}$ were measured over a period of 27 months with the objective of determining the likely

feedback effect that R_S in grasslands will have on rising atmospheric CO_2 concentration in response to soil warming and nitrogen addition.

3.2 Methods

3.2.1 Study site

This study was conducted at the Cass Warming Experiment at the University of Canterbury Cass Field Station in central, South Island, New Zealand (43.03 S, 171.75E, 590 m a.s.l.). The site was constructed in January 2009 in an area of tussock grassland. Soils at the site are classified as acidic allophane brown by the New Zealand Soil Classification System (Hewitt, 2010) and Typic Dystrochrept by USDA soil taxonomy (Soil Survey Staff, 2006). Prior to this study, vegetation and the top 200 mm of topsoil were removed, twenty 12.25 m^2 plots were laid out and 90 m of resistance heating cable (Argus Heating, Ltd., Christchurch, New Zealand) were placed in each plot to achieve a heating density of 76 W m^{-2} (with dummy cables added to unheated plots). The cables were then covered with 200 mm of topsoil and the native New Zealand tussock grasses *Festuca novae-zelandiae* (50 individuals per plot), *Poa cita* (50 per plot), *Chionochloa rigida* (22 per plot), and *Chionochloa flavescens* (12 per plot) were planted.

In each of 10 plots designated for the warming treatment, three thermocouples (Type-E, Campbell Scientific, Logan, UT, USA) were buried to a depth of 100 mm in a stratified design which captured a range of horizontal distances from heating cables (directly above, one quarter of the distance between two cables and the midpoint). In each of the control plots, one thermocouple was buried to 100 mm soil depth. Temperature was controlled to maintain a 3° C difference between the average of the three thermocouples in warmed plots and the nearest un-warmed plot. Warming was controlled and hourly average plot temperature was recorded using a datalogger (CR1000X, Campbell Scientific, Logan, UT, USA). An auxiliary weather station measured hourly average air and soil temperature at 100 mm soil depth, soil volumetric water content at 100 mm soil depth and precipitation.

In February 2009, a 50 kg $ha^{-1}y^{-1}$ nitrogen addition treatment was initiated. Nitrogen was applied as calcium ammonium nitrate in 10 kg N ha^{-1} amounts spread throughout the growing season. For each

plot, calcium ammonium nitrate was dissolved in 4 L water and distributed using a watering can over both plants and soil. The continuous 3° C warming treatment was initiated in July 2009. Nitrogen and warming treatments were applied in a factorial design resulting in five control plots, five soil warming plots, five nitrogen addition plots and five combined warming and nitrogen addition plots. Two plots, one each of the soil warming and combined warming and nitrogen addition treatments, were subsequently dropped from analyses due to malfunctioning heating cables.

3.2.2 Respiration measurements

Measurements of soil respiration were carried out over a 27 month period beginning in August 2009 (winter) and continuing through October 2011 (spring). Six 100 mm diameter polyvinyl chloride measurement collars were installed to a soil depth of 70 mm in each plot one month prior to the start of measurements. The rate of soil respiration in each collar was measured at 2-4 week intervals using a portable respiration system (SRC-1 and EGM-4, PP Systems, Hitchin, UK). A further two measurement collars were installed in each plot to a soil depth of 300 mm in order to exclude roots and provide an estimate of heterotrophic respiration ($R_{S,H}$). Measurement of these collars began in January 2010. Simultaneous with soil respiration measurement, soil temperature at 50 mm depth was measured with a thermocouple (Type-E, Omega Engineering, Ltd, Stamford, CT, USA) and soil water content in the top 50 mm of soil was measured with a soil moisture sensor (Theta Probe type ML1 and ML2, DeltaT Devices, Cambridge, UK).

3.2.3 Substrate addition

In order to assess levels of substrate limitation induced by warming and nitrogen addition treatments, as well as presence of roots, a substrate addition experiment was carried out in late-October 2011. In each of 16 plots representing four replicates for each treatment, two pairs of soil respiration measurement collars were selected: one pair with roots present and another pair with roots excluded. One collar from each pair was selected randomly for substrate addition. All collars were measured

immediately prior to substrate addition. Subsequent to initial measurement, the two collars from each plot selected for substrate addition were amended with 20 ml of 0.2 M sucrose solution (an amount approximately equivalent to 10 d carbon losses from $R_{S,H}$). In order to ensure that the sucrose solution infiltrated beyond the soil surface, the solution was injected with a syringe at a depth of 25 mm in 5 ml aliquots at four locations within the collar. The collars designated as controls were similarly treated with water. Soil respiration was then measured in each collar at 30 min, 1 h, 2.5 h, 4 h and then at 4 – 8 h intervals until the substrate response was no longer evident. Substrate induced respiration (S_I) was calculated for each pair of collars as the difference between respiration rates of the control and substrate-added collars as a proportion of the control rate.

3.2.4 Soil analyses

Soils were sampled in January 2010, March 2011 and March 2012. Three 54 mm diameter soil cores were taken to a depth of 100 mm in each plot and the soil was homogenized into a single sample. Roots were removed by 8 mm sieve and dried at 60° C. As the grass roots were very fine, in 2012 a subsample was removed from the whole sample and washed over a 650 µm sieve to obtain root biomass. Microbial biomass was estimated using the fumigation-extraction technique adapted from Vance et al. (1987). Remaining soil was dried at 60° C, passed through a 2 mm sieve to remove remaining roots and ground in a ball mill. Samples were then analysed for organic carbon and total nitrogen concentration on an elemental analyser (CNS2000, Leco, St. Joseph, MO, USA).

Between 20 September 2011 and 22 October 2011, plant available nitrogen was estimated using ion exchange membranes (PRS probes, Western Ag Innovations, Saskatoon, Canada). The PRS probes were installed to a depth of 100 mm at three locations in each plot. Following a one-month burial period, probes were removed, rinsed with deionized water and returned to Western Ag Innovations for analysis of NH_4^+ and NO_3^-.

3.2.5 Statistical analyses

The effects of warming and nitrogen addition on seasonal measurements of R_S, $R_{S,H}$ and soil water content were assessed using linear mixed-effects models conducted in the 'nlme' package (Pinheiro et al., 2012) in R version 2.12.1 (R Development Core Team, 2010). Each measurement of R_S, $R_{S,H}$ or soil water content was treated as a sample. Warming, nitrogen addition and measurement date, along with their interactions were included as fixed effects, while measurement collars nested within plots were included as random effects to account for the non-independence of multiple samples through time. Residual analyses were undertaken and log transformation was used for R_S and $R_{S,H}$, to correct for heteroscedasticity. The effects of warming and nitrogen on the proportion of R_S constituted by $R_{S,H}$ (fR_H) were similarly assessed treating plot averages of fR_H on a given date as a sample and evaluating random effects at the plot level.

Temperature responses of R_S and $R_{S,H}$ were fitted to an Arrhenius-type curve (Lloyd and Taylor, 1994), modified with a soil water content response function (Bahn et al. 2008):

$$R_S = R_{10} * \exp E_0 \left(\frac{1}{56.02} - \frac{1}{T_S - 227.13}\right) * \exp(-\exp(a - b * \theta) \qquad (3.1)$$

where T_S is soil temperature (° K), R_{10} is the basal respiration rate at 10° C (μmol m^{-2} s^{-1}), E_0 is the activation energy of enzymatic reactions (kJ mol-1), θ is the soil volumetric water content (m^3m^{-3}) and a and b are parameters which determine the shape of a sigmoidal response of respiration to soil water content.

Non-linear mixed-effects models (also conducted in the 'nlme' package for R) were used to fit Equation (3.1) initially to measurements of R_S, and subsequently to $R_{S,H}$. First, the effect of roots on the temperature response of R_S was investigated by testing how root presence (as a fixed effect) altered parameter values for R_{10} and E_0. Subsequently, temperature responses of R_S and $R_{S,H}$ were investigated in separate models, with the latter substituting $R_{S,H}$ in place of R_S in Equation (3.1). Warming and nitrogen addition, as well as their interactions, were investigated as fixed effects on R_{10} and E_0 for both the R_S and $R_{S,H}$ models. For all the above nonlinear models, random effects were evaluated at the level of

measurement collars within plots. Fixed and random effects on *a* and *b* were not evaluated, as few measurement dates occurred under water-limited conditions, and a generic moisture response curve which limited respiration beneath 0.2 m^3m^{-3} soil water content was deemed appropriate based on analysis of residuals of a temperature-only model.

Final fixed-effects structure was determined by first constructing a maximal model which included the presence of plant roots, warming, nitrogen and all interactions. A power variance function was fitted in order to correct for heteroscedasticity (Pinheiro and Bates, 2000). To account for autocorrelation in repeated measurements of the same collar, a first order autoregressive structure was used (Crawley, 2007). Fixed effects and interactions were removed iteratively based on their p-values and the best fit model was selected. During each step of this procedure, model comparisons were undertaken using a likelihood ratio test and minimisation of Akaikie's Information Criterion (AIC). Additionally, to test the effect of time on the temperature response of R_S, the data were bisected such that the first full measurement season was separated from the second. A season by warming interaction was then tested for significance.

Soil carbon content, soil nitrogen content, microbial biomass, plant available nitrogen and substrate induced respiration (S_I) were all assessed by multi-way ANOVA, with temperature and nitrogen treatments as well as their interaction as factors. For S_I, the maximum value of S_I recorded for each pair off collars over the measurement period was tested. For those variables that were measured repeatedly (soil carbon, soil nitrogen, microbial biomass carbon), a separate ANOVA was conducted for each time point.

3.3 Results

Average soil temperature over the entire 27 month measurement period was 9.6° C. Warming increased soil temperature by an average of 3.1 ± 0.2°C over the course of the experiment (Fig. 3.1). Soil water content varied seasonally, falling below 0.2 m^3m^{-3} periodically during summer and remaining above 0.40 m^3m^{-3} during the winter months. Warming significantly reduced soil volumetric water content ($F_{1,14}$

= 13.8, p = 0.002) by an average 0.01 m^3 m^{-3}. This reduction in soil water content was most evident in summer when water was limiting, with maximum reduction in soil water content of 0.04 m^3m^{-3} observed in February 2010 and 0.07 m^3m^{-3} in January 2011.

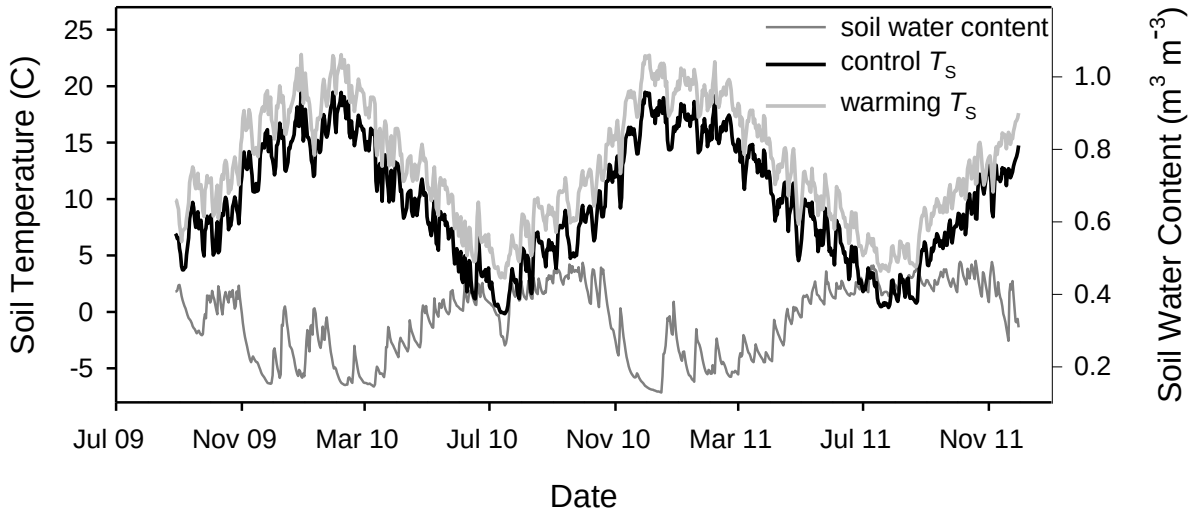

Figure 3.1: Seasonal soil temperature, T_S (° C), and soil water content, θ, at 100 mm.

3.3.1 Soil Respiration

Soil respiration showed a seasonal pattern driven primarily by seasonal temperature (Fig. 3.1, Fig. 3.2A). As such, measurement date was a significant effect in the linear mixed-effects model ($F_{29,2961}$ = 595.5, p < 0.0001). Warming increased R_S by 41% on average ($F_{1,14}$ = 54.3, p < 0.0001) and nitrogen addition increased R_S by 12% ($F_{1,14}$ = 9.1, p = 0.009), however, there was no significant interaction between warming and nitrogen on R_S ($F_{1,14}$ = 0.3, p = 0.862).

Heterotrophic respiration showed a similar response to seasonal temperature and, as such, measurement date was significant in predicting $R_{S,H}$ ($F_{23,726}$ = 171.9, p < 0.0001, Fig. 3.2B). Warming significantly increased $R_{S,H}$ by 37% ($F_{1,14}$ = 6.5, p = 0.022), but neither nitrogen ($F_{1,14}$ = 0.1, p = 0.814) nor the warming by nitrogen interaction ($F_{1,14}$ = 0.0, p = 0.847) had a significant effect on $R_{S,H}$. The average fR_H was 71% (Fig. 3.2C). This proportion was reduced to an average 59% by nitrogen addition, although

this reduction was of marginal significance ($F_{1,16} = 4.4$, $p = 0.051$) due to high variability in fR_H. Warming had no significant impact on fR_H and no apparent seasonal pattern was observed in fR_H.

Both R_S and $R_{S,H}$ were sensitive to soil water content, with a reduction in respiration rate below 0.2 m^3m^{-3} soil water content. This was particularly evident on 5 March 2010 and 12 December 2010, the driest measurement dates (Fig.3.2). As such, addition of the soil water content response function in Equation (3.1) resulted in a significant improvement in model fit over a temperature-only model ($\Delta AIC = 1350$, $p < 0.0001$).

Responses of R_S and $R_{S,H}$ to added substrate were highly variable, with values of S_I ranging from a 0.8 to 4.75 fold increase in respiration. There was no effect of presence of roots on S_I ($F_{1,15} = 0.5$, $p = 0.487$). For R_S, maximum S_I was significantly decreased by warming ($F_{1,12} = 5.2$, $p = 0.040$, Table 3.1). In contrast, nitrogen addition did not significantly affect S_I ($F_{1,12} = 2.4$, $p = 0.146$), nor was there any significant interaction with warming ($F_{1,12} = 0.0$, $p = 0.835$).

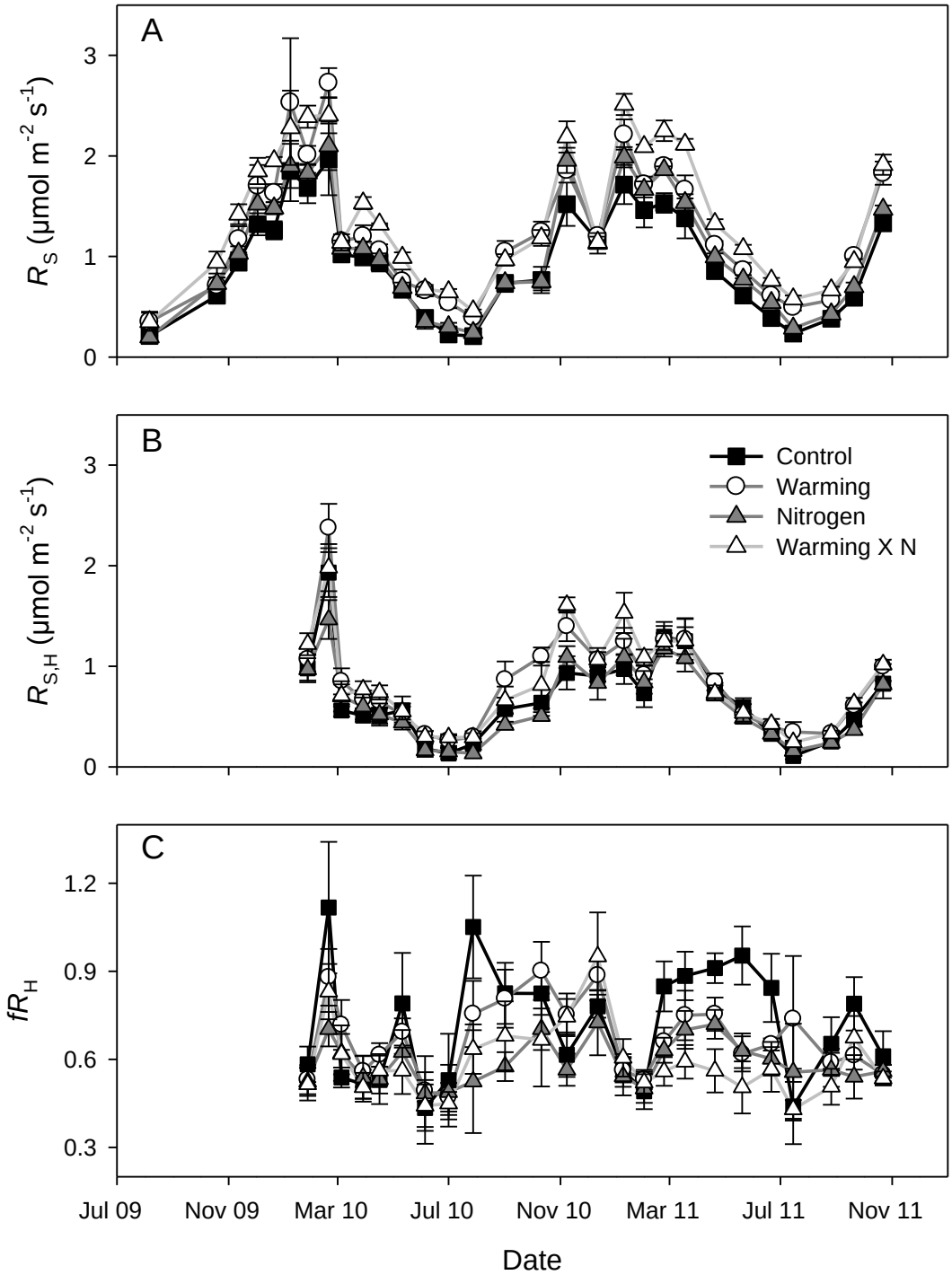

Figure 3.2: Seasonal rate of soil respiration, R_S (A), hetrotrophic respiration, $R_{S,H}$ (B), and the proportion of total R_S contributed by $R_{S,H}$ (fR_H) (C).

Table 3.1: Average soil organic carbon concentration, soil nitrogen concentration, microbial biomass, plant available nitrogen and substrate induced respiration (S_I) by treatment. Data shown are from the final soil samples collected 7 March 2012.

Response	units	Treatment			
		Control	Warming	Nitrogen	Warming × N
n	plot	5	4	5	4
Organic carbon	g kg^{-1}	41.7 ± 2.4	43.2 ± 0.3	44.9 ± 1.0	44.1 ± 0.7
Total nitrogen	g kg^{-1}	3.3 ± 0.2	3.4 ± 0.1	3.5 ± 0.1	3.5 ± 0.1
Microbial biomass carbon	mg kg^{-1}	639 ± 34	741 ± 47	655 ± 24	604 ± 25*
Plant available nitrogen	mg m^{-2}	7.9 ± 1.8	3.8 ± 0.5	11.8 ± 3.8	10.0 ± 3.9
Root biomass	g m^{-2}	389 ± 51	454 ± 27	518 ± 83	482 ± 30
S_I	unitless	1.91 ± 0.38	1.27 ± 0.14*	1.46 ± 0.28	0.93 ± 0.12

* indicates a significance level of: $p \leq 0.05$.

3.3.2 Temperature Responses of R_S and $R_{S,H}$

The presence of roots significantly increased R_{10} ($F_{1,3890} = 248.0$, $p < 0.0001$), however there was no significant effect of root presence on E_0 ($F_{1,3890} = 0.9$, $p = 0.340$). The best-fit model of the temperature response of R_S included warming and nitrogen addition as fixed effects on R_{10}. R_{10} was significantly increased by both warming ($F_{1,3072} = 16.1$, $p < 0.0001$) and nitrogen addition ($F_{1,3072} = 17.7$, $p < 0.0001$, Table 3.2). E_0 was unaffected by either warming or nitrogen and there were no significant interactions between warming and nitrogen for either R_{10} or E_0, so they were removed from the model. The inclusion of a warming by measurement season interaction for R_{10} resulted in small (0.02 μmol m^{-2} s^{-1}) and marginally significant decrease in R_{10} during the second year of warming ($F_{1,3067} = 3.3$, $p = 0.065$) Inclusion of the temperature by season interaction for E_0 resulted in a non-significant interaction term

($F_{1,3067}$ = 0.8, p = 0.347), indicating little effect of treatment time on the temperature response of R_S. For $R_{S,H}$, all treatments exhibited a single temperature response curve regardless of treatment.

Table 3.2: Table of parameters for the temperature response of soil respiration, R_S, and heterotrophic soil respiration, $R_{S,H}$, generated by fitting Equation (3.1) to measured data using a non-linear mixed effects models. Parameters supplied represent significant fixed effects in the final model.

Treatment	R_{10} (μmol m^{-2} s^{-1})	E_0 (kJ mol^{-1})	*a*	*b*
R_S - Control (intercept)	0.77 ± 0.03	326 ± 6	0.62 ± 0.05	12.36 ± 0.79
Warming	0.88 ± 0.04	-	-	-
Nitrogen	0.93 ± 0.04	-	-	-
Warming × Nitrogen	1.05 ± 0.04	-	-	-
$R_{S,H}$ - Control (intercept)	0.56 ± 0.03	331 ± 12	0.62 ± 0.05†	12.36 ± 0.79†

† fixed value, not fitted in the model.

3.3.3 Soil Analyses

Average soil organic carbon concentration was 43 g kg^{-1} and this was not affected significantly by either warming or nitrogen addition (Table 3.1). Likewise, total nitrogen concentration, which averaged 3.4 g kg^{-1}, was unaffected by treatment. Average microbial biomass carbon was 646 mg kg^{-1}. This did not change significantly under the main effects of warming and nitrogen addition, however, a significant interaction between warming and nitrogen addition was observed on the final soil sampling data ($F_{1,14}$ = 6.4, p =0.023) indicating a reduced microbial biomass under combined warming and nitrogen addition. Cumulative exchange of plant available nitrogen, as estimated from the one month burial of PRS probes, was 8 mg N m^{-3} on average and was not significantly different between treatments. At the time of the final soil sample, average root biomass in the top 100 mm of soil was 465 ± 26 g m^{-2}. Root biomass was highly variable and no treatment differences were detected.

3.4 Discussion

The soil respiration rates measured here fall within the range reported for temperate grasslands (Raich and Schlesinger, 1992). The relative contribution of $R_{S,H}$ to R_S of 71% for control plots was very close to the average for non-forest ecosystems (Hanson et al., 2000) and also agreed well with other grassland warming experiments (Zhou et al., 2007).

As expected, the 3°C warming treatment led to an increased rate of both R_S and $R_{S,H}$ over the 27 month measurement period. The average 41% increase in R_S due to warming falls well within the range of reported values for warming experiments (Rustad, 2001). Likewise, warming-induced enhancement of $R_{S,H}$ has been observed in other grassland warming experiments (Zhou et al., 2007). The fact that warming treatment had no significant impact on fR_H indicates that $R_{S,A}$ and $R_{S,H}$ were similarly sensitive to temperature. This is supported by the results of the temperature response curve fitting, in which similar values of E_0 were obtained for both R_S and $R_{S,H}$.

Apparently in contradiction to the analysis of fR_H, the temperature response analysis revealed a slightly higher basal respiration rate, R_{10}, for warmed soils. As this increase in R_{10} was only evident in R_S, and not $R_{S,H}$, we must assume that $R_{S,A}$ is responsible for the increase in R_{10}. Measurements of fR_H were highly variable and, as they were calculated from plot averages, subject to within plot temperature variation. Thus, our results are consistent with a slight increase in root activity in the warmed plots. As we found no differences in root biomass associated with warming, this increase in $R_{S,A}$ may be due to increased specific root respiration as a result of warming. Similarly, long-term warming has been associated with increased root exudation (Uselman et al., 2000), which may have stimulated rhizosphere microbial activity.

The acclimation of R_S to warming frequently observed in many long-running soil warming experiments (Luo et al., 2001, Melillo et al., 2002) was absent in the present study. It was expected that acclimation would result in a significant, negative warming by measurement season interaction (i.e., a decrease in R_{10} or E_0 relative to the control during the second year of warming). However, we observed only a small, marginally significant treatment by measurement season interaction effect for R_{10}. One

possible explanation for this lack of acclimation is the relatively short duration of this experiment. Physiological acclimation of roots and soil microbes should occur rapidly compared with the duration of our experiment, though changes to biomass and soil carbon pools may take longer. Another explanation is that acclimation has been linked to depletion of labile carbon substrates (Kirschbaum, 2004, Hartley et al., 2007). As the study site was recently cleared of vegetation and soil structure was disturbed, it is likely that the size of the labile carbon pool was reduced as a result of the disturbance. Our measurements would then reflect the temperature response of decomposition of more recalcitrant soil organic matter in the absence of a large labile carbon pool to which size adjustments can occur. As the system advances and labile carbon accumulates, acclimation may become evident. In partial support of this hypothesis, S_I was significantly higher in the control soil, indicating that labile substrates represent a greater limitation to R_S in the control plots. This is consistent with observations of other grassland warming experiments which showed higher labile carbon content in warmed soils due to greater belowground allocation and turnover of roots (Belay-Tedla et al., 2009).

The significant reduction in soil volumetric water content as a result of soil warming has potential implications for the effects of soil warming on R_S. R_S and $R_{S,H}$ were both observed to be water limited beneath a soil volumetric water content of 0.2 m^3m^{-3}. Thus, warming-induced soil drying may serve to mitigate warming-enhanced carbon losses to R_S, as has been shown in other warming experiments (Schindlbacher et al., 2012). However, the soil-drying effects observed here were small except when water was already limiting in all treatments. We suggest that soil-drying effects of warming are currently contributing little to the mitigation of warming effects due to the frequency of rainfall and the relatively short duration of periods of water limitation.

The finding that nitrogen addition increased R_S by 12% is consistent with past findings. Though an average 17% decrease in R_S in response to nitrogen addition has been noted in forests (Janssens et al., 2010), exceptions have been noted among young ecosystems, such as the current study site. Likewise, the response of R_S to nitrogen addition has been shown to be non-linear with respect to rate of application (Hasselquist et al., 2012).

The increase in R_S due to nitrogen addition can be attributed entirely to $R_{S,A}$, as $R_{S,H}$ remained unaffected by nitrogen addition. The analysis of fR_H confirmed that autotrophic contribution to R_S increased with addition of nitrogen. Likewise, nitrogen increased R_{10} for R_S, but had no effect on $R_{S,H}$. Similar to warming, we found no significant increase in root biomass in the fertilized treatment, though there was a trend for higher root biomass. As well, increases in root metabolic activity have been linked to tissue nitrogen concentration in grasslands (Bahn et al., 2006). Thus, it is likely specific root respiration rate contributed to this increase in R_S.

Contrary to expectations, plant-available nitrogen in the soil was not increased by warming or nitrogen addition. There may be several factors contributing to this result. First, nitrogen was applied to both the plant and soil. As a result, a portion of the nitrogen was likely intercepted by foliar uptake (Sparks, 2009). Additionally, the tussock grassland soils are subject to heavy leaching, likely decreasing the residence time of added nitrogen in soils. Further, the PRS probes used to estimate plant available nitrogen were inserted into soil with roots. Strong competition for nitrogen amongst roots may have contributed to the low level of plant available nitrogen in all treatments.

No interactive effects of warming and nitrogen addition were observed for R_S, $R_{S,H}$ or their respective temperature responses, indicating that effects of these separate global change drivers are additive. It has been suggested that global change drivers may interact, resulting in smaller effect sizes than those reported for single drivers (Leuzinger et al., 2011). However, few such instances have been noted for R_S (Wan et al., 2007, Contosta et al., 2011). The only significant interaction observed in this study was the negative interaction between warming and nitrogen on microbial biomass. Added nitrogen has been shown to result in decreased microbial biomass (Treseder, 2008). Our results suggest synergistic effects of warming and nitrogen addition in suppressing microbial biomass, though this did not generate a reduction in $R_{S,H}$.

Absent from this study is the inclusion of the rhizosphere priming effect in our estimates of $R_{S,H}$. The rhizosphere priming effect refers to the effect that living roots have on $R_{S,H}$ as a result of their impact on the physical and chemical environment within the soil (Kuzyakov, 2002). Priming effects have been

shown to influence both the rate and temperature response of $R_{S,H}$ (Uchida et al., 2010, Zhu and Cheng, 2011b, Graham et al., 2012), so this may represent a potential source of error in our determination of the contribution of $R_{S,H}$ to R_S. Priming effects have been shown to dampen the short-term response of $R_{S,H}$ to temperature in tussock grassland soils (Graham et al., 2012). However, in that study, when plants and soil were held at a constant temperature of 15° C, priming effects were absent. Only when the soil temperature was perturbed from the constant incubation temperature over a period of hours were priming effects observed. As such, use of the root exclusion method may be appropriate for evaluating longer-term, seasonal temperature responses of $R_{S,H}$, as in the present study.

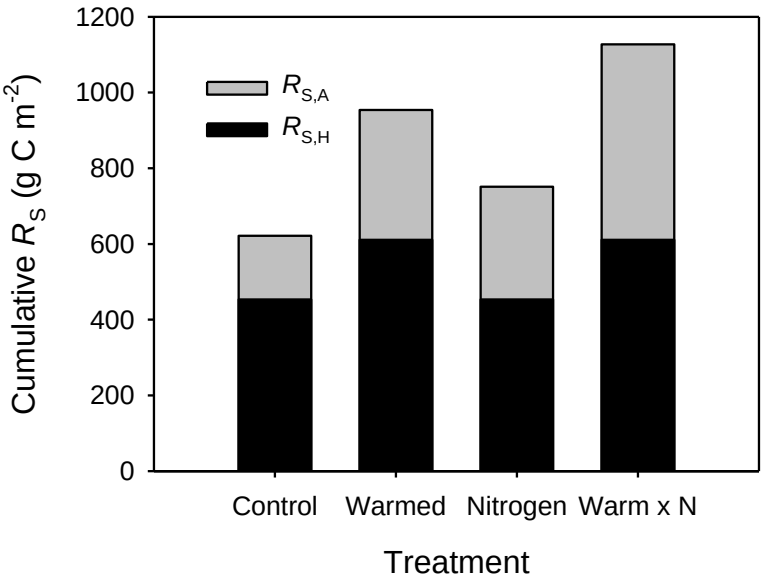

Figure 3.3: Modelled cumulative soil respiration, R_S, for the entire 27 month study period partitioned between autotrophic, $R_{S,A}$, and heterotrophic respiration, $R_{S,H}$.

Our results highlight the potential impacts of warming and nitrogen addition on the global carbon cycle, as we noted sustained increases in R_S in response to treatments. Over the course of the 27 month experiment, simulated cumulative CO_2 emissions, based on measured temperature response curves of R_S, were 621 g C m^{-2} for the control treatment (Fig. 3.3). Warming increased cumulative R_S to 953 g m^{-2}, nitrogen addition resulted in cumulative emissions of 750 g C m^{-2} and the combined effect resulted in

emissions from R_S of 1127 g C m^{-2}. While these represent substantial differences in emissions, the contrasting responses of autotrophic and heterotrophic respiration to the treatments must be considered. For nitrogen addition, the entire 129 g C m^{-2} additional carbon emissions can be accounted for by increased $R_{S.A}$. For warming, of the additional 332 g C m^{-2} carbon emitted, only 172 g can be accounted for by the increase in $R_{S.A}$. While increases in $R_{S,A}$ may have consequences for the carbon economy of plants, they are likely to be offset by increased primary production. However, increases in $R_{S,H}$ due to warming present the potential for sustained loss of stored soil carbon. Particularly if extrapolated to the 4.3 Mha of tussock grassland in New Zealand, the additional 70 g C $m^{-2}y^{-1}$ carbon losses to $R_{S,H}$ through warming represent a credible threat, as they amount to a positive feedback to climate change equivalent to 30% of New Zealand's current emissions due to fossil fuel combustion.

CHAPTER 4: EFFECTS OF SOIL WARMING AND NITROGEN ADDITION ON NET ECOSYSTEM CARBON BALANCE IN A NEW ZEALAND TUSSOCK GRASSLAND

4.1 Introduction

Terrestrial ecosystems are currently a net sink for 4.7 Pg C y^{-1}, partially mitigating the 8.7 Pg C yr^{-1} added to the atmosphere through anthropogenic CO_2 emissions (Le Quere et al., 2009). The ability of the terrestrial biosphere to continue to act as a carbon sink is largely dependent on the global balance between gross primary production and ecosystem respiration and its response to global change drivers such as climate warming and nitrogen deposition (Grace and Rayment, 2000). Global temperatures are expected to increase by 1.1 to 6.4°C over the next century (IPCC, 2007), while nitrogen deposition as a result of fossil-fuel burning and crop fertilisation, currently totalling 160 Tg N y^{-1}, is also expected to increase in the future (Gruber and Galloway, 2008). Both warming and nitrogen deposition could lead to increases in both gross primary production and ecosystem respiration, and potentially affect their balance. Coupled-climate models suggest that future climate warming scenarios could lead to a positive feedback response to increasing atmospheric CO_2 concentration from the terrestrial biosphere (Cox et al., 2000, Friedlingstein et al., 2006, Sitch et al., 2008), although large uncertainties in these predictions still exist.

Ecosystem respiration is the combination of above-ground plant respiration and soil respiration. Plant respiration is expected to increase with temperature due to increased maintenance respiration at warmer temperatures (Ryan, 1991). Likewise, soil respiration has been shown to increase in response to long-term warming (Rustad, 2001, Melillo et al., 2002, Zhou et al., 2007, Schindlbacher et al., 2009). However, acclimation of both plant and soil respiration to warming has been observed, leading to uncertainty in the response of ecosystem respiration to warming (Luo et al., 2001, Melillo et al., 2002, Atkin and Tjoelker, 2003). In particular, the heterotrophic component of soil respiration has been implicated as a potential positive feedback response to warming (Kirschbaum, 1995, Kirschbaum, 2000, Davidson and Janssens, 2006). While carbon losses from plants are constrained by photosynthesis, heterotrophic respiration may remain unconstrained, as soil organic matter is the largest terrestrial carbon pool.

Independent of respiration, warming may also enhance carbon uptake by plants. Warming has been shown to increase net primary productivity by 19%, on average, across biomes (Rustad, 2001).

These changes in net uptake have been shown to abate losses due to enhanced soil respiration (Wan et al., 2005, Wan et al., 2009, Melillo et al., 2011). Despite these trends, in general, the temperature sensitivity of net primary production has been shown to be lower than that for heterotrophic respiration (Kirschbaum, 2000).

The effects of nitrogen fertilisation on carbon balance are expected to be mediated primarily by increases in gross primary production, as nitrogen is known to increase photosynthetic capacity (Evans, 1989) and leaf area (Knops and Reinhart, 2000). Increases in forest carbon sequestration have already been documented in response to nitrogen fertiliser (Nadelhoffer et al., 1999, Magnani et al., 2007). Addition of nitrogen may also result in increased respiration, as tissue nitrogen concentration is related positively to plant respiration (Ryan, 1991, Pregitzer et al., 1998, Bahn et al., 2006). However, studies have also shown that soil respiration decreases with addition of nitrogen fertiliser in both forests and grasslands (Janssens et al., 2010, Yan et al., 2010).

As a model system, grasslands are both widespread and an important below-ground carbon sink (Scurlock and Hall, 1998, Conant et al., 2001). Additionally, models predict that grasslands will be sensitive to climate change drivers such as warming and nitrogen deposition (Thornley et al., 1991, Parton et al., 1993). In our study, we investigated the impacts of a 3°C soil warming and the addition of 50 kg N ha^{-1} y^{-1} on net carbon balance in a New Zealand tussock grassland. We employed a chamber-based approach to measure net ecosystem exchange (F_N), ecosystem respiration (R_E), soil respiration (R_S) and the heterotrophic component of soil respiration ($R_{S,H}$), along with physiological response curve analyses in order to produce annual estimates of F_N partitioned into its components. Measurements of F_N provide a useful approach to measuring carbon balance, as they integrate fluxes from all potential sources while precluding the need for biometric estimates of net primary production which are problematic, particularly below-ground (Verburg et al., 2004). By separating the components of carbon balance we can better evaluate the size and direction of potential plant and soil mediated feedbacks to rising atmospheric CO_2 concentration.

4.2 Methods

4.2.1 Site Description

This study was undertaken at a tussock grassland site in central South Island, New Zealand. Soils are a silt-loam classified as acidic allophane brown under the New Zealand Soil Classification System (Hewitt, 2010) and Typic Dystrochrept by USDA Soil Taxonomy (Soil Survey Staff, 2006) . The experimental setup consists of twenty, 12.25 m^2 plots planted with native New Zealand tussock grass species (*Chionochloa rigida, Chionochloa flavescens, Poa cita, Festuca novae-zelandiae*) in January 2009. A 3°C soil warming treatment (achieved by resistance heating cables buried at 200 mm soil depth) and the addition of 50 kg N ha^{-1} nitrogen fertiliser (as calcium ammonium nitrate) were applied in a factorial design. One warmed plot and one warmed and fertilised plot were dropped from analysis due to malfunction of heating cables resulting in a final count of five control plots, four warming plots, five added nitrogen plots, and four combined warming and nitrogen addition plots. The continuous warming treatment has been in place since July 2009. The addition of nitrogen fertiliser commenced in February 2009, being added in five 10 kg N ha^{-1} y^{-1} amounts distributed throughout spring and summer months when plant growth was most active. Further details describing the site are reported in de Sassi and Tylianakis (2012).

Mean air temperature over the measurement period from October (spring) 2010 to October 2011 was 9.4 ± 6.7° C, with a maximum mean daily temperature of 23.7°C reached in February and minimum of -3.0°C in August (Fig. 4.1). Average soil water content was 0.32 ± 0.10 m^3 m^{-3}, but showed seasonal variation, reaching a minimum of 0.13 m^3 m^{-3} in December 2010 and decreasing intermittently below the threshold for water limitation of 0.20 m^3 m^{-3} during summer and remaining above 0.40 m^3 m^{-3} in winter.

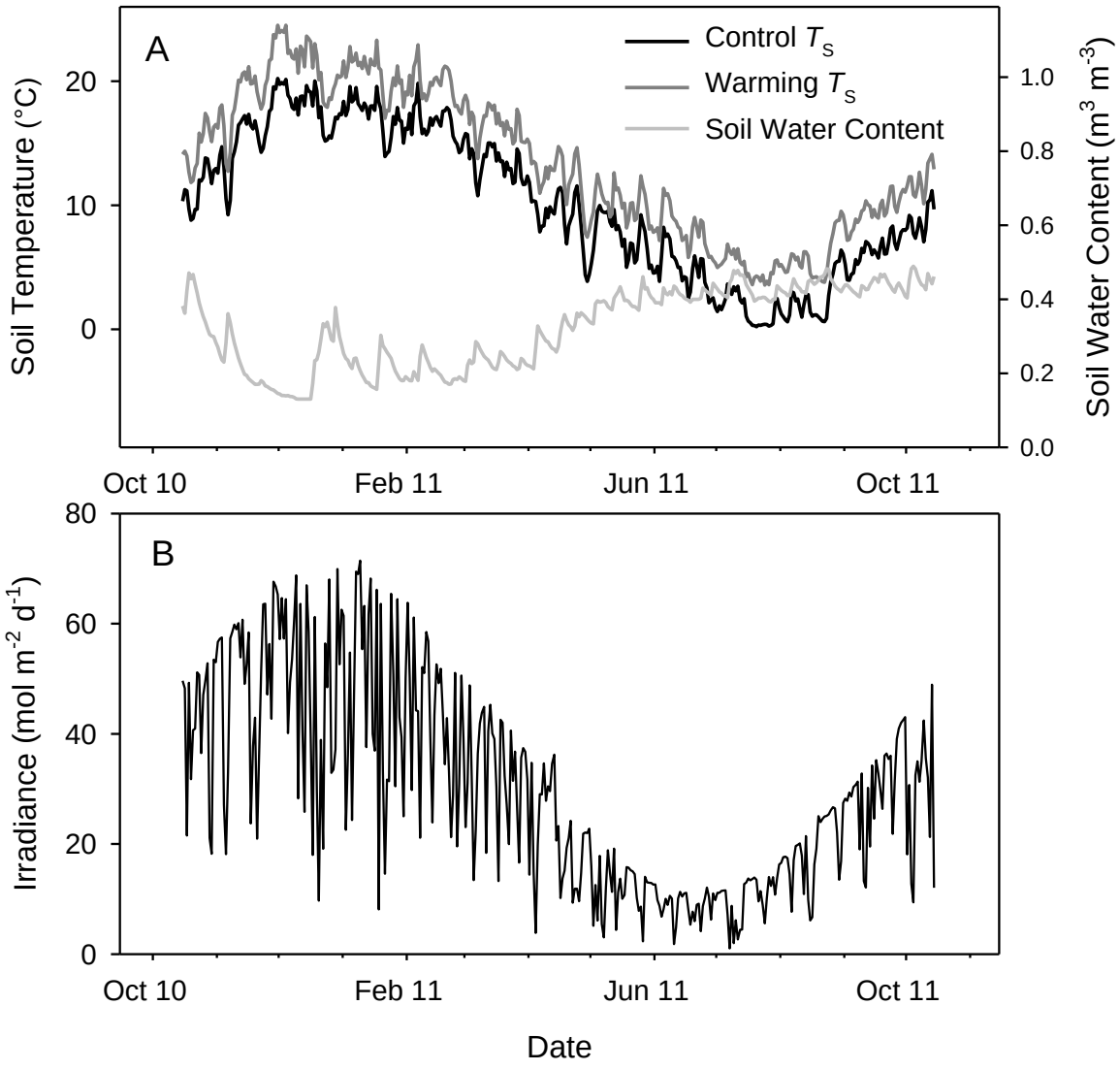

Figure 4.1: Daily mean values of (A) control and warmed soil temperature, T_S, at 50 mm soil depth, soil water content, θ, at 50 mm depth and (B) irradiance, Q, at the site between October 2010 and October 2011.

4.2.2 Net ecosystem exchange measurements

Net ecosystem carbon exchange is the balance between photosynthetic and respiratory processes and can be represented as:

$$F_N = R_{E,a} + R_{S,A} + R_{S,H} - P_G \qquad (4.1)$$

where $R_{E,a}$ is the above-ground component of ecosystem respiration (R_E), $R_{S,A}$ and $R_{S,H}$ are the autotrophic and heterotrophic components of soil respiration (R_S). P_G is gross primary production. We adopt the micrometeorological convention of a negative F_N representing net uptake of carbon by the ecosystem.

During the measurement period, net ecosystem CO_2 exchange (F_N) was measured monthly using a chamber-based approach (Shaver et al 2007, Street et al. 2007). A 0.42 m^2 aluminium chamber base was placed directly on the ground in each plot and sealed around the edges with a plastic skirt held down by a heavy chain. A 0.17 m^3 clear, Perspex® chamber was placed on top of the base, sealed by a closed-cell foam gasket, and CO_2 concentration in the chamber was measured using a portable photosynthesis system (LI-6400XT, LI-COR, Inc., Lincoln, NE, USA). Four small fans were mounted inside the chamber in order to maintain well-mixed conditions. A measurement time of 60 s was used to minimise chamber heating and the chamber was removed from its base between measurements to restore ambient temperature and CO_2 concentration. Irradiance (Q, 400-700 nm) was measured inside the chamber with a quantum sensor (LI-190,LI-COR, Inc., Lincoln, NE, USA) and air temperature with a thermocouple (Type-E, Omega Engineering, Ltd., Stamford, CT, USA) shaded from incident irradiance.

On each measurement date, the base was installed in a given plot, surrounding five tussocks, on average. One to two measurements of F_N were made under full-sun conditions. Subsequently, measurements were conducted at three different shade levels achieved by draping the chamber in one, two or three sheets of 30% shade screen in order to obtain a light response of F_N. Finally, a dark measurement was made by draping the chamber in a dark cloth, which excluded all light. This dark measurement was used as an estimate of ecosystem respiration (R_E). Following each light response curve, temperature and volumetric water content in the top 50 mm of soil were measured using a thermocouple (Type-E, Omega Engineering, Ltd., Stamford, CT, USA) and soil moisture sensor (Theta Probe type ML1 and ML2, Delta

T Devices, Cambridge, UK). In October 2011, one measurement of F_N was made at night in each plot between 2200 and 0200 hrs. These data were not included in the model formulation, but were reserved to assess the suitability of models based on daytime measurements of R_E using the shade cloth method to estimate R_E at night.

Simultaneous with measurements of F_N, soil respiration (R_S) was measured at eight locations within each plot using a portable respiration system (EGM-4 and SRC-1, PP-Systems, Hitchin, UK). At each of six locations, measurements were made by sealing the chamber on to 100 mm diameter polyvinyl chloride measurement collars, inserted to 70 mm depth at permanent sites prior to the start of the measurement period. At the additional two locations, measurement collars had been inserted to a depth of 300 mm in order to exclude roots. These deep collars served as an estimate of heterotrophic soil respiration ($R_{S,H}$).

4.2.3 Response curve fitting

In order to produce a model with which we could derive annual estimates of F_N, we used physiological response curves of CO_2 exchange based on temperature, irradiance and soil water content.

Temperature is the primary driver of respiration, thus respiration was modelled using an Arrhenius-type equation (Lloyd and Taylor, 1994):

$$R = R_{10} exp E_0 \left(\frac{1}{56.02} - \frac{1}{T-227.13}\right) \qquad (4.2)$$

where R represents either $R_{E,a}$, R_S or $R_{S,H}$, R_{10} is the basal rate of respiration at 10° C (μmol m^{-2} s^{-1}), E_0 is the energy of activation (kJ mol^{-1}), and T represents either air temperature, T_a, or soil temperature, T_S (° K). For the purposes of modelling, we subtracted our measurements of R_S from measurements of R_E such that R_S and $R_{E,a}$ could then be modelled separately, as we expected R_S to respond more strongly to T_S and $R_{E,a}$ to respond to T_a.

Photosynthetically active radiation is the primary driver of P_G, so we used a light-response function to predict P_G (Luo et al., 2000):

$$P_G = \frac{\alpha Q P_{max}}{\alpha Q + P_{max}} \quad (4.3)$$

where P_{max} is the rate of gross primary production under non-limiting light conditions, α is the canopy quantum yield, and Q is the measured irradiance. For the purposes of curve fitting, P_G at a given irradiance was estimated from our measurements of F_N by subtracting R_E, from the dark measurement for each light response curve.

Both P_G and R_E and its components were observed to be sensitive to soil water content (θ) below a critical value of 0.2 m^3 m^{-3}. Equations (4.2) and (4.3) were multiplied by the linear function:

$$\frac{\theta}{\theta_N} + b \text{ , for } (\theta < 0.2) \quad (4.4)$$

where θ_N is the optimal soil water content (m^3 m^{-3}) and b is an additional slope parameter. For $\theta > 0.2$, Equation (4.4) was fixed at a value of 1.

4.2.4 Biomass Measurements

In order to estimate above-ground biomass following the conclusion of the study, the circumference of each tussock at ground level, as well as maximum leaf extension, were measured for all tussocks in one half of each plot. Two randomly-selected tussocks of each species were then harvested at ground level, sorted into green leaves and other non-photosynthetic material, and dried at 60°C for 48 h. A subsample of fresh green leaves from each tussock was used for measurement of leaf area using a leaf area meter (LI-3100, LI-COR, Inc., Lincoln, NE, USA) prior to drying in order to establish specific leaf area. Dry mass was then used to establish linear relationships between leaf area, above-ground biomass and tussock volume, estimated from the circumference and leaf extension measurements. These relationships were then applied to the volume measurements for the remaining tussocks which were measured to produce estimates of tussock biomass and leaf area in each plot.

Biomass of inter-tussock vegetation, as well as litter, was estimated by harvesting all inter-tussock material from one 0.42 m^2 quadrat in each plot, sorting into live and dead material and drying at 60°C for 48 h. Root biomass was estimated from three 54 mm diameter soil cores, taken to 100 mm soil depth. Roots were washed free of soil over a 650 µm sieve and dried at 60°C for 48 h.

4.2.5 Statistical analyses

The effects of warming and nitrogen addition on seasonal measurements of F_N, R_E, R_S and $R_{S,H}$ were assessed using linear mixed-effects models conducted in the 'nlme' package (Pinheiro and Bates, 2000) of R version 2.12.0 (R Development Core Team, 2010). Each measurement of F_N (full-sun measurements only), R_E, R_S or $R_{S,H}$ was treated as a sample. Warming, nitrogen addition, measurement date and their interactions were treated as fixed effects, while plot number was included as a random effect to account for non-independence of multiple samples through time. For R_S and $R_{S,H}$, collar number was nested within plot as a random effect to account for repeated samples of the same collar through time. The effects of warming and nitrogen fertiliser addition on the proportion of R_E constituted by R_S were assessed similarly, treating proportions calculated from each simultaneous measurement of R_E and R_S as a sample.

The effects of warming and nitrogen on the temperature responses of $R_{E,a}$, R_S and $R_{S,H}$ were evaluated using non-linear mixed-effects models conducted in the 'nlme' package of R to fit Equation (4.2), selectively modified by Equation (4.4) when $\theta < 0.2$, to samples of $R_{E,a}$ (calculated as the difference between simultaneous measurements of R_E and R_S), R_S and $R_{S,H}$. To account for seasonal variation in temperature responses of $R_{E,a}$, R_S and $R_{S,H}$, samples were grouped into four seasons by measurement date, and a season factor with four levels was created. While a continuous seasonal response would be most appropriate, we chose to use calendar season as a grouping variable in order to ensure a range of temperatures adequate for fitting temperature response functions. Season, warming and nitrogen addition, as well as their interactions were investigated as fixed effects on R_{10} and E_0. Warming and nitrogen were included as fixed effects on θ_N and b. Plot number was treated as a random effect on R_{10} and E_0 for $R_{E,a}$

and collar number nested within plot structure was used for R_S and $R_{S,H}$. A power-variance function was fitted in order to correct for heteroscedasticity (Crawley, 2007). For R_S and $R_{S,H}$, a first-order autoregressive function was used to account for temporal autocorrelation in repeated measurements of the same collar (Crawley, 2007).

The effects of warming and nitrogen addition on the light responses of P_G were evaluated by fitting Equation (4.3) modified with Equation (4.4) as appropriate to measurements of P_G (calculated by adding measured R_E to each measurement of F_N for a light response curve). Season, warming, nitrogen addition and their interactions were investigated as fixed effects on P_{max} and α and warming, nitrogen addition and their interaction were investigated as fixed effects on θ_N and b. To account for non-independence of measurements of the same location at different irradiance, the individual measures comprising each light response curve were grouped within plots as a random effect.

The final fixed effects structure was determined by iteratively removing non-significant fixed effects. During each step of this procedure, competing models were compared using a likelihood ratio test, and removal of a variable was only accepted if the result lowered Akaike's Information Criterion.

To test the predictive utility of the final model of F_N, we used a linear regression between measured and modelled F_N. The efficacy of the model for estimating R_E at night was evaluated by comparing modelled F_N to independent measurements of F_N made at night. Modelled annual sums of F_N were generated by predicting components of carbon balance at hourly time-steps over the course of the year using continuously measured values of Q, T_S, T_A and θ. Standard errors for modelled values of F_N were calculated from the 95% confidence intervals of a linear regression between measured and modelled F_N, and these were used to conduct a one-tailed Student's t-test for evaluating treatment effects on estimated annual F_N.

The effects of warming and nitrogen addition on tussock biomass, inter-tussock biomass and litter were all assessed using multi-way ANOVA with warming and nitrogen treatments, as well as their interaction, as factors.

4.3 Results

4.3.1 Seasonal measurements of the components of carbon balance

Seasonal measurements of midday F_N followed the patterns of temperature and irradiance, with maximum rates of uptake (more negative F_N) occurring in the spring and summer and minimum rates in winter (Fig. 4.1, Fig. 4.2A). Reflecting this seasonal variation, date had a significant effect on F_N in the linear mixed effects model ($F_{12,167} = 39.3$, $p < 0.0001$). Warming had a significant main effect on F_N ($F_{1,14} = 32.6$, $p = 0.0001$), resulting in 41 ± 19% greater uptake of CO_2. Similarly, nitrogen addition increased CO_2 uptake significantly by 24 ± 5% ($F_{1,12} = 40.5$, $p < 0.0001$). Combined warming and nitrogen addition increased CO_2 uptake by 102 ± 25%, with no significant interaction between the two treatments ($F_{1,14} = 3.84$, $p = 0.070$).

Measurements of R_E exhibited a seasonal pattern driven by temperature (Fig. 4.2B). Similar to F_N, date was a significant effect in the linear mixed effects model ($F_{12,342} = 337.1$, $p < 0.0001$). Likewise, warming increased R_E significantly by 42 ± 10% ($F_{1,14} = 44.6$, $p < 0.0001$). Nitrogen addition increased R_E by 30 ± 4% ($F_{1,14} = 16.3$, $p = 0.001$) and combined warming and nitrogen increased R_E by 81 ± 14%, with no significant interaction ($F_{1,14}=0.36$, $p=0.556$).

On average, R_S comprised 46 ± 2% of R_E and this proportion was reduced significantly to 40 ± 2% by nitrogen addition ($F_{1,14}=5.6$, $p=0.031$). Seasonal measurements of R_S followed a similar pattern to R_E (Fig. 4.2C), with date significantly affecting R_S ($F_{13,1351} = 747.5$, $p < 0.0001$). Warming and nitrogen addition increased R_S significantly by 41 ± 7% ($F_{1,14} = 107.2$, $p < 0.0001$) and 17 ± 3% ($F_{1,14} = 12.8$, $p = 0.003$) respectively, and combined warming and nitrogen increased R_S by 60 ± 9%. No significant interaction was observed between the warming and nitrogen addition treatments for R_S ($F_{1,14} = 0.1$, $p = 0.747$). As a component of R_S, $R_{S,H}$ showed a similar seasonality to R_S, with date having a significant effect on $R_{S,H}$ ($F_{13,416} = 96.1$, $p < 0.0001$). Warming was the only treatment that significantly affected $R_{S,H}$ ($F_{1,14} = 6.3$, $p = 0.024$), increasing it by 37 ± 15%.

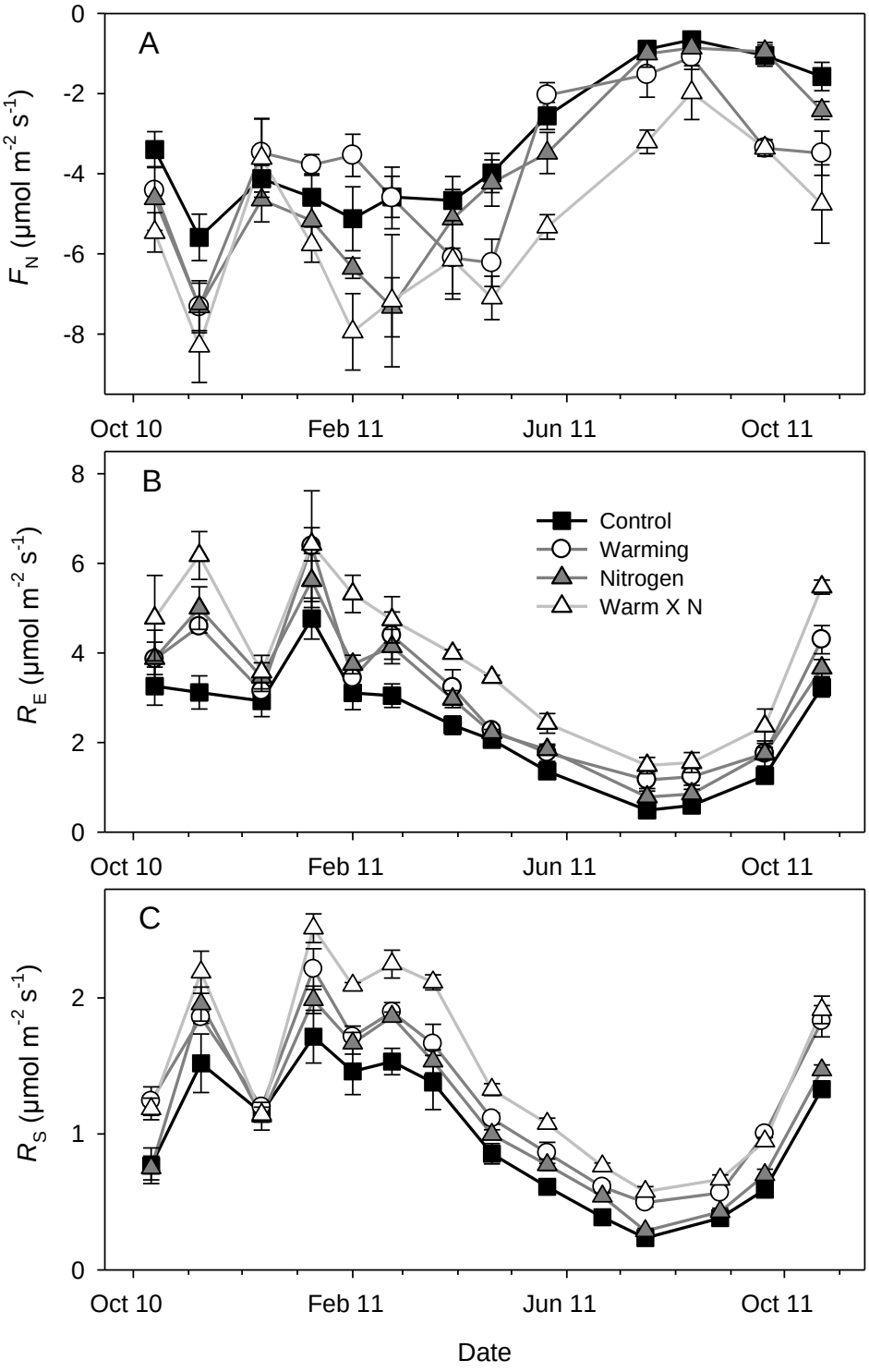

Figure 4.2: Seasonal measurements of midday (A) net ecosystem exchange, F_N, (B) ecosystem respiration, R_E, and (C) soil respiration, R_S for warming, nitrogen addition and the combined warming and nitrogen treatments.

4.3.2 Light and temperature response curves

Results of the non-linear mixed effects models indicated that the best-fit model of $R_{E,a}$ included season, warming and nitrogen addition, as well as two-way interactions between season and warming and season and nitrogen, as fixed effects on R_{10}. Season was included as a fixed effect on E_0, and warming as a fixed effect on *b*. Resultant parameter formulation showed that R_{10} was highest in spring, lowest in winter, and was highly variable in autumn (Table 4.1). Treatments resulted in an average 58% increase in R_{10} due to warming, a 49% increase in R_{10} due to nitrogen addition and a 106% increase due to the combined effects of nitrogen and warming. The E_0 parameter varied seasonally, with the highest temperature sensitivity (i.e. largest E_0) observed in spring and the lowest in winter. As with R_{10}, E_0 was poorly constrained in autumn. Warming significantly reduced the *b* parameter from 0.50 to 0.33.

Similarly, the best-fit model of R_S included season, warming, nitrogen addition and two-way interactions between season and warming and season and nitrogen as fixed effects on R_{10}. Fixed effects on E_0 included season, warming, nitrogen and all possible interactions. Fixed effects on θ_N and *b* included warming, nitrogen addition and their interaction. Parameter values indicated a seasonal pattern in which R_{10} was higher in spring and summer and lowest in winter, across the treatments (Table 4.2). E_0 was highest in autumn across the treatments. Warming resulted in an average 20% increase in R_{10} across seasons while nitrogen addition increased R_{10} by 23% and the combined warming and nitrogen treatment resulted in a 42% increase in R_{10}. Warming resulted in a 19% decrease in E_0 across seasons. Nitrogen addition resulted in a 58% increase in E_0 in the spring, but decreased E_0 relative to the control treatment for the remainder of the year. Combined effects of warming and nitrogen similarly increased E_0 by 15% in spring, but decreased E_0 relative to the control by an average of 28% across all other seasons. θ_N and *b* were both affected by warming, nitrogen addition and their interaction. These effects were observed primarily as a decrease in *b* from 0.47in the control treatment to 0.27 for warming, 0.14 for nitrogen addition and 0.15 in the combined warming and nitrogen treatment.

Table 4.1: Values of basal respiration at 10°C (R_{10}), activation energy (E_0) and soil water content response parameters (θ_N and b) estimated by fitting Equations (4.2) and (4.4) to samples of aboveground ecosystem respiration ($R_{E,a}$) for the control, warming, nitrogen addition and combined warming and nitrogen addition treatments. Values shown are means ± standard error.

Treatment	Season	Parameter values			
		R_{10} (μmol m^{-2} s^{-1})	E_0 (kJ mol^{-1})	θ_N (m^3 m^{-3})	b
Control	Spring	0.87 ± 0.17	195 ± 45	0.26 ± 0.06	0.50 ± 0.11
	Summer	0.39 ± 0.08	380 ± 47	0.26 ± 0.06	0.50 ± 0.11
	Autumn	0.62 ± 0.20	158 ± 134	0.26 ± 0.06	0.50 ± 0.11
	Winter	0.31 ± 0.04	133 ± 23	0.26 ± 0.06	0.50 ± 0.11
Warming	Spring	1.29 ± 0.24	195 ± 45	0.26 ± 0.06	0.33 ± 0.10
	Summer	0.59 ± 0.10	380 ± 47	0.26 ± 0.06	0.33 ± 0.10
	Autumn	0.83 ± 0.25	158 ± 134	0.26 ± 0.06	0.33 ± 0.10
	Winter	0.62 ± 0.06	133 ± 23	0.26 ± 0.06	0.33 ± 0.10
Nitrogen	Spring	1.24 ± 0.23	195 ± 45	0.26 ± 0.06	0.50 ± 0.11
	Summer	0.53 ± 0.09	380 ± 47	0.26 ± 0.06	0.50 ± 0.11
	Autumn	0.88 ± 0.28	158 ± 134	0.26 ± 0.06	0.50 ± 0.11
	Winter	0.54 ± 0.05	133 ± 23	0.26 ± 0.06	0.50 ± 0.11
Warming × Nitrogen	Spring	1.67 ± 0.30	195 ± 45	0.26 ± 0.06	0.33 ± 0.10
	Summer	0.73 ± 0.12	380 ± 47	0.26 ± 0.06	0.33 ± 0.10
	Autumn	1.09 ± 0.33	158 ± 134	0.26 ± 0.06	0.33 ± 0.10
	Winter	0.84 ± 0.07	133 ± 23	0.26 ± 0.06	0.33 ± 0.10

The best-fit model of $R_{S,H}$ included season, warming and their interaction as fixed effects on R_{10} and season as a fixed effect on E_0. The seasonal patterns in R_{10} and E_0 observed for $R_{S,H}$ were similar to those observed for R_S (Table 4.3). Overall, warming increased R_{10} by 11% across seasons, although this effect was seasonally dependent, being greatest in spring (33% increase), while decreasing R_{10} by 21% in autumn.

Table 4.2: Values of basal respiration at 10°C (R_{10}), activation energy (E_0) and soil water content response parameters (θ_N and b) estimated by fitting Equations (4.2) and (4.4) to measurements of soil respiration (R_S) for each treatment. Values shown are means ± standard error.

Treatment	Season	Parameter values			
		R_{10} (μmol m^{-2} s^{-1})	E_0 (kJ mol^{-1})	θ_N (m^3 m^{-3})	b
Control	Spring	0.91 ± 0.04	196 ± 19	0.36 ± 0.06	0.47 ± 0.06
	Summer	0.94 ± 0.06	240 ± 30	0.36 ± 0.06	0.47 ± 0.06
	Autumn	0.78 ± 0.04	437 ± 49	0.36 ± 0.06	0.47 ± 0.06
	Winter	0.64 ± 0.04	292 ± 31	0.36 ± 0.06	0.47 ± 0.06
Warming	Spring	1.23 ± 0.06	161 ± 22	0.24 ± 0.02	0.27 ± 0.06
	Summer	1.12 ± 0.09	190 ± 31	0.24 ± 0.02	0.27 ± 0.06
	Autumn	0.81 ± 0.04	359 ± 52	0.24 ± 0.02	0.27 ± 0.06
	Winter	0.77 ± 0.04	230 ± 33	0.24 ± 0.02	0.27 ± 0.06
Nitrogen	Spring	0.97 ± 0.04	310 ± 22	0.22 ± 0.02	0.14 ± 0.05
	Summer	1.30 ± 0.08	167 ± 30	0.22 ± 0.02	0.14 ± 0.05
	Autumn	1.01 ± 0.04	361 ± 43	0.22 ± 0.02	0.14 ± 0.05
	Winter	0.75 ± 0.05	278 ± 32	0.22 ± 0.02	0.14 ± 0.05
Warming × Nitrogen	Spring	1.28 ± 0.06	225 ± 25	0.23 ± 0.15	0.15 ± 0.05
	Summer	1.49 ± 0.11	187 ±32	0.23 ± 0.15	0.15 ± 0.05
	Autumn	1.03 ± 0.04	347 ± 52	0.23 ± 0.15	0.15 ± 0.05
	Winter	0.88 ± 0.05	165 ± 34	0.23 ± 0.15	0.15 ± 0.05

The best-fit non-linear mixed effects model for P_G included season, warming and nitrogen addition, as well all interactions, as fixed effects on P_{max} and α and warming as a fixed effect on b. As a result, P_{max} showed a seasonal pattern in which the highest values were observed in summer and the lowest in winter (Table 4.4). Warming increased P_{max} by 31% on average, with the greatest effect taking place in winter. Nitrogen addition likewise increased P_{max} by 31%, with the greatest effect in spring. Combined warming and nitrogen addition resulted in an 87% increase in P_{max}, with the greatest effect observed in winter. Seasonal patterns of α showed the highest values occurring in autumn and the lowest in winter (Table 4.4). Similar to P_{max}, warming increased α by an average of 58% with the greatest effect taking place in winter. Nitrogen addition increased α by an average of 19% across all seasons and the

combined warming and nitrogen treatment resulted in a 104% increase in α with the greatest effect taking place in winter. Warming decreased b from 0.85 to 0.71.

Table 4.3: Values of basal respiration at 10°C (R_{10}), activation energy (E_0) and soil water content response parameters (θ_N and b) estimated by fitting Equations (4.2) and (4.4) to measurements of for heterotrophic soil respiration ($R_{S,H}$) for each treatment. Values shown are means ± standard error.

Treatment	Season	Parameter values			
		R_{10} (μmol m^{-2} s^{-1})	E_0 (kJ mol^{-1})	θ_N (m^3 m^{-3})	b
Control	Spring	0.63 ± 0.04	178 ± 21	0.61 ± 0.19	0.57 ± 0.06
	Summer	0.74 ± 0.06	172 ± 29	0.61 ± 0.19	0.57 ± 0.06
	Autumn	0.71 ± 0.04	427 ± 42	0.61 ± 0.19	0.57 ± 0.06
	Winter	0.40 ± 0.04	278 ± 27	0.61 ± 0.19	0.57 ± 0.06
Warming	Spring	0.84 ± 0.05	178 ± 21	0.61 ± 0.19	0.57 ± 0.06
	Summer	0.82 ± 0.07	172 ± 29	0.61 ± 0.19	0.57 ± 0.06
	Autumn	0.56 ± 0.04	427 ± 42	0.61 ± 0.19	0.57 ± 0.06
	Winter	0.49 ± 0.04	278 ± 27	0.61 ± 0.19	0.57 ± 0.06
Nitrogen	Spring	0.63 ± 0.04	178 ± 21	0.61 ± 0.19	0.57 ± 0.06
	Summer	0.74 ± 0.06	172 ± 29	0.61 ± 0.19	0.57 ± 0.06
	Autumn	0.71 ± 0.04	427 ± 42	0.61 ± 0.19	0.57 ± 0.06
	Winter	0.40 ± 0.04	278 ± 27	0.61 ± 0.19	0.57 ± 0.06
Warming × Nitrogen	Spring	0.84 ± 0.05	178 ± 21	0.61 ± 0.19	0.57 ± 0.06
	Summer	0.82 ± 0.07	172 ± 29	0.61 ± 0.19	0.57 ± 0.06
	Autumn	0.56 ± 0.04	427 ± 42	0.61 ± 0.19	0.57 ± 0.06
	Winter	0.49 ± 0.04	278 ± 27	0.61 ± 0.19	0.57 ± 0.06

Table 4.4: Values of maximum gross primary production (P_{max}), canopy quantum yield (α) and soil water content response parameters (θ_N and b) estimated by fitting Equations (4.3) and (4.4) to samples of (P_G) for each treatment. Values shown are means ± standard error.

Treatment	Season	Parameter values			
		P_{max} (μmol m^{-2} s^{-1})	α (μmol CO_2 μmol $photon^{-1}$)	θ_N (m^3 m^{-3})	B
Control	Spring	14.59 ± 1.37	0.0080 ± 0.0009	0.53 ± 0.19	0.85 ± 0.11
	Summer	15.63 ± 1.26	0.0109 ± 0.0010	0.53 ± 0.19	0.85 ± 0.11
	Autumn	9.89 ± 1.50	0.0139 ± 0.0015	0.53 ± 0.19	0.85 ± 0.11
	Winter	4.66 ± 1.35	0.0030 ± 0.0010	0.53 ± 0.19	0.85 ± 0.11
Warming	Spring	16.78 ± 1.42	0.0125 ± 0.0011	0.53 ± 0.19	0.71 ± 0.10
	Summer	17.70 ± 1.44	0.0140 ± 0.0011	0.53 ± 0.19	0.71 ± 0.10
	Autumn	11.61 ± 1.66	0.0187 ± 0.0017	0.53 ± 0.19	0.71 ± 0.10
	Winter	8.22 ± 1.55	0.0064 ± 0.0012	0.53 ± 0.19	0.71 ± 0.10
Nitrogen	Spring	21.13 ± 1.78	0.0100 ± 0.0010	0.53 ± 0.19	0.85 ± 0.11
	Summer	21.45 ± 1.54	0.0119 ± 0.0010	0.53 ± 0.19	0.85 ± 0.11
	Autumn	11.82 ±1.54	0.0161 ± 0.0014	0.53 ± 0.19	0.85 ± 0.11
	Winter	5.66 ± 1.40	0.0038 ± 0.0011	0.53 ± 0.19	0.85 ± 0.11
Warming × Nitrogen	Spring	21.27 ± 1.52	0.0157 ± 0.0012	0.53 ± 0.19	0.71 ± 0.10
	Summer	24.80 ± 1.72	0.0173 ± 0.0012	0.53 ± 0.19	0.71 ± 0.10
	Autumn	18.86 ± 1.95	0.0232 ± 0.0017	0.53 ± 0.19	0.71 ± 0.10
	Winter	11.78 ± 1.72	0.0088 ± 0.0012	0.53 ± 0.19	0.71 ± 0.10

4.3.3 Net ecosystem exchange estimates

The model of F_N, combining the temperature responses of $R_{E,a}$ and R_S and the light response of P_G predicted 85% of the variability in F_N across treatments and levels of irradiance (Fig. 4.3). The slope of the relationship between measured and modelled F_N was 0.98 ± 0.01 ($p < 0.001$, $R^2 = 0.85$), not significantly different from a 1:1 fit. Modelled F_N indicated accumulation of carbon throughout spring and summer months, with the site becoming a carbon source in late autumn through winter (Fig. 4.4). Cumulative annual F_N for control, warming, nitrogen addition and combined treatments were -108, -59, -86 and -105 g C m^{-2} $y^{-1,}$ respectively (Fig. 4.5), although only the warming treatment differed significantly from the control ($t = 1.86$, $p \leq 0.05$). Mean modelled night-time R_E was within 14% of means for

independently measured night-time R_E for all treatments, suggesting that our model, based on estimates of R_E obtained using the shade-cloth method, provides reasonable estimates of R_E at night (Fig. 4.6).

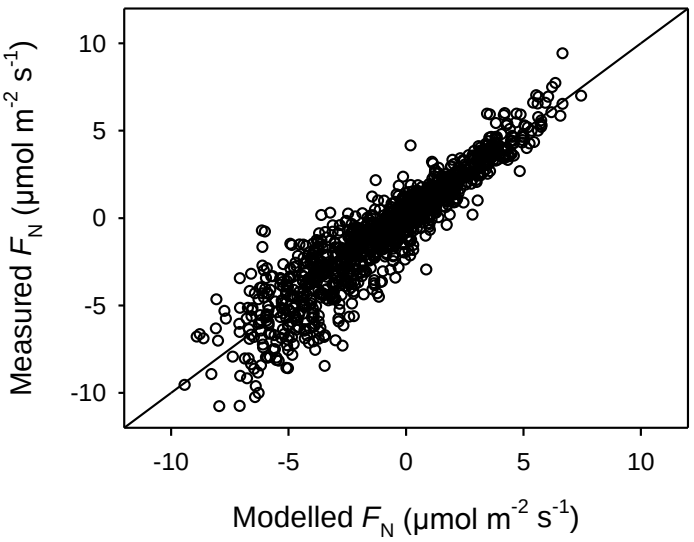

Figure 4.3: Modelled versus measured values of net ecosystem exchange around the 1:1 line.

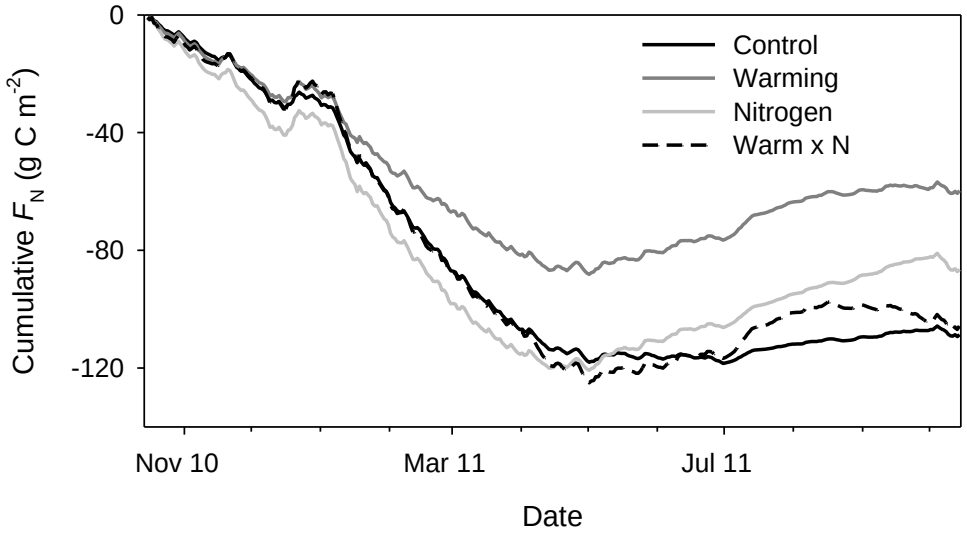

Figure 4.4: Modelled daily cumulative net ecosystem exchange, F_N, for warming, nitrogen addition and the combined warming and nitrogen treatments between October 2010 and October 2011.

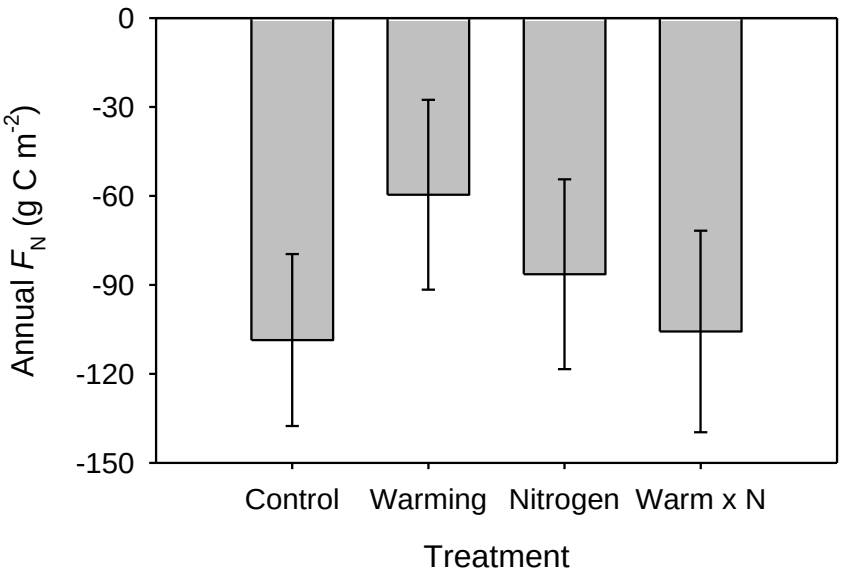

Figure 4.5: Annual modelled net ecosystem exchange, F_N, for the warming, nitrogen addition and combined warming and nitrogen treatments. Error bars represent estimated 95% confidence intervals.

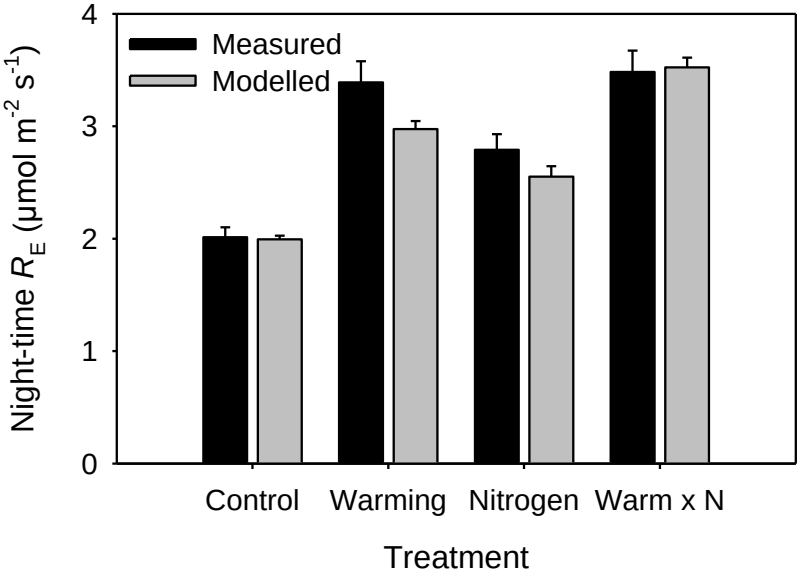

Figure 4.6: Comparison of measured versus modelled ecosystem respiration (R_E) by treatment for night-time measurements made in October 2011.

4.3.4 Biomass measurements

Total biomass estimates ranged from 825 ± 55 g m^{-2} in the control treatment to 1222 ± 32 g m^{-2} in the combined warming and nitrogen addition treatment (Table 4.5). Warming significantly increased total biomass ($F_{1,14} = 4.7$, $p \leq 0.05$), as did nitrogen addition ($F_{1,14} = 15.2$, $p \leq 0.01$), with additive effects in the combined warming and nitrogen treatment. The effect of warming on biomass was driven primarily by a 21% increase in tussock biomass and a 258% increase in inter-tussock vegetation biomass. In contrast, the effect of nitrogen addition on biomass was attributable to a 19% increase in tussock biomass and a 125% increase in litter. Warming and nitrogen addition increased leaf area significantly by 32% ($F_{1,14} = 17.7$, $p \leq 0.001$) and 20% ($F_{1,14} = 5.8$, $p \leq 0.05$) respectively.

Table 4.5: Estimates of biomass in tussocks, inter-tussock vegetation, roots and litter and leaf area by treatment. Values shown are means ± standard error.

Biomass Compartment		Treatment			
		Control	Warming	Nitrogen	Warm × Nitrogen
Tussocks	(g m^{-2})	350 ± 32	424 ± 32**	419 ± 16*	518 ± 30
Intertussock vegetation	(g m^{-2})	25 ± 5	92 ± 26*	58 ± 13	80 ± 21
Roots	(g m^{-2})	389 ± 51	454 ± 27	518 ± 83	482 ± 30
Litter	(g m^{-2})	59 ± 6	80 ± 10	133 ± 22**	141 ± 21
Leaf Area	(m^2 m^{-2})	0.31 ± .02	0.41 ± .02***	0.36 ± .01*	0.49 ± .03
Total Biomass	(g m^{-2})	825 ± 55	1052 ± 52*	1129 ± 99**	1222 ± 32

Symbols indicate significance of effect of warming, nitrogen, and warming × nitrogen interaction. Levels are *$p \leq 0.05$, **$p \leq 0.01$, ***$p \leq 0.001$

4.4 Discussion

Our tussock grassland site was a net sink for carbon, regardless of warming and nitrogen addition treatments. The estimates of cumulative net ecosystem exchange (F_N) ranged from -59 to -108 g C m^{-2}, which fall within the range reported for other temperate C_3 grasslands (Flanagan et al., 2002, Hunt et al., 2004, Gilmanov et al., 2007). Likewise, our annual estimates of gross primary production (673 to 1131 g C m^{-2}, Fig. 4.7) and ecosystem respiration (564 to 1026 g C-m^{-2}) fall within the reported range for temperate grasslands (Gilmanov et al., 2007). In contrast, measurements of F_N in another New Zealand tussock grassland showed smaller annual F_N (9 to -41 g C m^{-2}), despite having biomass, leaf area and climate similar to our site (Hunt et al., 2004). We attribute the greater sink activity at our site to the fact that, at the time of our measurements, the site was in its third year of development and grasslands are known to accrue carbon rapidly following disturbance (Conant et al., 2001, Matamala et al., 2008). We expect that our site will become a more marginal sink as biomass and litter accumulate and biotic constraints to F_N become more important.

As expected, soil warming led to enhanced ecosystem respiration (R_E). All components of R_E were responsive to temperature, with warming having proportionately similar effects on above- and below-ground components, such that both $R_{E,a}$ and R_S increased by 45% relative to the control treatment. Increases in R_E, were driven not only by direct temperature effects on respiration (i.e., mediated by E_0), but also by increases in basal respiration rates (R_{10}) of both above-ground (58% increase in R_{10}) and soil respiration (20% increase in R_{10}) in response to warming. The increase in R_{10} for above-ground respiration is consistent with the larger biomass recorded in the warmed treatment. However, increased metabolic activity at warmer temperatures also likely contributed, as increases in R_{10} were proportionately larger than the change in biomass. Increases in metabolic activity may also have contributed to the increase in R_{10} for soil respiration. No significant changes in root biomass were observed as a result of warming, so we expect that an increase in R_{10} must have been driven, in part, by increased root activity. Warming-induced increases in heterotrophic respiration accounted for only 34% of the total effect of soil warming

on R_E, indicating that plant respiration was primarily responsible for the observed increases in R_E (Fig. 4.7)

As with warming, R_E increased under nitrogen fertilization, though this was driven entirely by increases in both above- and below-ground plant respiration, which was underpinned by increases in basal respiration of both $R_{E,a}$ (49% increase in R_{10}) and R_S (23% increase in R_{10}). However, unlike soil warming, nitrogen fertilization had no effect on heterotrophic respiration. Rather, we expect that increased root metabolic activity as well as biomass contributed to elevated autotrophic respiration, as increased tissue nitrogen concentration has been linked previously to specific respiration rates in plants (Pregitzer et al., 1998, Bahn et al., 2006).

The combined effects of warming and nitrogen addition on R_E were largely additive (although interactive effects of treatments were observed for the temperature response of soil respiration, they were small in comparison to the main effects). As a result, annual accumulation of R_E was 81% greater in the combined warming and nitrogen addition treatment (Fig. 4.7). Due to additive effects of warming and nitrogen addition on above-ground respiration and the autotrophic component of R_S, increases in $R_{S,H}$ as a result of warming accounted for only 19% of the total increase in R_E.

Increases in R_E were counterbalanced by enhanced gross primary productivity (P_G) in the warming and nitrogen addition treatments, as well as the combined treatment. Warming increased P_G (by 30%) primarily by increasing quantum yield (α, 58% increase), as well as by increasing light saturating gross primary production (P_{max}, 31%). These increases in photosynthetic parameter values are consistent with the increased leaf area as a result of warming. Increased leaf area is known to increase interception of irradiance and therefore quantum yields (Ruimy et al., 1995, Luo et al., 2000). Soil warming also appears to have alleviated temperature limitation of photosynthesis. The effects of warming on α and P_{max} were greatest in winter, supporting this hypothesis. Likewise, nitrogen mineralisation generally increases with soil warming (Rustad, 2001), leading to increased nitrogen availability as an explanation for enhanced P_G. Measurements of plant available nitrogen did not indicate any increase in nitrogen

mineralisation with either warming or addition of nitrogen. However, these measurements were conducted over only one month and were subject to competition with plant roots (see Chapter 3).

As expected, addition of nitrogen increased annual P_G by 24%. This increase was driven by an increase in both α (19%) and P_{max} (31%). There is a well-established relationship between leaf nitrogen concentration and photosynthetic capacity (Evans, 1989) and increased leaf nitrogen as a result of addition of fertiliser is the expected to increase production. Nitrogen addition also increased leaf area, which likely contributed to the increase in P_G.

Combined warming and nitrogen addition had synergistic effects on both photosynthetic parameters, increasing P_{max} by 87% and α by 104% relative to the control. The resulting 68% increase in cumulative P_G was likely due to the combined effects of alleviated temperature limitation of P_G by warming, increases in photosynthetic capacity due to nitrogen addition and additive effects of warming and nitrogen on leaf area.

Despite enhanced carbon uptake, the warming treatment was a significantly poorer carbon sink over the course of the year, with annual F_N reaching 54% of that of the control. The source of this imbalance was likely due to warming-enhanced heterotrophic soil respiration. These results agree with findings from a forest site, which indicated soil warming as a net source of CO_2 to the atmosphere as a result of increased heterotrophic respiration, despite increases in net primary production (Melillo et al., 2011). However, grassland sites have often showed neutral effects of ecosystem warming on net carbon balance (Wan et al., 2005, Xia et al., 2009).

Surprisingly, nitrogen addition had no significant effect on annual estimates of F_N. Nitrogen addition has generally been shown to increase net carbon uptake as a result of increased net primary productivity (Cheng et al., 2009, Xia et al., 2009). However, there exist examples where the respiratory cost of supporting larger biomass offsets gains through nitrogen-enhanced P_G (Lai et al., 2002). We suggest that this was the case in our study, because modelled cumulative F_N showed that the nitrogen treatment gained carbon at the greatest rate throughout spring and summer months (Fig. 4.4). However in autumn and winter, when growing conditions were less favourable, nitrogen addition enhanced carbon

losses. Though nitrogen addition alone had no impact on net carbon balance, it did serve to mitigate the negative impacts of warming on carbon balance due to synergistic effects of warming and nitrogen addition on P_G.

Our results support the hypothesis that tussock grasslands will act as a positive feedback to climate change. We observed a significant 49 g C m^{-2} reduction in net carbon uptake over the course of a year in response to soil warming. At present for New Zealand, national-scale models indicate the 4.3 Mha of tussock grasslands are having a near-neutral effect on carbon emissions (Tate et al., 2000). As our site was planted within two years prior to our measurements and was actively accruing carbon, our results should not be directly generalised to all tussock grasslands in New Zealand. It is likely that tussock grassland ecosystems at equilibrium, varying between source and sink years, will experience more source years with climate warming as a result of warming-enhanced heterotrophic soil respiration. Small changes in ecosystem carbon balance of these systems will result in large changes to national and global-scale carbon emissions because of the extensiveness of grasslands. Increases in nitrogen deposition may serve to alleviate the effects of warming on net carbon balance. However, this effect will be mediated by the timing and rates of nitrogen deposition.

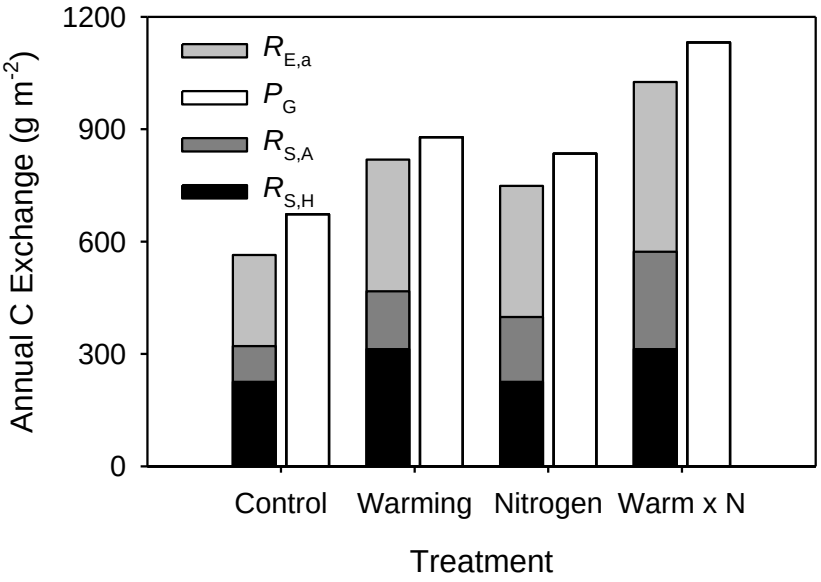

Figure 4.7: Annual modelled gross primary production, P_G, and components of ecosystem respiration, aboveground respiration, $R_{E,a}$, and autotrophic and heterotrophic soil respiration, $R_{S,A}$ and $R_{S,H}$, for warming, nitrogen addition and combined warming and nitrogen treatments.

CHAPTER 5: GENERAL DISCUSSION

5.1 Overall Results Pertaining to Thesis Objectives

This thesis has addressed the question of whether *tussock grasslands will act as a positive feedback to rising atmospheric CO_2 concentration*. The effects of temperature on CO_2 exchanges within tussock grassland were investigated at scales from the rhizosphere to the whole ecosystem. As well, nitrogen addition was investigated as a potential counterbalance to warming-enhanced carbon losses from the soil.

At the outset of this thesis, the objectives were to:

1. Determine the role of autotrophic and heterotrophic respiration in regulating the short-term response of soil respiration to temperature;
2. determine the seasonal-scale drivers of soil respiration and its heterotrophic component and investigate the impact of long-term soil warming and nitrogen addition on the response of soil respiration to these drivers; and
3. determine the net effect of soil warming and nitrogen addition on ecosystem carbon balance by:
    a. measuring net carbon exchange and partitioning these exchanges into autotrophic and heterotrophic components of ecosystem respiration and gross primary productivity; and
    b. modelling these processes in order to produce a time-integrated estimate of ecosystem carbon balance.

In pursuing these objectives, the studies here provide consistent evidence, across scales, for the temperature dependence of soil respiration. The microcosm study in Chapter 2 showed soil temperature to be an important driver of total soil respiration. However, these results also revealed the importance of plant roots in determining the response of heterotrophic respiration to temperature due to the temperature dependence of rhizosphere priming effects. Subsequently, field-scale studies in Chapter 3 and Chapter 4

strongly suggest that climate warming will result in a positive feedback to rising atmospheric CO_2 through enhanced heterotrophic soil respiration. While nitrogen addition did increase soil respiration under field conditions, it did not affect heterotrophic soil respiration, suggesting little likelihood of a soil-driven response to increased nitrogen deposition. Further, in Chapter 4, though nitrogen addition did increase gross primary production, this did not translate to increased ecosystem uptake of carbon. While nitrogen addition alone showed little potential as a counterbalance to rising CO_2, synergistic effects of nitrogen addition and soil warming on gross primary production did offset warming-enhanced carbon losses through heterotrophic respiration. As a result, nitrogen deposition may serve to mitigate positive feedback effects of warming in tussock grasslands.

5.2 Drivers of Soil Respiration

Temperature, soil water content, and the presence of plant roots were shown to be, by far, the most important variables regulating soil respiration in tussock grassland. In microcosms of tussock grass, soil respiration nearly doubled over a 10° C temperature increase. Similarly, in the field-based study, soil respiration showed sensitivity to seasonal temperature equivalent to a doubling of soil respiration over 10° C. The autotrophic component comprised 70% of total soil respiration in tussock grass microcosms, while in the field study autotrophic respiration was 30% of total soil respiration. This difference can be attributed primarily to the smaller soil volume and higher root density in the microcosms relative to the field. However, in both systems, autotrophic respiration was an important component of soil respiration.

The relative temperature sensitivities of autotrophic and heterotrophic soil respiration to temperature have been a topic of some debate in the literature (Boone et al., 1998, Högberg et al., 2001, Högberg, 2010, Luo and Zhou, 2010). In both the field and microcosm experiments in this thesis, the sensitivity of total soil respiration to temperature was similar to that of root-free soil, suggesting that autotrophic and heterotrophic components of soil respiration respond similarly to temperature. However, when heterotrophic respiration was measured in the presence of roots, it showed a much lower sensitivity to a short-term temperature increase than did autotrophic respiration and root-free soil respiration, with

heterotrophic respiration increasing by only 20% over a 10° C increase in temperature (Chapter 2). These results highlight the importance of plants as a driver of heterotrophic respiration through rhizosphere priming effects, as has been shown in other such experiments (Uchida et al., 2010, Zhu and Cheng, 2011b).

The finding that plant roots affect the response of heterotrophic soil respiration to temperature has important implications for the modelling of temperature effects on soil carbon turnover. Laboratory incubations have been regarded as an unbiased approach to measuring the temperature response of heterotrophic respiration (Kirschbaum, 2000). However, the results of the partitioning experiment reported in Chapter 2 suggest that such root exclusion methods would overestimate temperature effects on heterotrophic respiration in tussock grassland substantially. The observed decrease in the temperature sensitivity of heterotrophic respiration was likely driven by the use of root-derived substrates to fuel temperature-associated increases in microbial activity, following the preferential substrate use hypothesis (Kuzyakov, 2002). However, this does not represent a generality, as priming has been shown to increase the sensitivity of heterotrophic respiration to temperature in other similar experiments (Zhu and Cheng, 2011b).

Chapters 3 and 4 of this thesis go on to use the root exclusion method in the field to quantify the response of heterotrophic respiration to seasonal-scale temperature changes and long-term soil warming. Rhizosphere priming effects would therefore represent an important source of error in the estimation of autotrophic and heterotrophic contributions to total soil respiration. However, in defence of these measurements, the temperature responses measured in Chapter 2, including priming effects, were only short-term (hours). Further, at 15° C, the temperature at which the microcosms had been incubated for five months, priming effects were absent. A potential explanation for these results is that, at a constant growing temperature, heterotrophic and rhizosphere microbial activity may reach an equilibrium, at which priming effects are less important. Thus, priming effects would have greater implications for diurnal temperature shifts and less so over the longer-term seasonal temperature progression. The large variation in total soil respiration in response to seasonal temperature (Fig 3.2) strongly suggests a temperature

response of heterotrophic respiration greater than that measured in Chapter 2 including priming effects. The effects of seasonal-scale temperature changes on rhizosphere priming effects is a clear avenue for future research, as so much of the literature on temperature responses of heterotrophic respiration is underpinned by the assumption that root-free soils adequately reflect the processes of an intact system (Subke and Bahn, 2010).

In addition to temperature and the presence of plants, soil water content was another environmental variable that affected soil respiration in the field. Both total soil respiration and heterotrophic respiration were reduced when soil volumetric water content fell below 0.2 m^3m^{-3}. This was evident in soil respiration rates measured in March 2010 and December 2010, when soil volumetric water content in the top 50 mm of soil fell to 0.14 and 0.07 m^3m^{-3} respectively (Fig 3.2). Accordingly, the model of soil respiration in Chapter 3, which predicted respiration rate from soil temperature, was fitted with a soil water content response function (Equation 3.1). While the application of this function was well-founded based on the observations, it was parameterised using relatively few measurements under water-limiting conditions. As such, the response of soil respiration to soil water content does represent a potential weakness in the model of soil respiration. Similarly, methodological differences between the survey measurements of soil water content that accompanied soil respiration measurements, which integrated water content over 0-50 mm soil depth, and the continuous measurement of soil water content in the field, which occurred at 100 mm soil depth and did not account for warming-enhanced soil drying, may also represent a source of uncertainty in modelling soil respiration. However, water-limiting conditions represented only 17% of the study period, so errors in predicting the response of soil respiration to soil water content likely contributed little to the outcomes of Chapter 3. Future studies may include controlled environment experiments to test the response of total soil respiration and heterotrophic respiration to soil water content, as these responses will likely be important in predicting the response of soil respiration to future changes in precipitation (Schindlbacher et al., 2012).

5.3 Soil Respiration and Soil Warming

Soil respiration increased by 41% following a 3° C soil warming treatment in a tussock grassland. This confirms the finding of many warming experiments across biomes, which have resulted in an increase in soil respiration (Rustad, 2001, Melillo et al., 2002, Zhou et al., 2007, Schindlbacher et al., 2009). The increase in total soil respiration observed in this thesis was shown to be driven by increases in both autotrophic and heterotrophic components of soil respiration. The basal rate of soil respiration increased by 14%, suggesting that the increase in soil respiration was driven not only by direct effects of temperature (which would not affect the basal rate), but also by indirect effects such as changes in root biomass and activity. No significant change in root biomass was observed to be associated with soil warming. However, root biomass is variable, and a greater sampling effort may be necessary in order to quantify such changes. Further research is needed on root processes, including rates of production and turnover, as changes in carbon inputs from roots to the soil in response to warming will be related strongly to dynamics of the soil carbon pool.

Heterotrophic soil respiration increased by 37% in response to 3° C soil warming, as has been shown in other warming experiments (Zhou et al., 2007, Schindlbacher et al., 2009). As warming had no effect on the temperature response of heterotrophic respiration, it is hypothesised that this effect was largely the result of direct effects of temperature on respiration rate. As discussed previously, measurements of heterotrophic respiration in the field utilised root-free measurement collars. Rhizosphere priming effects, unaccounted for by root-free measurements, may have contributed to the increase in the basal rate of total soil respiration. As shown in Chapter 2, priming effects are generally relatively small and negative in this tussock grass system, however, warming has been shown previously to increase root exudation (Uselman et al., 2000). More research is necessary to determine how root exudation responds to soil warming in tussock grassland and how this would affect rhizosphere priming.

This effect of soil warming on soil respiration did not diminish over the 27 month measurement period, indicating no evidence of acclimation of soil respiration to temperature, which has been reported previously (Luo et al., 2001, Melillo et al., 2002). Meta-analysis has shown that, on average, soil

respiration is significantly increased during the first three years of experimental warming, with the effect becoming subsequently non-significant (Rustad, 2001). As such, the field experiment used in this thesis may not have been sufficiently long-term to reveal signs of acclimation. As well, the disturbance history of the site may have contributed to the observed lack of acclimation. Construction of the Cass Soil Warming Experiment involved removal of vegetation and topsoil from the site and later homogenisation and redistribution of the soil. This major soil disturbance likely contributed to substantial soil carbon losses, particularly from the most labile component. In fact, measurements of organic carbon concentration indicated a loss of approximately 20 g kg^{-1} of carbon (a 30% loss) compared to adjacent undisturbed soil. It is plausible that the site is currently accumulating carbon following the disturbance, as has been shown in other grasslands recovering from disturbance (Conant et al., 2001, Matamala et al., 2008). This is supported by the results of the substrate addition experiment in Chapter 3, which showed a smaller response of soil respiration to added substrate in the warming treatment. This may be the result of warming-enhanced root production and turnover, and its role in rebuilding the labile soil carbon pool. As the labile carbon pool is an important driver of soil respiration and adjustments in the size of the labile carbon pool are a likely mechanism for the acclimation response (Kirschbaum, 2004, Hartley et al., 2007), acclimation may still become apparent as the total soil carbon pool approaches a steady-state.

5.4 Soil Respiration and Nitrogen Addition

Addition of 50 kg N ha y^{-1} increased soil respiration by 12% at the tussock grassland field site. A meta-analysis of forest nitrogen addition experiments showed that, on average, soil respiration decreases as a result of nitrogen fertilisation (Janssens et al., 2010). However, exceptions in which soil respiration increased in response to added nitrogen were noted for young, rapidly growing systems due to high belowground allocation of carbon. This is likely the case for the recently planted tussock grassland field site.

The observed increase in soil respiration was attributed to an increase in activity and turnover of roots, as no effects of nitrogen addition were shown on heterotrophic soil respiration. Similar to warming,

no significant change in root biomass was observed with nitrogen addition. However, more intensive sampling of root biomass would be needed to demonstrate this conclusively. Likewise, as with soil warming, estimates of heterotrophic soil respiration did not include rhizosphere priming effects. One explanation put forward for negative priming effects, such as those observed in Chapter 2, is that roots are the source of the highest quality substrate, thus microbes preferentially use the carbon and nutrient rich substrates supplied by the roots, reducing the rate of soil organic matter decomposition (Kuzyakov, 2002). Addition of nutrients would therefore either have little effect on rhizosphere priming or further supress soil organic matter decomposition as nitrogen addition increased root activity, but have no measurable effect on soil nitrogen concentration. However, negative priming effects have also been explained as competition between the rhizosphere and soil microbes for mineral nutrients (Schimel et al., 1989). Thus, increases in nitrogen supply may increase heterotrophic respiration by alleviating nitrogen limitation to microbial activity. More research on the effects of nitrogen addition on rhizosphere priming effects in this system will be needed.

5.5 Net Carbon Balance in Tussock Grassland

The tussock grassland field site was shown to be a net sink for between 59 and 108 g C m^{-2} y^{-1}. As tussock grasslands are generally considered to be near-neutral in their impact on the atmosphere (Tate et al., 2000), this strong sink activity is attributable to the fact that this system was planted recently and is still accruing carbon, as grasslands have been shown to rapidly accumulate carbon in biomass and soils following disturbance (Conant et al., 2001, Matamala et al., 2008). On an annual basis, soil warming and nitrogen addition both increased carbon uptake via gross primary production by 30% and 24% relative to the control. The effect of warming was assumed to be due to the more favourable growing conditions conferred by soil warming, based on the finding that carbon uptake by gross primary production was enhanced to a greater extent in winter. However, soil warming has also been shown to increase nitrogen mineralisation, thereby decreasing nitrogen limitation to net primary production (Rustad, 2001, Melillo et al., 2011), which may have contributed to warming-enhanced carbon uptake. The effects of nitrogen

addition on gross primary production were attributed to the alleviation of nitrogen limitation to net primary production.

When modelled over the course of a year, soil warming and nitrogen addition increased ecosystem respiration by 45% and 32% respectively. Both warming and nitrogen increased above ground and autotrophic soil respiration. This finding is most likely related to the 27% and 36% increases in total plant biomass as a result of soil warming and nitrogen addition respectively. Only soil warming increased heterotrophic soil respiration, as discussed previously.

As a result of enhanced heterotrophic respiration, soil warming reduced net carbon uptake significantly from 108 g C m^{-2} y^{-1} to 59 g C m^{-2} y^{-1}, despite warming-enhanced gross primary production. Similarly, despite enhanced carbon uptake, nitrogen addition had no effect on net carbon balance, even in the absence of increased heterotrophic respiration. Both nitrogen fertilisation and soil warming clearly stimulated net primary production since the site was established, as plant biomass is greater in these treatments relative to the control. However, the results of Chapter 4 indicate that, by the third year of growth, net ecosystem uptake was no longer enhanced by nitrogen addition. This may reflect the respiratory cost of carrying increased biomass through unproductive parts of the season, as has been shown for forests under nitrogen addition (Lai et al., 2002). Additionally, the failure of nitrogen to increase net carbon uptake may suggest an asymptote in the response of biomass to warming and nitrogen. Biotic effects, such as self-shading, may become limiting to net primary production as plant biomass increases. This would have important consequences for net carbon uptake of tussock grasslands under climate change, as heterotrophic respiration is likely to remain elevated, unconstrained by plant activity.

As with other studies of the impacts of multiple global change drivers on carbon cycling (Wan et al., 2007, Contosta et al., 2011), few interactive effects of warming and nitrogen addition were observed throughout this thesis. Warming and nitrogen addition did interact to increase gross primary production in the combined warming and nitrogen fertilisation treatment. The alleviation of nitrogen limitation by nitrogen addition, along with the more favourable growing conditions conferred by soil warming,

increased gross primary production by 68% relative to that of the control. As a result, warming enhanced-heterotrophic respiration was offset in the combined driver treatment, and the net carbon uptake did not differ significantly from the control.

The previously discussed results on net ecosystem carbon balance depended strongly on the performance of the model of net ecosystem exchange, which was based on temperature responses of respiration and light responses of gross primary production. One feature of this model that may represent a source of error is that it does not include coupling between gross primary production and respiration. Photosynthesis has been shown to be an important source of respiratory substrate for autotrophic respiration (Atkin and Tjoelker, 2003) and an important driver of heterotrophic respiration (Gomez-Casanovas et al., 2012). However, the model of net ecosystem exchange explained 85% of the variability in the observations of net ecosystem exchange, and proved effective in predicting net ecosystem exchange outside the conditions that were used to parameterize the model (i.e., night-time respiration). Thus, we expect that the model integrated these effects.

Another potential source of uncertainty lies in the response of both photosynthesis and respiration to soil water content. Both processes were shown to be limited when soil water content fell below 0.2 m^3m^{-3}. However, these responses were not well parameterised in the model of net ecosystem exchange due to the small number of measurement dates when water limiting conditions occurred. As discussed previously, water limiting conditions were limited to a small portion of the year in the study, however, a well parameterised response of net ecosystem exchange to soil water content will be essential for predicting the response of tussock grasslands to future climate conditions, which will likely include increased drought frequency (IPCC, 2007).

5.6 Conclusions

In response to the question, *will tussock grasslands act as a positive feedback to rising atmospheric CO_2 concentration,* the evidence compiled here suggests, *yes.* The finding that net carbon uptake was reduced by 49 g C m^{-2} y^{-1} in response to soil warming suggests that tussock grasslands will act

as a positive feedback which, if extrapolated to the 4.3 Mha of tussock grassland in New Zealand, may be equivalent to 2 Tg C y^{-1}, or 20% of New Zealand's current annual fossil-fuel emissions. Reported here is an additional 70-86 g C m^{-2} y^{-1} in heterotrophic respiration as a result of a 3° C soil warming which strongly indicates losses to soil carbon as a mechanism for the observed reduction in net carbon sink activity. These results confirm the results of a survey of tussock grasslands across a climatic gradient which suggested reduced carbon storage in soils with increasing temperature (Tate, 1992).

Although addition of 50 kg N ha^{-1} y^{-1} nitrogen fertiliser resulted in the accumulation of an additional 120 g C m^{-2} in plant biomass relative the control, by the third year after planting, nitrogen addition alone had no significant impact on net carbon balance, suggesting that *tussock grasslands will not act to offset rising atmospheric CO_2 in response to increasing nitrogen deposition.* Despite having little individual effect on carbon balance, the coincidence of increasing nitrogen deposition and warming may produce a counterbalancing effect, as interactive effects of warming and nitrogen addition on carbon uptake were shown here to mitigate warming-enhanced heterotrophic respiration. However, this mitigating effect would be dependent on deposition of nitrogen over the estimated 4.3 Mha of tussock grassland in New Zealand. Atmospheric nitrogen deposition is not expected to increase markedly in New Zealand (Phoenix et al., 2006). Although, fertiliser input to New Zealand's ecosystems is currently equivalent to 12 kg N ha^{-1} y^{-1} and is likely to increase (Parfitt et al., 2006). However, these increases will likely represent intensification of use of smaller areas of land, rather than broader fertiliser application. Thus the mitigating effects of nitrogen addition may be limited to a relatively small area nationally.

While my findings strongly indicate losses of soil carbon as a result of soil warming, further research is needed on processes within the soil including root production, exudation and turnover as these are important *in situ* carbon inputs to the soil. Further, rhizosphere priming effects can be an important component of the temperature response of heterotrophic respiration and should be included in studies of soil organic matter turnover in order to improve models and improve predictions of the response of the soil carbon pool to future global change.

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