

**Development of a soil respiration
isotopic sampling system**

A thesis submitted in partial fulfilment of the
requirements for the Degree
of Master of Science in Biological Sciences
at the University of Canterbury

by S. J. Murray

University of Canterbury

2014

Abstract

The rate of carbon turnover in soil is a balance between the input of carbon by plants through their roots and associated fungi and the loss of carbon due to plant and microbial respiration, oxidation and leaching. Soil carbon dynamics are notoriously difficult to measure, and being able to separate total soil respiration into its autotrophic and heterotrophic components would help understanding of carbon cycling processes. Where autotrophic respiration originates from roots and their associated mycorrhizal fungi, using newly fixed carbon, and heterotrophic respiration originates from the breakdown of older soil organic matter.

By calculating the $\delta^{13}\text{C}$ signature of respired CO_2 (the ratio of the abundances of C isotopes ^{12}C and ^{13}C) it is possible to determine whether it is of heterotrophic or autotrophic origin. In this study a 6 chamber, constant CO_2 concentration measuring apparatus was developed to determine both the rate of CO_2 efflux and to collect undisturbed CO_2 samples for isotope analysis. This apparatus was tested using live soil samples with different $\delta^{13}\text{C}$ values (-22 ‰ to -27 ‰) and respiration rates ($2 - 8 \mu\text{mol m}^{-2} \text{s}^{-1}$) obtained from various locations in New Zealand. Testing involved taking samples using the respiration apparatus, then incubating the same samples in a bag, and then comparing the two. There was no difference between the results from the soil respiration apparatus and the bags ($R^2=0.96$, $p=0.0002$).

Twelve microcosms including soil and grass were extracted from a newly converted dairy farm and placed into in growth cabinets. Diurnal courses of partitioned soil respiration were made over 24 hours with constant soil temperature to eliminate temperatures effect on soil respiration. Half were then covered with 90% shade cloth for 12 days to test if a reduction in light (and therefore newly fixed carbon) would have any effect on soil respiration. There was a significant reduction in soil respiration, yet no detectable change in the $\delta^{13}\text{C}$ of soil respired CO_2 under heavily shaded treatment. There was however there was a shift towards heterotrophic dominated respiration. This shows that while *L. perenne* is resilient to surrounding conditions it is susceptible to change if exposed to different conditions for prolonged periods of time. The use of this new technique in the field will allow improved understanding of factors effecting soil C efflux.

Acknowledgments

I would like to firstly like to thank the University of Canterbury and Professor Matthew Turnbull for providing me this opportunity and the funding which has enabled me to pursue this thesis. I would also like to thank Matthew for his consistent support and guidance through this entire thesis, even from the other side of the world.

I would like to say a huge thank you to Dr. John Hunt, without whom this project and thesis would never have been accomplished. Thank you John for the countless hours you have spent teaching and guiding me throughout this entire process. Your patience and willingness to share your wisdom and experiences has been exceptionally appreciated.

I would like to thank Dr. David Whitehead of Landcare Research (Lincoln) and the entire Greenhouse Gas Team, Dr. Johannes Laubach, Dr. Ellen Cieraad, Dr. Matti Barthel, Anitra Fraser, Graeme Rogers and Tony McSeveny, all of whom welcomed me in to their team and were always on hand to offer advice and support and help me with any queries I might have had. Thank you very much it has been greatly appreciated.

I would also like to thank my fellow students at Landcare Research, Anna Zakharova and Gabriel Moinet for their constant support and advice.

Thank you to Synlait Farms LTD for letting me use their fields for samples and initial research.

I would like to say a massive thank you to my family, Grandad, Mum, Dad and my sister Jessica. Their love and constant support been a source of inspiration through the last 5 and a half years, especially through some very difficult times. And a special thank you to Grandad whose extra help went a long way to elevating a lot of stress in my final year.

Thank you to my flatmate Matthew for awesome graphical support.

Table of Contents

Abstract.....	i
Acknowledgments	ii
Table of Contents	iii
List of tables and figures	vi
Chapter 1 Introduction.....	1
1.1 Global climate change and carbon.....	1
1.2 Soil complexity	2
1.3 Soil Respiration.....	3
1.4 Soil carbon isotopes	5
1.5 Introduction of techniques to measure $\delta^{13}\text{C}$	7
1.6 Thesis objectives	8
Chapter 2 Soil Respiration Isotope Sampling System (SRISS)	9
2.1 Introduction.....	9
2.2 Overview of methods	9
2.3 Respiration System development and use in this thesis	12
2.4 Respiration System Components and Build	13
2.5 Soil Isotope Respiration Program	17
2.6 Measurement of $\delta^{13}\text{C}$	18
2.7 SRISS Testing.....	19
2.8 Respiration system challenges and solutions	23
2.9 Summary	24
Chapter 3 Sample site and acquisition.....	25
3.1 Site description.....	25
3.2 Soil samples	27
3.3 Sample growing condition	27

3.4 Sample species cover	29
Chapter 4 24 hour response experiment.....	30
4.1 Introduction.....	30
4.2 Methods.....	31
4.2.1 24-hour experiment.....	31
4.2.2 Soil respiration and isotope analysis.....	31
4.3 Results and Discussion	32
4.3.1 Soil temperature	32
4.3.2 Soil efflux.....	33
4.3.3 $\delta^{13}\text{C}$ Analysis	35
4.3.4 Conclusion	38
Chapter 5 Impact of reduced photosynthesis on soil CO₂ efflux and $\delta^{13}\text{C}$ of Rs.....	39
5.1 Introduction.....	39
5.2 Methods.....	41
5.2.1 Soil pasture samples.....	41
5.2.2 Simulation of cloud cover	41
5.2.3 Measurement procedure.....	42
5.2.4 End-member analysis.....	43
5.3 Results and Discussion	43
5.3.1 Temperature	43
5.3.2 Soil respiration rates	44
5.3.3 Respiration analysis	47
5.3.4 $\delta^{13}\text{C}$ measurements	49
5.3.5 Root and soil end members.....	51
5.3.6 $\delta^{13}\text{C}$ Analysis	51
5.3.7 Conclusion	53
Chapter 6 Conclusion	56

6.1: Overview of findings	56
6.1.1: SRISS Overview	56
6.1.2: 24-hour experiment overview	57
6.1.3: Impact of reduced photosynthesis on soil CO ₂ efflux and δ ¹³ C of R _S overview...58	
6.2: Significance of findings	59
6.3: Future applications.....	59
References.....	61
Appendix I	67

List of tables and figures

Table 3.1: Distribution of plant species for all sample pots.	29
Table 5.1: Average end member measurement of respired $\delta^{13}\text{CO}_2$ for shaded and light treatments (n=6). The average value was calculated from all samples taken (n=18).	51
Figure 2.1: All components in their final installation form	14
Figure 2.2: Schematical drawing of the dynamic system used for this thesis. Solid lines represent gas flow and direction. Dotted lines represent data transfer and communication....	16
Figure 2.3: Testing the SRISS. Rings are identical to those used in soil/pasture experiments, however are glued and fully sealed onto plastic base. This provided a closed environment where the only gas in the system was either from the soil itself or CO_2 -free air coming from the SRISS.	20
Figure 2.4: A comparison between the $\delta^{13}\text{C}$ of soil respired CO_2 obtained from bag incubations and $\delta^{13}\text{C}$ from the soil sampled using the SRISS. All values are the mean \pm SEM (n = 6). The black line is the linear regression ($R^2 = 0.96$). Dotted line represents the 1:1 line.	21
Figure 2.5: Respiration rates from soil samples used for SRISS tests. Averages (n=6) \pm SEM. (a) LCR crop soil, (b) LCR shrubland soil, (c) Beacon farm soil, (d) West Coast rainforest soil, (e) degraded tussock grassland soil. Solid points are the corresponding $\delta^{13}\text{C}$ for that sample using the SRISS method. All data are averages (n=6) \pm SEM.	22
Figure 3.1: Pin (A) indicating the location of Beacon Farm on the Canterbury plains (Google Inc. 2009).	25
Figure 3.2: Satellite photograph of Beacon Farm (Google Inc. 2009).	26
Figure 3.3: Closer view of Beacon Farm where research sites are located (Google Inc. 2009).	26

Figure 3.4: Samples growing in growth chamber with SRISS efflux chambers placed on the rings.....28

Figure 4.1: Soil temperatures measured at three different depths (20 mm, 70 mm, 150 mm). Temperature was taken every 30 minutes from experiment start for 24-hours. The hours of light were from 0 – 10 hours and 20 – 24 hours, shaded area indicates lights-off period.33

Figure 4.2: Soil respiration rates from 6 pots inside a growth chamber, sampled every 6 minutes for 24 hours. The hours of light were from 0 – 10 hours and 20 – 24 hours, shaded area indicates lights-off period.33

Figure 4.3: Average respiration rate (n=6) ± SEM of all samples over 24 hours. Each measurement was made 6 min apart. The hours of light were from 0 – 10 hours and 20 – 24 hours, shaded area indicates lights-off period.....34

Figure 4.4: $\delta^{13}\text{C}$ (‰) measurement over the 24 hours. The hours of light were from 0 – 10 hours and 20 – 24 hours, shaded area indicates night-time (lights-off) period.35

Figure 4.5: $\delta^{13}\text{C}$ (‰) cabinet measurement over the 24 hours. The hours of light were from 0 – 10 hours and 20 – 24 hours, shaded area indicates night-time (lights-off) period.....36

Figure 4.6: Normalised $\delta^{13}\text{C}$ data. Cabinet (ambient) has been shown in red for clarity. The hours of light were from 0 – 10 hours and 20 – 24 hours, shaded area indicates lights-off period.37

Figure 5.1: Shade cloth construction used to simulate shaded cloud cover.....42

Figure 5.2: Day-time soil temperature recordings. Temperature was measured at 50 mm into the soil in representative pots. Results are the mean (n=3) ± SEM.44

Figure 5.3: Night-time soil temperature recordings. Temperature was measured at 50 mm into the soil in representative pots. Results are the mean (n=3) ± SEM.44

Figure 5.4: Soil respiration rates for day-time respired CO₂, all points are averages (n=6) ± SEM. Shaded measurements have been shifted by 0.05 for error bar clarity. Vertical dotted lines indicate watering events.45

Figure 5.5: Soil respiration rates for night-time respired CO₂, all points are averages (n=6) ± SEM. Shaded measurements have been shifted by 0.05 for error bar clarity.46

Figure 5.6: Respiration rate difference (shade – light) of both day and night respired CO₂, all points are averages (n=6) ± SEM. Night-time measurements have been shifted by 0.05 for error bar clarity.46

Figure 5.7: Soil respiration rates for day-time respired CO₂, all points are averages (n=6) ± SEM. Triangle symbols are the predicted soil respiration rates if the shaded soil temperature was raised to the average light treatment soil temperatures for the corresponding day. Predictions were calculated with equation (2). Shaded measurements have been shifted by 0.05 for error bar clarity.....47

Figure 5.8: Soil respiration rates for night-time respired CO₂, all points are averages (n=6) ± SEM. Triangle symbols are the predicted soil respiration rates if the shaded soil temperature was raised to the light soil temperatures. Predictions were calculated with equation (2). Shaded measurements have been shifted by 0.05 for error bar clarity.48

Figure 5.9: Gross Ecosystem Production (GEP) measured at different photosynthetic available radiation (PAR) levels. Values are averages (n=30) ± SEM.48

Figure 5.10: Day-time δ¹³C of soil respired CO₂ from both shade and light treatments. All measurements are averages (n=6) ± SEM. Light treatment measurements have been shifted by 0.05 for error bar clarity.....49

Figure 5.11: δ¹³C measurements of night-time respired CO₂ from both shaded and light pots. All measurements are averages (n=6) ± SEM. Light treatment measurements have been shifted by 0.05 for error bar clarity.....50

Figure 5.12: Difference (shade – light) of δ¹³C of soil respired CO₂ between light and shaded treatments. All measurements are averages (n=6) ± SEM. Day measurements have been shifted by 0.05 for error bar clarity.....50

Figure 5.13: Partition of day-time δ¹³C. Lines are linear regression. Dotted line represents light with solid line representing shaded.....52

Figure 5.14: Partition of night-time $\delta^{13}\text{C}$. Lines are linear regression. Dotted line represents light with solid line representing shaded.52

Figure 5.15: Difference between light and shaded treatments (shaded – light) for partitioning of $\delta^{13}\text{C}$. Lines are linear regression. Dotted line represents night with solid line representing day.....53

Chapter 1 Introduction

1.1 Global climate change and carbon

Soils contain over 1500 Gt of carbon, which amounts to two thirds of the terrestrial carbon pool. Thus soils are of increasing importance in the study of global climate change (Amundson, 2001). Soil is a dynamic, fluctuating, biological entity, which has the potential to be either a carbon source or carbon sink for atmospheric carbon (Midwood & Millard, 2011). Soil is vastly heterogeneous, in both space and time, and encompasses carbon from very recent root exudate (removed from the atmosphere just minutes ago), to persistent humified material which could be up to a millennium old (Amundson, 2001; Barthel *et al.*, 2014). Understanding the factors which can cause change to the soil system are therefore extremely important in understanding how soil can contribute to mitigation or exacerbation of global climate change in the future (Lal, 2009; Schipper *et al.*, 2010).

Since most developed and developing nations are striving to increase the productivity of their soils, the planet is going through an unprecedented rate of change that is having a direct effect on the atmospheric levels of CO₂ and hence climate (Ciais *et al.*, 2013). Global warming is now recognised as a major threat to natural and socio-economic systems and our current way of life (Kirschbaum *et al.*, 2012). Global warming is recognised as a significant part of global climate change. Each of the last three decades have been successively warmer than any other preceding decade since 1850 (Ciais *et al.*, 2013). Carbon accumulation in the atmosphere is recognised as a major contributing factor towards this global increase of temperature (Smith *et al.*, 2008). The soil carbon pool is also in constant flux with the atmosphere, with soil-carbon accumulation and loss happening continually both during the day and night (Janzen, 2006). As CO₂ accumulation in the atmosphere is considered detrimental to the global climate, sequestering carbon in soil is a positive outcome (Janzen, 2006). Therefore studies into the storage, retention and accumulation of carbon in soil are now becoming increasingly important.

A major potential to change the amount of carbon stored in New Zealand soils, is a change in the use of land. Studies have shown that soil use and disturbance, such as tillage and crop use, significantly reduce the carbon retention ability of soils which is a significant issue with land use change (Kirschbaum *et al.*, 2012; Wright & Hons, 2005; Zakharova *et al.*, 2014). Two significant areas where land use change has the potential to significantly alter soil carbon storage ability are (1) deforestation with the conversion to farming land and (2) the conversion

of farmland from traditional dry farming to intensified farming (DairyNZ, 2012; Dynes *et al.*, 2010).

In the last two decades Canterbury, in the South Island of New Zealand, has seen a significant change in land use and farming practices. Many farms have moved from traditional dry farming practices with sheep or deer, to more intensive farming practices with dairy stock (DairyNZ, 2012). This has involved a massive change in the way farmers are now managing their pastures with increasing levels of nutrient and water input, a change in pasture species, cultivation regimes, stocking densities and animal effluent (Dynes *et al.*, 2010; Lambie, 2012). All these effect soil processes and the storage and release of CO₂.

As part of being a signatory to the Kyoto Protocol, New Zealand is required to provide a detailed report of greenhouse gas emissions each year to the Intergovernmental Panel on Climate Change (IPCC). By reducing the amount of greenhouse gas emissions from soil (and other areas) there is the potential for considerable monetary gain in the form of carbon credits for New Zealand and other nations (Ciais *et al.*, 2013; Kirschbaum *et al.*, 2012). The control and mitigation of soil carbon loss is of huge benefit to science, society and the economy.

1.2 Soil complexity

Soil is a dynamic system made up of both organic and inorganic components. The organic parts that reside in the soil are grouped together as soil organic matter (SOM) (Amundson, 2001). Soil also has an above ground terrestrial component which includes the plants and trees whose root systems reside in the soil surrounded by the SOM (Amundson, 2001). As both are living entities, respiration from these components is an important factor and where most of the carbon exchange happens (Lal, 2009). Other mechanisms of loss of carbon include, leaching into ground water and oxidation (Kindler *et al.*, 2011; Lorenz *et al.*, 2006; Tivet *et al.*, 2012). The abundance of microbial species from a huge range of taxa in soil make it an ever changing system which can react in many different ways to changing environmental factors (Sejian *et al.*, 2011).

The rate of carbon turnover in soil is a balance between the input of carbon by plants through their roots (photosynthate exudate) and mycorrhizae and the loss of carbon due to plant and microbial respiration. Leaching and oxidation contribute to carbon loss however their contribution is relatively minor (Melillo *et al.*, 2002). Many factors can influence the rate of

carbon turnover in soil including temperature (Davidson & Janssens, 2006; Melillo *et al.*, 2002), moisture (Millard *et al.*, 2008), addition of nutrients (for example in the form of fertilisers) and photosynthesis (Kelliher *et al.*, 2005; Uchida *et al.*, 2010a; Uchida *et al.*, 2010b).

When considering soil carbon turnover, one of the main aspects is understanding where the carbon is originating from. Plants as a result of photosynthesis add carbon to soil, while microbes feed off both newly supplied carbon and older carbon from the SOM pool. However, when microbes respire carbon from the SOM carbon pool (SOM breakdown) this carbon cannot so easily be replaced by plants (Post & Kwon, 2000). Therefore it is important to understand what factors can influence soil respiration and how they occur.

1.3 Soil Respiration

Soil respiration (R_S) can be broken down into two parts; autotrophic respiration (R_A) from roots and their associated mycorrhizal fungi, using newly fixed carbon, and heterotrophic respiration (R_H) due to the breakdown of older SOM (Millard *et al.*, 2008). Therefore, in regards to global climate change, when looking at soil respiration in the ideal situation, respired carbon would mostly come from R_A , with very little coming from R_H . However this would adversely affect decomposition and the release of nutrients. By being able to distinguish between R_A and R_H it can be ascertained from what carbon pool a sample of respired CO_2 has originated from. This will help in determining if the soil is responding to land use practices by being a net sink or source of CO_2 (Melillo *et al.*, 2002). It is also important to determine what factors influence R_H and R_A with the end goal of moderating R_H to encourage storage of C in the soil as much as possible.

Soil respiration originates from multiple processes, including R_A by plant roots (and their associated microbes) and R_H during SOM matter decay. R_A represents the rapid turnover of a labile proportion of the total soil C pool such as direct exudate as a result of photosynthesis (Melillo *et al.*, 2002), while R_H represents the slower turnover of much larger C pools which can be divided into a number of sources (Kuzyakov, 2006). These include decomposition of plant residues and the turnover of SOM which occurs at a basal rate, however this can be increased by the presence of plant roots - this is referred to as the priming effect (Fontaine *et al.*, 2003).

There are many factors that can influence soil respiration and can be broadly be associated into the following: temperature, sunlight, water, and solid inputs (for example, leaf litter, fertiliser and animal excretions). Understanding how each of these factors can influence soil respiration and carbon turnover will give us valuable information on how these systems are likely to react to an ever changing climate and anthropogenic land-use change.

It is widely accepted that R_s increases in response to increasing soil temperature, which globally, could result in a positive feedback to rising atmospheric CO_2 concentrations (Bond-Lamberty & Thomson, 2010; Lloyd & Taylor, 1994). Experimental evidence has shown there is an exponential relationship between soil temperature and soil respiration rates in a well-watered system, dryer soils potentially result in lower respiration rates (Höglind *et al.*, 2011; Lloyd & Taylor, 1994). These studies have also shown that at lower prevailing temperatures, R_s is more sensitive to changes in temperature (Lloyd & Taylor, 1994). However, it has also been shown that plant species and soil types have an effect on soil responses to temperature (Bernacchi *et al.*, 2001; Graham *et al.*, 2012; Uchida *et al.*, 2010b). R_A has been shown to be the predominant factor in the response of R_s to changes in temperature (Boone *et al.*, 1998; Graham *et al.*, 2012; Ryan, 1991; Sage & Kubien, 2007). However studies have also shown that the temperature response for soil respiration varies significantly with plant and soil type (Bergeron *et al.*, 2007; Vargas & Allen, 2008; Zeng *et al.*, 2014). While there is a definite response in soil respiration to temperature, this shows the origin of this response is highly variable. This effect can be mitigated by keeping the soil temperature as constant as possible to reduce interaction effects with other treatments.

Sunlight has a major influence on soil respiration (Höglind *et al.*, 2011). Sunlight in most circumstances increases the photosynthetic rates of plants which thus in turn will affect soil respiration rates by influencing the amount of photosynthate exudated from the roots, and thus directly affecting R_A (Lötscher & Gayler, 2005; Saarinen *et al.*, 2011). Sunlight (solar radiation) also has an effect on soil temperature, which itself has a direct effect on the respiration rates of soil (Lloyd & Taylor, 1994). Therefore it is important whether carrying out experiments in growth cabinets or in the field to consider light saturation and sunlight hours as these have a direct effect on soil respiration rates.

Soil moisture content also has a significant effect on soil respiration. Studies have shown that both high and low soil moisture content can have an inhibitory effect on soil respiration rates otherwise at moderate levels have a promotional effect on soil respiration (Davidson *et al.*,

2000; Wan *et al.*, 2007). However it has been noted that these observations were not independent of temperature recordings, temperature and moisture effects on soil respiration are not mutually exclusive (Wan *et al.*, 2007). The soil moisture content is often altered by high intensity farming where water application is significantly increased to produce a higher yield of pasture which will influence both R_H and R_A . The water holding capacity of soil (and thus its impact on availability) plays a prevalent role in R_S (Davidson *et al.*, 2000; Wan *et al.*, 2007).

Additional factors related to changing farming practices - such as fertiliser, and animal excretions can also cause changes in the respiration rates of soil. Most of these inputs revolve around the input of nitrogen into the soil. Nitrogen is essential for plant growth, however the effect it has on soil respiration varies considerably with plant species and soil type (Janssens *et al.*, 2010; Lee & Jose, 2003; Pregitzer *et al.*, 2000). While plants need nitrogen to grow, many studies have shown a reduction in soil respiration rates when nitrogen in various forms is added (Bowden *et al.*, 2004; Janssens *et al.*, 2010; Lee & Jose, 2003; Pregitzer *et al.*, 2000; Ramirez *et al.*, 2010; Ryan & Law, 2005). This is likely caused by several mechanisms, firstly the addition on nitrogen to soil causes a shift in the carbon allocation in plants to above ground growth at the expense of root growth, this has the effect of less photosynthate exudate being excreted by the roots and thus causes a reduction in R_A (Litton *et al.*, 2007; Lötscher & Gayler, 2005). Secondly this also causes a shift in the microbial population structure and feeding regime, an increase in soil nitrogen can also cause a shift from carbon assimilation to nitrogen fixing in the microbes thus reducing the amount of SOM being decomposed and therefore a reduction in R_H (Janssens *et al.*, 2010). Studies have shown both these combined cause a reduction in R_S when nitrogen has been added to the soil, irrelevant of soil type (Ramirez *et al.*, 2010).

Soil respiration is an exceptionally complex system with many factors which can have an influence on respiration rate. Soil carbon storage has the potential to have massive effects on global climate change, therefore understanding how different factors affect soil respiration and potentially how to directly control these factors needs to be a top priority in the study of global climate change.

1.4 Soil carbon isotopes

Carbon has three naturally occurring isotopes (^{12}C , ^{13}C , and ^{14}C). ^{12}C and ^{13}C are stable isotopes, while ^{14}C is radioactive and undergoes decay over time. The natural abundance of ^{12}C

in the earth's atmosphere is ~98.89% and ^{13}C is ~1.11%. $\delta^{13}\text{C}$ is a measurement of the ratio of ^{12}C and ^{13}C compared to an internationally recognised standard (Pee Dee Belemnite). Since the amount of ^{13}C is so much smaller than ^{12}C in the environment, $\delta^{13}\text{C}$ are presented as units in per mil (‰). A full review on the calculation and derivation of $\delta^{13}\text{C}$ was carried out by Brugnoli and Farquhar (2000).

There are many techniques that have been used to measure and determine the rate of carbon turnover in soil, but only a few recent studies have used $\delta^{13}\text{C}$ of respired carbon to separated R_S into R_H and R_A components (Millard *et al.*, 2010; Millard *et al.*, 2008). This approach is based on the premise that the older SOM has a different $\delta^{13}\text{C}$ than the newer photosynthate. It has been known since the 1940s that the $\delta^{13}\text{C}$ of soil respired CO_2 of plants which have been growing in different environments can differ significantly (Nier & Gulbransen, 1939; Wickman, 1952).

The carbon in C_3 plants is more depleted in ^{13}C (approximately -27 ‰) compared with C_4 plants (-12 ‰). This is a result of their different initial photosynthetic pathways (Bowling *et al.*, 2008). C_3 plants more actively discriminate against $^{13}\text{CO}_2$ molecules, consequently resulting in a significant depletion in ^{13}C in the photosynthate transported to the roots. This is because most physical and biochemical processes favour the lighter isotope, leaving the product more depleted in the heavier isotope. There are two main discriminating steps - firstly during CO_2 diffusion from the outside air into the leaves of the plant through its stomata and then during carboxylation by ribulose-1,5-bisphosphate carboxylase (or oxygenase) (Brugnoli & Farquhar, 2000). Consequently this depletion of ^{13}C leads to a measureable difference between the $\delta^{13}\text{C}$ of the CO_2 in the air and that fixed by the plant and respired via R_A (Högberg, 1997). In C_4 and CAM plants less fractionation occurs, this is due to rubisco in these plants being in a relatively closed system which is unable to expel $^{13}\text{CO}_2$ as easily and this increases its utilisation (Gannes *et al.*, 1998).

Respired CO_2 originating from SOM (R_H) is enriched in ^{13}C relative to CO_2 derived from fresh photosynthate (R_A). By separating the soil into its roots and SOM components and measuring the $\delta^{13}\text{C}$ of CO_2 respired from them separately, the isotopic 'end-members' for R_A (respired CO_2 from photosynthate) and R_H (respired CO_2 from SOM) can be determined, an example of this can be found in Millard *et al.* (2010). The $\delta^{13}\text{C}$ of CO_2 respired from in intact soil in the field can be measured to the proportion of respired CO_2 that has originated from the R_H or R_A .

While doing this the rate in which CO₂ is being respired can be measured and quantified to determine if there are any changes in rate or trends.

These measurements can give us important insights into how changing the conditions that plants and soils are subjected to, for example increasing water application or converting forest land into pasture, can affect the respiration balance of the soil. By being able to determine the change in $\delta^{13}\text{C}$ of a specific soil sample over time, a better understanding of how these changes will affect the global carbon budget can be obtained and a better plan to predict the effects of land use change to the environment can be determined.

1.5 Introduction of techniques to measure $\delta^{13}\text{C}$

To be able to assess the $\delta^{13}\text{C}$ signature of a soil sample, a gas sample from the soil needs to be obtained. The net exchange of CO₂ over a large landscape can be measured using a micrometeorological technique called eddy covariance (Goulden *et al.*, 1996). However normal eddy covariance is less sensitive to measuring changes in the $\delta^{13}\text{C}$ and determining whether changes in net exchange are due to photosynthesis or respiration in an area smaller than a field, especially compared to efflux chamber methods (Midwood & Millard, 2011). Also, when taking gas samples from soil with the goal of measuring $\delta^{13}\text{C}$, it is imperative that there be no contamination from the outside air. This would cause a significant shift in the $\delta^{13}\text{C}$ value measured, as air is typically -8.5 ‰, whereas soil respired CO₂ samples can be as low as -31 ‰ (Ghashghaie & Badeck, 2014). It is also exceedingly important there is little to no disturbance to the soil sample being measured. It has been shown that as soon as a soil sample has been exposed to the outside environment, for example disturbed from digging or from a core sample being taken, this exposure can drastically change the $\delta^{13}\text{C}$ of the respired CO₂ in a matter of minutes (Midwood & Millard, 2011; Zakharova *et al.*, 2014). Therefore in order to obtain a soil respiration sample for isotopic analysis in its purest form, it needs to be obtained in a manner which doesn't disturb the soil and avoids contamination from the atmosphere.

It is widely considered that the most effective way of collecting gas samples for isotopic analysis involves the use of ground chambers that sit directly on top of the sample to be measured or on top of a ring previously inserted into the soil (Le Dantec *et al.*, 1999; Rochette *et al.*, 1997). There are four types of chamber systems that can be used to collect gas samples from undisturbed soil for isotopic analysis - closed, open, dynamic and forced diffusion systems (Midwood & Millard, 2011; Risk *et al.*, 2011). All these systems have benefits and

problems when measuring soil respiration and collecting samples for isotopic analysis. Each chamber type has its situational use and any decision should be based on the experimental setup itself (Midwood & Millard, 2011). This will be discussed in more detail in Chapter 2.

Chamber systems are however limited in they cannot measure the $\delta^{13}\text{C}$ signature of a gas sample by themselves, although there are now cheaper field instruments that can be used e.g., Picarro isotope analysers. Gas samples are usually collected and then transported to a laboratory to have their isotopic signature measured. There are three main methods to measure the $\delta^{13}\text{C}$ signature of a gas sample. Isotope ratio mass spectrometry (IRMS), tuneable diode laser spectroscopy (TDLS) and cavity ring down spectroscopy (CRDS). A comparison of these can be found in Midwood and Millard (2010).

1.6 Thesis objectives

The work in this thesis was conducted in two parts. First, to deconstruct and rebuild a previously non-operational, 4-chamber, dynamic soil respiration system into an operational 6-chamber system. This involved constructing a new housing for the system, the addition of new instruments to accommodate the increase of capacity, a complete rewiring and also several major modifications to the program that was running the system.

The second part of this thesis was to use the system to investigate factors which could influence the respiration rate and $\delta^{13}\text{C}$ of soil-pasture respired CO_2 from a Landcare Research site on a dairy farm in the South Island, New Zealand.

The specific objectives of this thesis were to:

- Construct a 6-Chamber, dynamic, Soil Respiration Isotope Sampling System (SRISS) which can be used to faithfully determine the total soil respiration rates of CO_2 and capture CO_2 samples for $\delta^{13}\text{C}$ measurement both in the lab and in the field.
- Test the performance of the SRISS over a range of soils with different $\delta^{13}\text{C}$ content and respiration rates.
- Determine the changes in $\delta^{13}\text{C}$ of respired CO_2 over a 24 hour period.
- Determine the effect of lowered photosynthate (reduced light) input on both total soil respiration rates, changes in respired $\delta^{13}\text{C}$ and partitioning soil respiration into their R_A and R_H components.

Chapter 2 Soil Respiration Isotope Sampling System (SRISS)

2.1 Introduction

One of the most important aspects in the measurement of $\delta^{13}\text{C}$ of R_s , is the ability to faithfully collect the gas diffusing out of the soil in a manner which causes the least disturbance to the established $\delta^{13}\text{C}$ and CO_2 gradients. It has been shown that even a slight disturbance, including slight changes in the pressure inside the chamber, can have a large effect on the $\delta^{13}\text{C}$ gradient and efflux (Le Dantec *et al.*, 1999; Midwood & Millard, 2011; Risk *et al.*, 2009; Zakharova *et al.*, 2014). This can lead to either an over or underestimation of both the flux and the $\delta^{13}\text{C}$ of R_s . Therefore it is imperative that, when collecting soil respired gas samples, there is as little soil disturbance and pressure fluctuations as possible.

Soil respiration has been measured for several decades, with the first measurements made in the late 1950s (Stevenson, 1956; Wieringa & Kerkhof Mogot, 1957). Methods for sampling the CO_2 respired from the soil have evolved, uncovering a number of problems and opportunities, with new methods proposed every year [for example, passive towers and *in situ* buried semi permeable tube] (Ryan & Law, 2005; Schaeffer *et al.*, 2008). I will look at these approaches and compare their advantages and disadvantages with the end goal of trying to obtain the more difficult goal of measuring both soil CO_2 efflux and the measurement of $\delta^{13}\text{C}$ of the soil respired CO_2 .

2.2 Overview of methods

One of the least disruptive approaches for the measurement of CO_2 efflux and $\delta^{13}\text{C}$ of a soil sample is to create a chamber system that is placed over the soil, make measurements, take samples and can be removed while causing very little soil disturbance. There are four main approaches that can be used to achieve this, each with their own advantages and disadvantages; open systems, closed systems, dynamic systems and forced diffusion chambers (Midwood & Millard, 2011; Risk *et al.*, 2011). These systems typically consist of a control module which controls and measures gas flow, a CO_2 analyser, and chambers which are either inserted a few centimetres into the soil or placed onto a collar which has been previously installed. These systems can consist of either one chamber or up to eight chambers, all managed by the control module.

Closed chambers are the most widely used method for measuring R_s (Midwood & Millard, 2011). Closed system chambers work by using a sealed chamber that is pushed 2-3cm into soil, creating a seal in the soil, or they can sit on a ring already inserted into the soil to create the seal. Once sealed, the chambers recirculate the air between the chamber and the analyser with the soil respiration efflux is determined by measuring the change in CO_2 concentration over a set period of time inside the chamber. This is an effective technique, especially when repeat measurements are required over a significant length of time. Many commercial respiration measuring devices use this approach, for example, SRC-1 Soil Respiration Chamber (PPSystems, Amesbury, MA, USA), LI-8100A (LI-COR, Lincoln, NB, USA), and SRS-SD1000 (ADC, Hoddesdon, Herts, UK). However, one of the main drawbacks to using a closed system is that, as the CO_2 builds up in the chamber the diffusion from the soil slows down due to a reduction in the concentration gradient, thus creating an underestimation of the efflux. There are ways to counter this problem, including mathematical programmes which predict and adjust for this effect or by absorbing the CO_2 as it enters the chamber. Both these solutions, while effective, can lead to other issues, including mathematical error or distorted data. Closed chambers can be very useful to measure differences between sites, however for an accurate measurement of $\delta^{13}C$ from a single site over a prolonged period of time this type of system is undesirable because they distort the $\delta^{13}C$ concentration and also don't preserve the soil respired CO_2 as it is mixed with ambient air already in the chamber. Closed systems usually require a lot of power and are often large and bulky to move.

Open chamber systems are, as the name suggests, open to the outside environment. These chambers have been traditionally used to make continuous measurements over a significant period of time (weeks to months). Open chambers work by drawing or pumping air through a chamber which is on the soil surface, then accurately measuring the flow rates and CO_2 concentrations (Fang & Moncrieff, 1998). The efflux is calculated by measuring the difference in concentration of CO_2 between the in-flow and the out-flow from the chamber. The benefit of having an open system like this is there is no need to compensate for any feedback due to CO_2 building up inside the chamber. However it has been shown that changes in pressure inside the chamber, especially due to wind, affect the measured efflux (Le Dantec *et al.*, 1999) and therefore is a major challenge in using this type of system for isotope sampling. This can be overcome by choosing a site with very little to no wind, or alternatively, shielding the chambers in some way. However, this may be undesirable as it may interfere with the natural air flow at the measurement site and thus create a distortion in data.

Dynamic chambers have the potential to avoid a number of the issues described above. Dynamic chambers work on the same principal as closed chambers, however sample gas is not reticulated and thus measurement of the efflux is achieved by accurate and simultaneous measurement of the flow rates and concentration of CO₂, by doing this the sample gas is also able to be captured for $\delta^{13}\text{C}$ analysis. These chambers work by controlling the concentration of CO₂ inside the chamber so it is as close to atmospheric concentrations as possible and also by keeping the pressure as close to atmospheric as possible. This is achieved by having a covering (lid) over the chamber such as a closed system would, and flowing CO₂ free air into the chamber at a strictly controlled rate and pumping air out at a slightly lower controlled rate (a difference of ~10-20 mL min⁻¹). This creates a slight positive pressure system, and an installed chimney allows pressure relief. This creates an environment where both the pressure and concentration of CO₂ inside the chamber are as close to atmospheric conditions as possible. This alleviates the CO₂ build up seen in closed systems and pressure changes which cause a change in efflux as observed in open systems.

Forced diffusion chambers are based on a traditional flow-through dynamic chamber, using a single point measurement technique. They use water resistant gas permeable diffusion membranes, to maintain equilibration by diffusive means which could potentially replace pump and controller as seen in dynamic systems. This is based on the principal that free air diffusion and thermal convection provide sufficient mixing in the chamber (Risk *et al.*, 2011). Forced diffusion has the advantage of being more reliable than traditional dynamic chamber systems as there are fewer mechanical parts where potential issues could arise and has very low power consumption. Therefore it could be possible to place a forced diffusion chamber system out in the field for a significant length of time with the power consumption occurring only as measurements need to be made (Risk *et al.*, 2011). This type of chamber however is still in its infancy and needs further testing before it can be used widely.

Although the dynamic system is an improvement on other approaches, and offers the greatest benefits while controlling most of the potential problems as presented in open and closed systems, they are exceptionally complicated and require a significant amount of time and resources to build and operate. Potential problems can arise in windy conditions if the chimney system is not sufficiently protected. Another major drawback of using a dynamic system is the need for CO₂-free air. CO₂-free air can be obtained in two ways: (i) A cylinder of compressed CO₂-free air, which can be large, heavy and logistically difficult to move from one place to another, severely limiting transportation options of the entire system; (ii) a CO₂ scrubber

system to remove CO₂ from the air. This is a viable option but does require regular changing of chemicals. While these drawbacks could be significant, this system still potentially offers the most reliable efflux data in a greater number of scenarios of the four systems described. The system developed and used for this thesis was a dynamic system.

Once the soil respired CO₂ gas has passed through the dynamic system, been analysed to determine the soil efflux, the gas is then collected in evacuated, sealed Teldar[®] bags (Keika Ventures, Chapel Hill, NC, USA) and can then be analysed to determine its $\delta^{13}\text{C}$ signature. There are three main methods to measure the $\delta^{13}\text{C}$ signature of a CO₂ gas sample. Isotope ratio mass spectrometry (IRMS), tuneable diode laser spectroscopy (TDLS) and cavity ring down spectroscopy (CRDS). A comparison of these methods can be found in Midwood and Millard (2010). For the experiments described in this thesis, a TDL (TGA 100A, Campbell Scientific, Logan, UT, USA) was used for all $\delta^{13}\text{C}$ measurements.

2.3 Respiration System development and use in this thesis

The Soil Respiration Isotope Sampling System (SRISS) built for the research described in this thesis is an extension of a previously developed four chamber dynamic system. The new system included two additional chambers and a complete rebuild including rewiring, re-plumbing, a modified program to control the system and a new housing. With six chambers and more sophisticated automatic CO₂ concentration control, this system has the potential to be used in a greater variety of applications than the previous version.

The old, pre-existing soil isotope respiration device was deconstructed and rebuilt at Landcare Research, Lincoln over several months. All parts were calibrated and tested on site. A significant part of the build was the development and testing of the program which runs the SRISS. This was done using CRBasic developed by Campbell Scientific (Logan, UT, USA). The programme was based on previous algorithms used on the four chamber system, however a number of additions were made to enable the use of six chamber and for more automatic control. The system is housed in a custom built wooden box and will eventually be housed in a solid plastic travel case to ensure protection and portability.

Once it was completed, testing was undertaken to ensure the system collected a true representation of the $\delta^{13}\text{C}$ of the respired samples. This is an extremely sensitive test as the difference between the respired $\delta^{13}\text{C}$ and the $\delta^{13}\text{C}$ of air is only 15-20 ‰. Since the TGA has a

repeatable precision of about 0.3‰, any contamination from outside air can easily be detected, or effects of pressure changes. Testing was carried out using soil samples from five different locations in New Zealand, covering a wide range in $\delta^{13}\text{C}$ signatures. A number of problems were diagnosed during this test, most of which were caused by leakage from the pumps. The pumps either caused an inward leak of outside air resulting in samples being contaminated, or they had a deleterious feedback on the accurate measurement and control by the mass flow controllers.

Once completed and tested, this system was used to determine the effect of photosynthate starvation on soil respiration on pasture soil cores. Beyond this work, the system has the potential to be used in many more applications in the study of $\delta^{13}\text{C}$ and R_s (see Future Applications, Chapter 6).

2.4 Respiration System Components and Build

The components of the SRISS can be grouped into four main sections.

- Measurement and control - carried out by a sophisticated datalogger.
- CO_2 concentration measurements - made with an infra-red gas analyser.
- Gas flow - measured and regulated by mass flow controllers (MFC). Three-way solenoids, and diaphragm pumps are used to tightly control gas flow and its destination.
- Electrical input/output. This involves signal and electrical control of the system - it has three four-channel analogue output modules, and one sixteen channel DC controller. These control the direct signal to all the gas flow components. Also 12 VDC power, fuses and distribution.

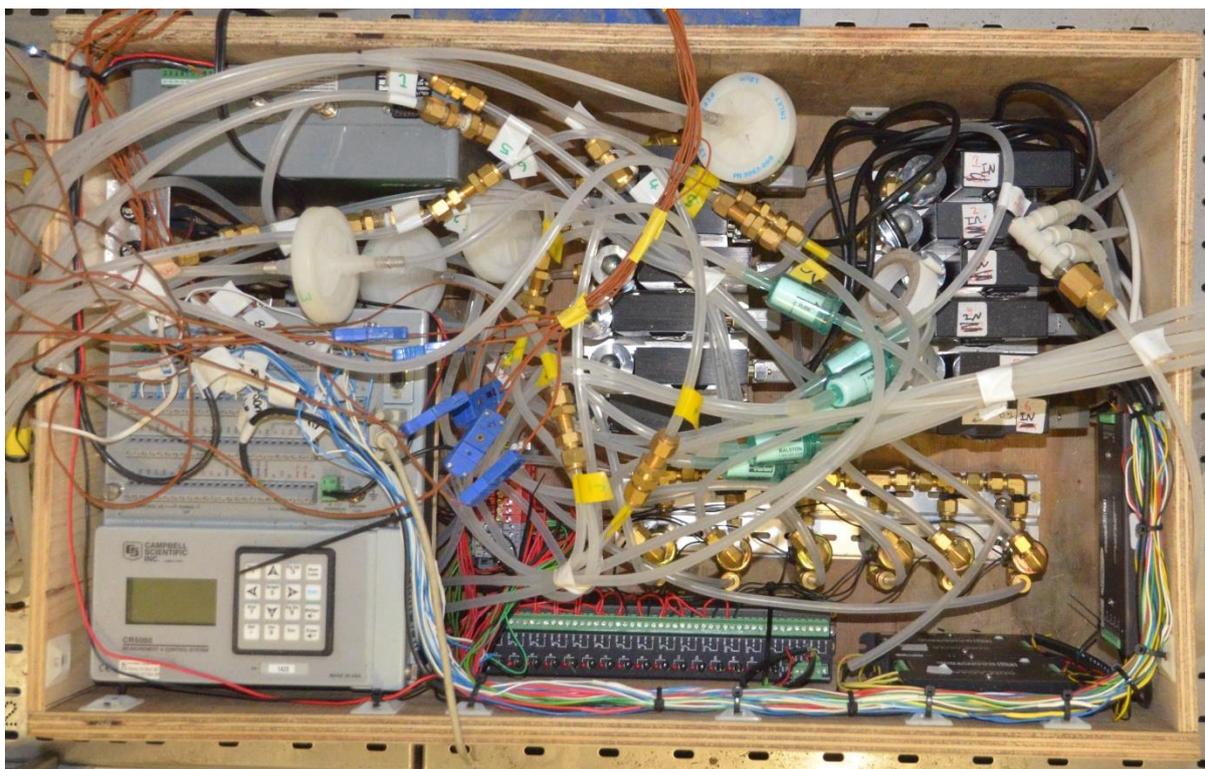


Figure 2.1: All components in their final installation form

The entire system uses 0.25 inch tubing (Bev-A-Line), all connections were made using Swagelok brass fittings to ensure stability and airtightness.

The datalogger (CR5000, Campbell Scientific Inc., Logan, UT) controls all the solenoid switching, flow control actions of the entire system as well as recording all data. While the system was running, the datalogger was continuously connected to a computer to provide continuous and instantaneous graphs which allowed the user full interaction and the ability to modify parameters on the fly. A custom program was written by the author to achieve a sophisticated, high level of control and was coded using CRBASIC (Campbell Scientific, Logan, UT, USA). The control program used was a substantially modified version of an old program originally written by Dr. John Hunt of Landcare Research, Lincoln.

The infrared gas analyser (LI-840A, LI-Cor Inc., Lincoln, NB, USA) measures the CO₂ and water vapour concentration leaving the chambers. It is an absolute non-dispersive infrared analyser based on a single path, dual wavelength infrared detection system. An internal 1 sec low pass filter was applied to the concentrations to ameliorate sensor noise. This analyser has low precision (typically <1% of reading, with <1 ppm RMS noise at 370 ppm) but it is small, has low power requirements and is robust, making it immune to vibration and useful in field locations. The LI-840A was calibrated at the start of each experiment. CO₂-free air was used to set the zero, and the span was set using a cylinder of known CO₂ concentration in air supplied

by BOC Special Application Mixture (BOC Gases New Zealand Limited, Auckland, New Zealand) and calibrated by The National Institute of Water and Atmospheric Research (NIWA). The water vapour concentration was also calibrated, using a magnesium perchlorate desiccation system to set the zero point and a dew point generator (LI-610, LI-Cor Inc) to set the span. The CO₂ analyser uses the water vapour measurements to internally calculate the dry air concentration of CO₂. The measurements made by the analyser were recorded by the datalogger.

Precise airflow to and from the chambers was measured and regulated by twelve mass flow controllers (MFC). It is essential that the flow to and from each chamber is accurately controlled and recorded as the efflux is calculated from these measurements. There were some unfortunate mechanical failures with some of the original mass flow controllers. Originally all the mass-flow controllers were 0.2 L min⁻¹ (OMEGA FMA-5410 0.0-200 mL min⁻¹, Stamford, CT, USA) and the deviation from this will be discussed in a later section. Eventually, six 0.2 L min⁻¹ were used to control the gas flow to the chambers and the other six were 2 L min⁻¹ (OMEGA FMA5510) used to control the gas flow from the chambers. The mass flow controllers receive an input signal (0-5000 mV) from the datalogger via an analogue to digital (A-D) convertor (SDMAO4, CSI), this set the flow rate, and the MFC produces an output analogue signal (0-5000 mV), proportional to the mass flow rate, which is recorded by the datalogger. The accurate recording of the flow rate, especially for the gas flow to the chamber, is vital because the efflux of CO₂ exchange with the soil is calculated from this value along with the CO₂ concentration. The feedback with the signals sent to and from the MFCs was critical in the success of this chamber system and allowed constant minute control of the mass-flow controllers. The use of subroutines in the program allowed for the quick changing of flow controllers and their associated calibration constants.

CO₂ free air was provide from a cylinder of compressed atmospheric air (“medical air”, BOC, Christchurch, NZ). This should not be confused with what BOC sells as “compressed air” which is reconstituted from only nitrogen and oxygen. The air was scrubbed by passing it through soda-lime reducing the CO₂ concentration to <1 ppm. The pressure from the gas bottle was used to push the CO₂-free air through the scrubber and MFCs to the chambers. Pumps were used to extract the gas from the chambers and push it through either the CO₂ analyser or to the gas collection bags. The pumps were upstream of the outlet mass flow controllers as mass flow controllers do not work well under vacuum (Figure 2.2). This also created positive pressure between the mass flow controller and the pumps. With the pumps unable to have their

flow rate controlled other than using the MFC, the advantage of having positive pressure between the mass flow controller and the pumps is it theoretically negates any chance of contamination from the outside air. This was proven experimentally.

Filters (9933-05-DQ-10, Parker, Cleveland, OH, USA) were installed between the pumps and the receiving MFCs. These served two purposes, firstly they remove particles from the chamber sampled gas which is preferable when dealing with soil systems as mass flow controllers can be easily damaged by dust and dirt. They also created a small buffer volume that reduced the airflow pulsating at its resonance frequency, which allows for better flow control.

After exiting the chamber, the gas flow was directed by solenoid valves to either the CO₂ analyser or the gas collections bags. These valves were controlled by a 16 channel switch controller (SDM-16AC, CSI), on instruction from the datalogger.

The system requires approximately 135W of power at 12 VDC from either a battery or mains power. The system is designed for autonomous control and was left running for 24 hours at a time. However, it can potentially run for weeks with only minimal checking.

Construction took several months, all components are housed internally with only the power cable, gas input, tubes leading to and from the chambers and the tubes leading to the gas capture bags protruding from the encasement. This has been done to protect the valuable contents, provide easy transport options in the future, and reduce the possibility of damage in the field.

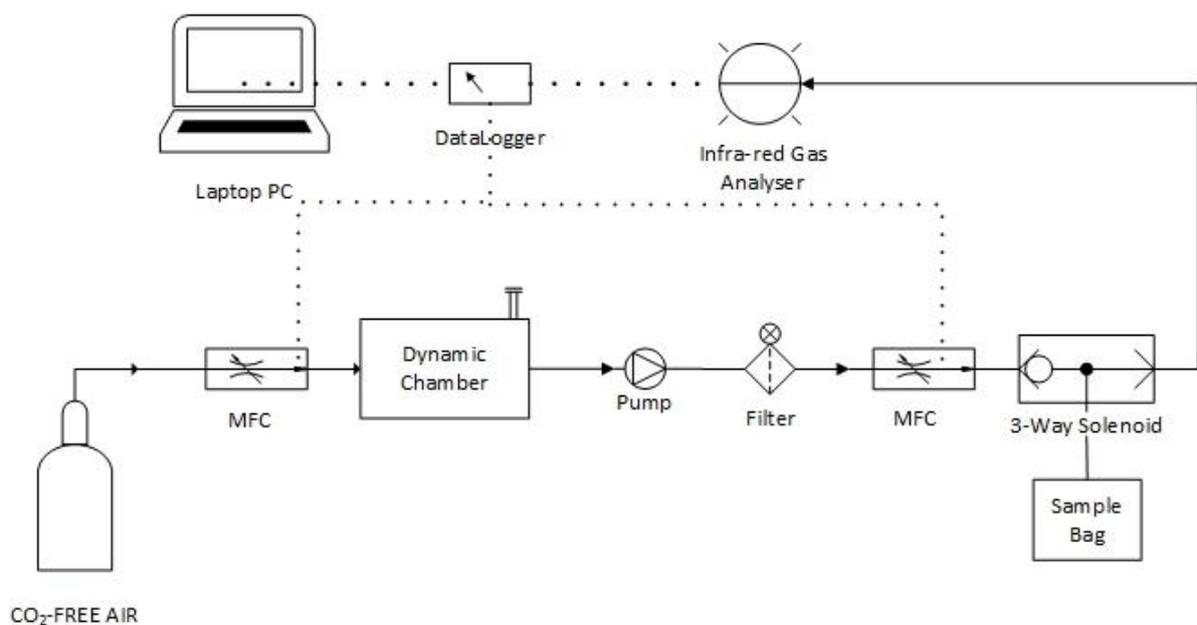


Figure 2.2: Schematical drawing of the dynamic system used for this thesis. Solid lines represent gas flow and direction. Dotted lines represent data transfer and communication.

2.5 Soil Isotope Respiration Program

The program consisted of an algorithm which was developed by Dr. John Hunt, Landcare Research for a previous experiment (Millard *et al.*, 2010). However, while based on this original algorithm, the final programme structure, interface and control subroutines were all coded for this thesis by the author, and they were included in the programme to increase efficiency, accuracy and autonomy (*6chamberconstant*, Appendix I)

An initialisation subroutine was written, so that every time the program was started, for instance at the start of a new experiment, all settings revert to a predetermined state. In the event of loss of power, this subroutine allows the original settings to be implemented and control to continue automatically. Power cuts occurred a couple of times during the experimental phase, and the growth cabinet would completely turn off. This reduced the respiration rates and required time for the respiration to return to an equilibrium rate. The pre-set, constant, initial settings on the SRISS effectively allowed the system to restart at an appropriate setting and reduced overshoot of CO₂ concentrations.

An automatic CO₂ concentration control subroutine was written to enable the program to keep the CO₂ concentrations in each chamber at a predetermined value, which was normally 20 ppm higher than ambient atmospheric concentrations. This was achieved by determining a desired set point and a dead-band. When the concentration is more than the dead-band maximum, the flow rate of CO₂-free air was incrementally increased by a desired step, and when it was lower than the minimum, the flow was decreased. The dead-band was set up as ± 10 ppm from the chamber concentration set point and step increments was determined experimentally. The major advantage of this subroutine was it allowed for autonomous running of the SRISS. This subroutine can be manually switched on and off and set points and bandwidth can be changed on the fly, depending on the respiration rate of soil being measured it took between 10 and 30 min to reach the desired set point with undisturbed control being ± 5 ppm.

All the pumps can be switched on and off via the control interface on the attached computer, as can the direction of flow from the three-way solenoids, to allow for sampling of air from each chamber for isotope analysis.

The program calculated soil sample efflux (R_s , $\mu\text{mol m}^{-2} \text{s}^{-1}$) using the following formula:

$$\text{Efflux} = \frac{\left(\left(\frac{([\text{CO}_2] \cdot \text{Flowrate}(\text{sample}))}{10^6} \right)}{R \times T} \right) \left(\frac{1}{\text{surface area (ring)}} \right)}{60\text{sec}} \quad (\text{Equation 1})$$

Where CO_2 concentration is in $\mu\text{mol}/\text{mol}$, flow rate is mL min^{-1} (by dividing by 10^6 it is converted to μmol), T is temperature in Kelvin, R is the ideal gas constant and the surface area is m^2 .

By using the change in concentration and the flow rate of CO_2 -free gas into the sample chamber (as mentioned in equation), and knowing the surface area of the soil exposed in the chamber, the above formula calculates the instantaneous CO_2 efflux from the soil. The instantaneous CO_2 efflux is calculated by the datalogger each second in $\mu\text{mol}(\text{CO}_2) \text{ m}^{-2} \text{ s}^{-1}$ and then it was also converted to $\text{g}(\text{CO}_2) \text{ m}^{-2} \text{ h}^{-1}$ for comparison with the output from another instrument (SRC-1 Soil Respiration Chamber, PP-Systems, Amesbury, MA, USA)

2.6 Measurement of $\delta^{13}\text{C}$

For these experiments, the $\delta^{13}\text{C}$ signature of CO_2 was measured with a TGA100A (TGA) trace gas analyser tuneable diode laser (Campbell Scientific Inc, Logan, UT, USA). Since the late 1990s tuneable diode lasers have been the preferred choice for carrying out $\delta^{13}\text{C}$ research (Bowling *et al.*, 2003). The TGA system can be used to measure concentrations of trace gases in air. It uses a lead-salt tuneable diode laser that operates in the temperature range of 80-140 K, and is cooled using liquid nitrogen. The laser is both temperature stabilised and current controlled at the same time to scan across predefined and well characterised absorption lines of the isotopologues of CO_2 ($\text{C}^{12}\text{O}^{16}\text{O}^{16}$, $\text{C}^{12}\text{O}^{16}\text{O}^{18}$, $\text{C}^{13}\text{O}^{16}\text{O}^{16}$) and outputs the mixing ratios (Barbour, 2007). A full description can be found in Bowling *et al.* (2003). The formula used to calculate the $\delta^{13}\text{C}$ ratio is described in the introduction of Barbour *et al.* (2007). All operations, measurements and calculations, were controlled by a datalogger (CR1000, CSI), running a program written by Dr. John Hunt, and includes an automatic calibration system and giving real-time calibrated values.

Respired gas was captured in 1 L sealed Tedlar[®] bags that had been flushed three times with CO_2 -free air and then evacuated completely. The closed bags were attached to the outlet nozzles of the respiration system and gas lines were purged for 30 s. This was to ensure no trace amounts of atmospheric gas were present. The bags were then opened and filled with approximately 400 mL of sample respired gas. The bags were filled sequentially approximately one minute apart. Once a bag was filled it was immediately transferred to the TGA for analysis. The bags were connected to the TGA and opened for 15 s to flush the tubes. The TGA then

analysed the gas for 35 s, recording every second, it then averaged the last twenty measurements and calculated the SEM.

The TGA100A does have a small amount of drift resulting in changes in the offset and gain of the instrument. Since isotopic ratio measurements require the highest accuracy possible, the TGA is recalibrated before every sample. Before measuring each bag the TGA measured the CO₂ isotopologues in two standards with known concentrations. The two cylinders of air were mixed by BOC NZ. Samples were sent away to NIWA for analysis of the CO₂ concentration and the $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ of the CO₂.

2.7 SRISS Testing

Once the design and construction was completed, the SRISS was tested to ensure that it could take a representative sample of soil respired CO₂ and perform the required measurements. These tests were also done to ensure that any gas sample that passed through the SRISS was not isotopically altered in any way, either by internal isotope fractionation, or by mixing with external air. An experiment was devised to tackle both of these potential problems.

When carrying out tests and also during the experimental phase of this work, the sampling system was housed in a waterproof box, and kept inside the growth chamber. This kept all tubing at the same temperature as the growth cabinet, avoiding condensation in the gas tubes.

Intact soil samples were obtained from five sites in NZ with different climates and vegetation to provide a large range of different but realistic $\delta^{13}\text{C}$ signatures. The soil samples were collected from a dry, reverting pasture/shrubland (Landcare Research, Lincoln), a broadleaf rainforest (West Coast, South Island), a very dry, degraded tussock grassland (Alexandra), a new converted dairy farm (Canterbury plains) and soil with crops of various species (Landcare Research, Lincoln). Once soil samples were returned to the laboratory, they were thoroughly mixed to ensure that any major $\delta^{13}\text{C}$ signature influences were distributed evenly throughout the sample. Six samples of 300 g each were placed in the experimental cylinders (Figure 2.3) and the chambers were then placed on top creating an airtight seal. After the CO₂ concentration in the chamber reached an equilibrium of about 400 ppm, the chambers were kept at a constant concentration for 30 min before taking gas samples for further analysis. Further 300 g soil samples were then placed in six clear, sealable Tedlar[®] bags and placed in the same conditions as the soil samples in the testing pots. When the bags collecting gas samples from the SRISS

were close to capacity, the open Tedlar[®] bags were then vacuum sealed and flushed with CO₂ free air and incubated in the same conditions as the respiration soil samples, until the CO₂ concentration reached 400 ppm, following the method of Zakharova (2014). Once enough gas was collected in all the Tedlar[®] bags the $\delta^{13}\text{C}$ signature was determined by the TGA. This was done to compensate for any change in the $\delta^{13}\text{C}$ signature due to exposure of the soil to well oxygenated air (Zakharova *et al.*, 2014). The bag respiration measurements were considered to be a true representation of the $\delta^{13}\text{C}$ of the respired CO₂, and the SRISS measurements were compared to them.

It was hypothesised that if the sampling system was airtight and working correctly then there would be no difference between the $\delta^{13}\text{C}$ signature determined by the SRISS and the $\delta^{13}\text{C}$ signature of respired CO₂ collected during soil incubation in sample bags.



Figure 2.3: Testing the SRISS. Rings are identical to those used in soil/pasture experiments, however are glued and fully sealed onto plastic base. This provided a closed environment where the only gas in the system was either from the soil itself or CO₂-free air coming from the SRISS.

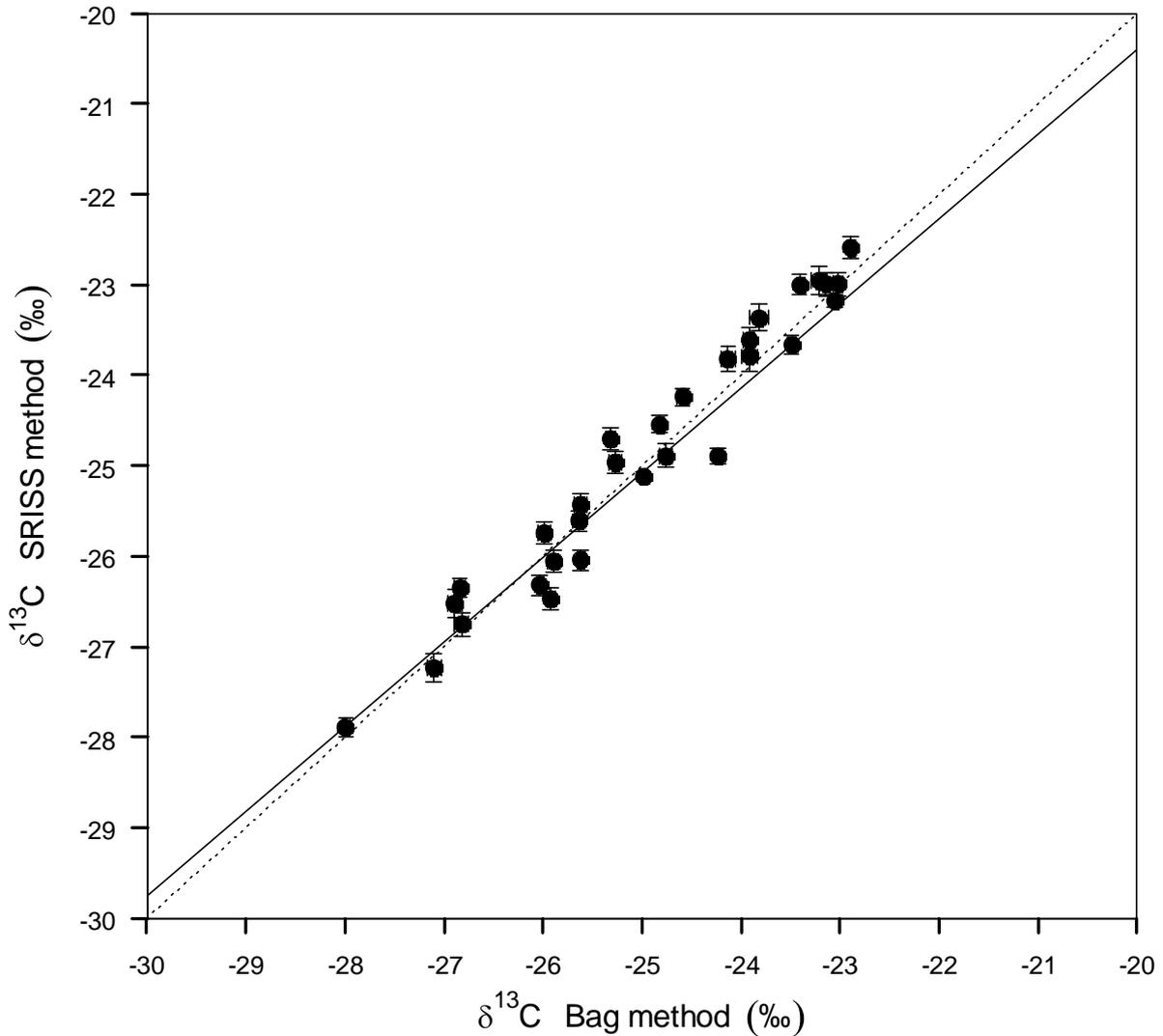


Figure 2.4: A comparison between the $\delta^{13}\text{C}$ of soil respired CO_2 obtained from bag incubations and $\delta^{13}\text{C}$ from the soil sampled using the SRISS. All values are the mean \pm SEM ($n = 6$). The black line is the linear regression ($R^2 = 0.96$). Dotted line represents the 1:1 line.

$\delta^{13}\text{C}$ ranged from -22.9 ‰ to -28.1 ‰ (Figure 2.4). This is as big as the range in C_3 soils anywhere in the world and certainly covered all values expected in New Zealand's natural and cultivated C_3 dominated landscapes. There was a very strong relationship ($R^2=0.96$) between the $\delta^{13}\text{CO}_2$ derived from the bag incubations and those produced by the SRISS with no significant difference ($p=0.0002$) between the two different methods.

Three of the samples used in the testing had their $\delta^{13}\text{C}$ manipulated so the SRISS could be tested with a wide range of $\delta^{13}\text{C}$ values. Studies have shown when a sample of soil is left in the open for periods more than 7 minutes the $\delta^{13}\text{C}$ of the respired CO_2 from the sample increases over time (Zakharova *et al.*, 2014). This method was used on three of the samples as otherwise the $\delta^{13}\text{C}$ of the samples used would have been too similar for testing. Therefore these samples do not represent the diversity of $\delta^{13}\text{C}$ from respired CO_2 in New Zealand soils.

An advantage of the soils being from different regions and soil types and the modification

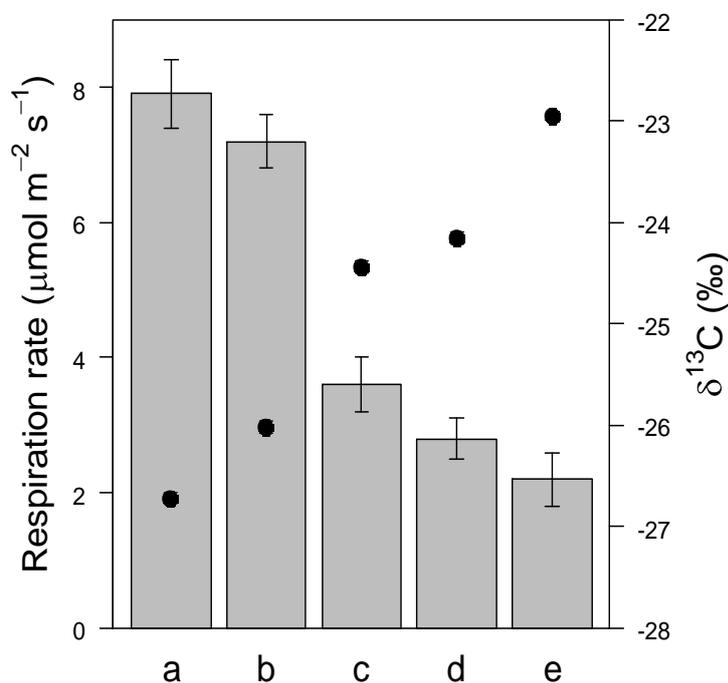


Figure 2.5: Respiration rates from soil samples used for SRISS tests. Averages (n=6) ± SEM. (a) LCR crop soil, (b) LCR shrubland soil, (c) Beacon farm soil, (d) West Coast rainforest soil, (e) degraded tussock grassland soil. Solid points are the corresponding δ¹³C for that sample using the SRISS method. All data are averages (n=6) ± SEM.

some of them underwent, the respiration rates they presented were varied (Figure 2.5). The soils tested had an efflux ranging from very low 2.2 µmol m⁻² s⁻¹ and up to 7.9 µmol m⁻² s⁻¹ (Figure 2.5). Previous studies of New Zealand soils have shown ranges that are between 1.6 and 9.2 (Millard *et al.*, 2010) (G. Moinet, personal communication, April 8, 2014). The lower the respiration rate, the higher the chance for the SRISS to be susceptible to outside atmospheric influences and thus contaminated soil respired gas samples. It would be highly improbable that the SRISS would

be making measurements on soils with respiration rates lower than 2.0 µmol m⁻² s⁻¹. However since the SRISS was able to accurately measure the δ¹³C of soil respired gas samples with a flow rate as low as 1.9 µmol m⁻² s⁻¹ it shows that the SRISS can take accurate measurements with a wide range of soil respiration rates.

Therefore, it can be concluded that the SRISS gives a true and consistent measurement of the δ¹³C of the respired CO₂, with no bias or offset due to leaks or fractionation. From these results it can be concluded that any gas samples that pass through the tubing, pumps and mass flow controllers will not have their δ¹³C signatures modified by the system. Also there was no infiltration of the chamber by atmospheric air, contaminating the gas samples, and that there was not influence of the slight internal pressure effecting the δ¹³C diffusion from the soil. This shows that the SRISS works correctly and as intended, and therefore it can be confidently said that any δ¹³C values collected using this system will be a true and accurate representation of the soil respired CO₂ values.

2.8 Respiration system challenges and solutions

Several issues that needed to be overcome presented themselves during the building and initial testing of the SRISS. These required significant time and effort to diagnose and rectify. Interestingly, all these challenges revolved around the pumps and the disturbance they presented with pulsating at a resonant frequency.

1. The first major issue was a noticeable reduction of 4-6 ‰ in the $\delta^{13}\text{C}$ signature of respired CO_2 between initial incubated bag samples and the samples obtained from the respiration sampling system. The initial thought was that there was a leak, allowing surrounding ambient -8.5 ‰ air into the gas sampling line. Several attempts were made to rectify this by retightening all connections and making sure all gas lines were air tight. Pure food grade CO_2 was used as a tracer to find the leaks. To do this, clean outside air with a CO_2 concentration of around 390 ppm was pumped through the respiration sampler system, and a fine stream of pure CO_2 was directed through a needle and targeted at all possible contamination locations. Direct, 1 sec readout of the CO_2 concentration in a graphical form allowed me to detect a CO_2 spike from around the pumps. It was discovered that the change in CO_2 was due to inward contamination through the seals in the pumps. The pumps at this stage were located between the MFCs and the solenoid valves. This created a negative pressure zone in which air from the outside environment was able to enter the system and contaminate the samples resulting in the observed reduction of 4-6‰. Several avenues were considered to rectify this issue, including looking at ways to better seal the pumps. In the end it was decided that the simplest way was to re-plumb the pumps to be positioned before the MFCs in the system. The added advantage of this was it helped prevent air entering the system and potentially contaminating gas samples resulting in erroneous measurements.

2. The second significant problem was the effect of the pump on the stability of the MFCs. Due to the oscillating frequency of the pumps, this created control issues. Since MFCs rely on a constant source of positive pressure to operate properly, having a pump pushing gas into them with an oscillating pressure and flow caused them to increase gas flow to compensate for the pressure lows. The pumps couldn't be placed in a different position as this would lead to negative pressure in the MFC. The solution devised for this was to include inline filters. This created a short-term buffered volume than dampened the oscillations. As the gas entered the filter, the expanded space would slow the flow down and thus would buffer the gas so it left at a more constant pressure.

3. Once the buffer filter was installed, the 0-200 mL min⁻¹ MFCs still allowed gas flow at an inconsistent and much higher rate than programmed. This presented a major problem because for the system to work as intended the gas flow from the chambers has to be slightly less than that of the gas flow into the chambers. If the outlet flow is higher than the inlet flow, atmospheric air is drawn into the chamber and this changes the $\delta^{13}\text{C}$ values. The 0.2 L min⁻¹ MFCs were replaced with 2 L min⁻¹ MFCs. Whilst they had lower precision they were less affected by the pulsating action of the pumps. Once calibrated, testing confirmed that the 2 L min⁻¹ MFCs were able to handle the oscillating pressure of the pumps through the filters and kept a constant flow of gas at a controllable and constant rate.

2.9 Summary

Isotopic sampling systems are difficult and expensive to build and take a long time to test. There are many individual components that can lead to small leaks. Since there is a large difference in the $\delta^{13}\text{C}$ of atmospheric and respired gas, any small leak can have detrimental effects on the determination of the true values of the respired $\delta^{13}\text{C}$. While the SRISS took longer than expected to build and be successfully tested, the end result was a well characterised system that can faithfully capture the $\delta^{13}\text{C}$ of *in-situ* soil respired CO₂.

Being a dynamic system it has the potential to be used in a wide variety of applications, including long term measurements in both laboratory growth cabinet environments and out in the field, similar to the conditions found in Millard et al. (2008). With its portability and future installation of a more advanced and robust CO₂ scrubber to alleviate the need to rely on CO₂ free air tanks, this system can be used in a wide variety of ecosystems and has the potential to be transported to remote areas where the measurements made by this system were previously not possible.

A number of problems presented themselves during the testing phases. These all revolved around the pumps and their pulsating action and the effect this had on the MFC. However, by redirecting the flow to create a positive pressure system and replacing the intake MFC's from 0.2 Lmin⁻¹ to 2 Lmin⁻¹ these problems were overcome and presented no further issues in testing. This SRISS is now fully tested and functioning and can be used with confidence in a range of areas where accurate soil respiration efflux and $\delta^{13}\text{C}$ signature measurements are essential.

Chapter 3 Sample site and acquisition

3.1 Site description

Beacon Farm is located outside of Dunsandel, 8.6 km north east of the Rakaia River on the Mid-Canterbury plains, South Island, New Zealand ($43^{\circ} 36' 1.41''$ S, $171^{\circ} 55' 57.46''$ E) (Figure 3.1 and 3.2). The land was formerly used as a deer farm and was not irrigated. It has recently been converted to a fully intensified dairy farm. However part of the farm has been left in its non-irrigated state. This site provides an opportunity to study the effects of intensification and land-use change on the soil characteristics and how they are influenced by these changes in farming land management. Landcare Research with the cooperation of the farm owners Synlait Farms Ltd has set up two sites at Beacon Farm, one in the intensified area and the other in the non-intensified area, hereafter called Wet and Dry sites respectively. Figure 3.3 shows the location of the dry and wet sites relative to each other.



Figure 3.1: Pin (A) indicating the location of Beacon Farm on the Canterbury plains (Google Inc. 2009).



Figure 3.2: Satellite photograph of Beacon Farm (Google Inc. 2009).



Figure 3.3: Closer view of Beacon Farm where research sites are located (Google Inc. 2009).

3.2 Soil samples

Twelve intact soil pasture samples of 260 mm diameter and 250 mm depth were taken from random positions along the outer edge of the wet paddock on Beacon Farm. Samples were taken by digging around the sample location, inserting a PVC ring and extracting the sample as whole and as undisturbed as possible. Once the sample was extracted a mesh covering was adhered to the underside of the pot allowing for water drainage but no soil to escape. The samples were then taken back to Landcare Research (Lincoln) and fully immersed in a water bath. Once fully saturated, the samples were left to drain to field water holding capacity and placed in a growth cabinet (HGC 1514, Weiss Gallenkamp Limited, Loughborough, United Kingdom) for six weeks (Figure 3.4).

3.3 Sample growing condition

The microcosms received an average of $600 \mu\text{mol m}^{-2} \text{s}^{-1}$ photosynthetically active radiation (PAR, 400-700 nm) for fifteen hours a day at 80% relative humidity. The position of sample pots was randomised once every week to ensure even light distribution. Day time temperature was set to 17°C and night time was set to 21°C. This temperature regime offset the daytime thermal warming and kept the soil temperature at 20 mm below the surface at a constant 21°C, with a maximum difference of 1°C between day and night.

For the first 3 weeks all pots were watered to full saturation every second day. After that, PVC sample rings of 110mm diameter were inserted into each pot to a depth of 30 mm to support the SRISS efflux chambers. Once the rings were inserted, all green foliage inside each ring was cut at the soil surface. This was done because when sampling R_s there needs to be no leaves in the sample area as this will affect $\delta^{13}\text{C}$ and respiration recordings. This cutting was continued until no new regrowth occurred in the middle of the sampling rings.

Each sample pot was then watered twice a week by filling up the central ring, to encourage root growth under the ring. The sample pots were then left for another three weeks to fully stabilise. During this stabilisation period the grass outside the ring was cut and maintained at the height of the inserted rings. This was to ensure that plants were kept in a juvenile form and prevented flowering.

Once the stabilisation period was completed each pot was assessed for species dominance. The biomass and growth rate characteristics of each sample pot was used to determine the allocation

of treatments in later experiments. It was important the plants were in a consistent day and night regime allowing for consistently stable measurements to be recorded during the measuring phase. The stabilisation continued for longer than expected due to several major power cuts at Landcare Research, after each power-cut the pots were given sufficient time to recover.



Figure 3.4: Samples growing in growth chamber with SRISS efflux chambers placed on the rings.

3.4 Sample species cover

The percentage cover of each species in each pot was estimated, and all species in the pot identified. All pots were dominated by ryegrass (*Lolium perenne L.*) with ~92% cover, *Agrostis capillaris L.* had the next highest coverage (6%), and the other 8 species registered a presence, but no substantial cover (Table 3.1).

Table 3.1: Distribution of plant species for all sample pots.

Plant Species	Cover
GRASS	
<i>Lolium perenne L.</i>	92%
<i>Agrostis capillaris L.</i>	6%
FLOWERING PLANTS	
<i>Achillea millefolium L.</i>	>1%
<i>Taraxacum officinale Weber</i>	>1%
HERBS	
<i>Cerastium glomeratum Thuill</i>	>1%
<i>Lamium purpureun L.</i>	>1%
<i>Hypochaeris radicans L.</i>	>1%
<i>Dichondra repens J.R.Forst.</i> & <i>G.Forst.</i>	>1%
<i>Trifolium subterranium L.</i>	>1%
CREEPING CLOVER	
<i>Trifolium dubium Siloth</i>	>1%

Chapter 4 24 hour response experiment

4.1 Introduction

Perennial ryegrass (*Lolium perenne* L.), is a high performance grass and the basis of the sheep and dairy industries in both New Zealand, the U.K. and continental Europe (Höglind *et al.*, 2011). Understanding what physiological changes occur during the transition from day to night by determining the changes in the efflux of soil respired CO₂ and the changes in the $\delta^{13}\text{C}$ signature over diurnal periods is important as it increases our understanding of the underlying causes of these changes and therefore has the potential for a large benefit for primary production. The aim of the work described in this chapter was to use the SRISS to gather information on the soil efflux rate and the $\delta^{13}\text{C}$ signature of a dairy soil-pasture over a period of 24 hours.

While there have been a number of studies measuring soil CO₂ efflux and $\delta^{13}\text{C}$ of plants and trees both in the dark and daytime light situations (Cernusak *et al.*, 2013; Comont *et al.*, 2012; Gilmanov *et al.*, 2003; Höglind *et al.*, 2011; Israeli *et al.*, 1996; Klumpp *et al.*, 2005), there has been very few investigations involving constant measurement of both $\delta^{13}\text{C}$ and soil respiration over a period of 24 hours or more. After the successful construction and testing of the SRISS, using soils with different $\delta^{13}\text{C}$ values, further testing over significant periods of time (for example 24 hours or more) was required. This provided the opportunity to also use the SRISS on field samples under controlled conditions with the aim of using it to detect changes in the soil CO₂ respiration rates and changes in the $\delta^{13}\text{C}$ signature.

Soil temperature is a major influencing factor in the heterotrophic respiration rate in soils, and a number of studies have shown there is an exponential rise in respiration as temperature increases (Barbour *et al.*, 2011; Höglind *et al.*, 2011; Lloyd & Taylor, 1994). With such a pronounced effect caused by temperature, separating the effect of light and temperature in this study it was very important. In order to achieve this it was vital to keep the soil at a constant temperature as possible throughout the experiment. Heat from the lamps in the growth cabinets can cause significant increases in the temperature of soil samples, especially at more shallow levels and thus create unwanted artefacts in total soil respiration. To minimise this effect, the air temperature during the day periods when the lights were on was decreased to 16 °C and during the light-off periods increased to 21 °C.

Measurements of the soil efflux from twelve pots are used to determine the variance through time and comparisons between day and night respiration. Confirmation of consistent

measurements of the soil efflux rate will also be beneficial for later experiments, where the most stable times for soil respiration and gas sampling for $\delta^{13}\text{C}$ analysis will need to be identified. Hourly measurements of the respired $\delta^{13}\text{C}$ provided not only an indication of the proportion of heterotrophic to autotrophic respiration, but also a good indication for the best time to gather diurnal measurements for later experiments. It is hypothesised that during the hours in which the lights are off the $\delta^{13}\text{C}$ of soil respired CO_2 will rise and thus signify a shift to more heterotrophic dominated soil respiration. This would be due to a significant reduction in recent photosynthate being respired by autotrophic organisms. With the temperature being kept as constant as possible this will result in an increase in the proportion of heterotrophic respiration which will result in an increase in the $\delta^{13}\text{C}$ of the soil respired CO_2 .

4.2 Methods

4.2.1 24-hour experiment

Each sample pot was numbered and a random number generator was used to assign sample pots that would be used in this experiment. The six pots were then placed in random positions in the growth cabinet and not moved again for the duration of the experiment. The conditions of the growth cabinet were set at an average of $600 \mu\text{mol m}^{-2} \text{s}^{-1}$ PAR and 80% relative humidity. The six SRISS efflux chambers were placed on the pots one hour after the lights were turned on. In order to make the chambers CO_2 free for the start of measurements, each chamber was flushed with 200 mL min^{-1} of CO_2 -free gas for one minute, during this time no suction was applied, making the chimney the only place the gas could escape from inside the chambers. Once the flush was completed the SRISS resumed normal operations and were allowed to stabilise for an hour. The SRISS was programmed to keep the concentration of CO_2 in each chamber at 400 ppm plus or minus 5 ppm, which was approximately equal to those in the growth cabinet. Once stabilised, gas samples were collected from each chamber every hour for 24 hours and isotopically analysed. The SRISS was consistently recording instantaneous CO_2 soil efflux.

4.2.2 Soil respiration and isotope analysis

The SRISS was housed inside the growth cabinet to reduce problems with condensation and eliminate the need to enter the growth cabinet. Previous measurements had indicated that

entering the growth cabinet influenced the $\delta^{13}\text{C}$ signature. To collect and analyse the $\delta^{13}\text{C}$ signal in the soil respired CO_2 , Tedlar[®] bags (Keika Ventures, Chapel Hill, NC, USA) were attached to each end of the tubes from the SRISS ten minutes before the scheduled measurement time. The sample air from the sampling system was directed to the bags until they were full (approx. 10 minutes), and isotopically analysed following the method of Zakharova *et al.* (2014). After each sampling the ambient $\delta^{13}\text{C}$ value of the growth cabinet was also measured using a direct tube line from the cabinet directly to the TGA, the inlet for these measurements was approximately 1000 mm above the grass leaves. Soil temperature from three representative pots at random locations in the growth cabinet used three temperature probes at 20 mm, 70 mm and 150 mm measured temperature at 30 min intervals and along with total soil efflux was also measured constantly by the SRISS.

4.3 Results and Discussion

4.3.1 Soil temperature

The average soil temperature at all depths changed less than 1 °C throughout the 24 hours (Figure 4.1). The more abiotic variables that can be controlled, the more information can be gathered to ascertain whether the SRISS is functioning correctly and obtaining accurate readings. It was important to keep the temperature as stable as possible as it has been shown many times in the past that temperature can play a major effect on the respiration rates of soil microbes and thus it is conceivable that this could have an effect on the $\delta^{13}\text{C}$ signature of samples (Barbour *et al.*, 2011; Höglind *et al.*, 2011; Lloyd & Taylor, 1994). This temperature stability was a huge advantage to reduce abiotic influences on soil respiration rates and possible influences on the $\delta^{13}\text{C}$ signal.

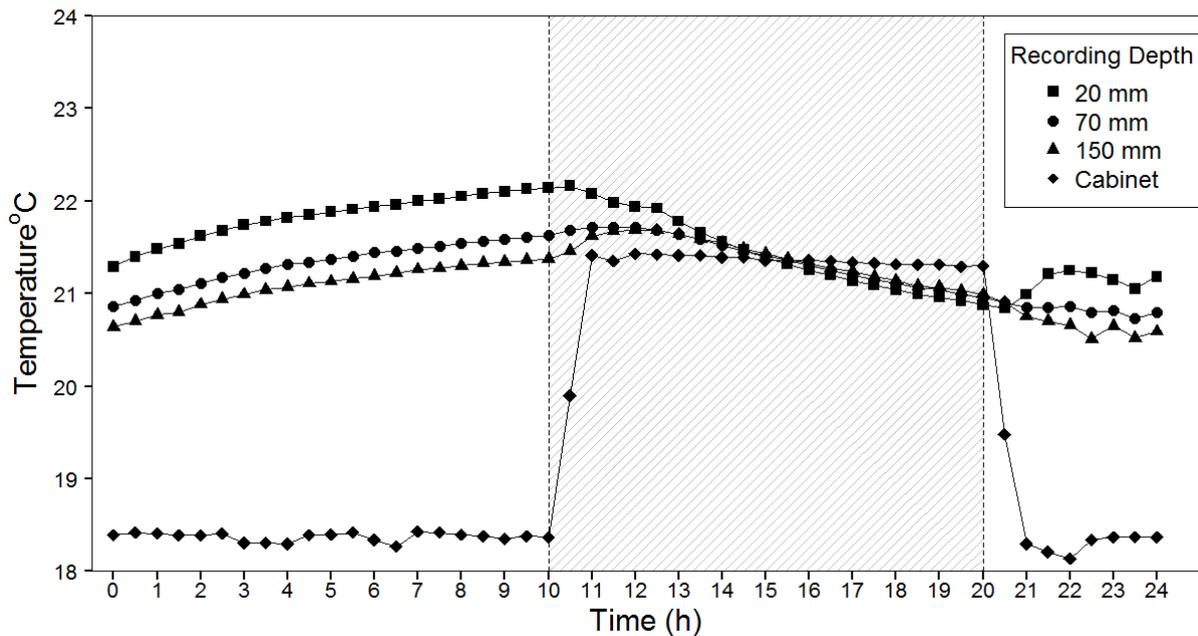


Figure 4.1: Soil temperatures measured at three different depths (20 mm, 70 mm, 150 mm). Temperature was taken every 30 minutes from experiment start for 24-hours. The hours of light were from 0 – 10 hours and 20 – 24 hours, shaded area indicates lights-off period.

4.3.2 Soil efflux

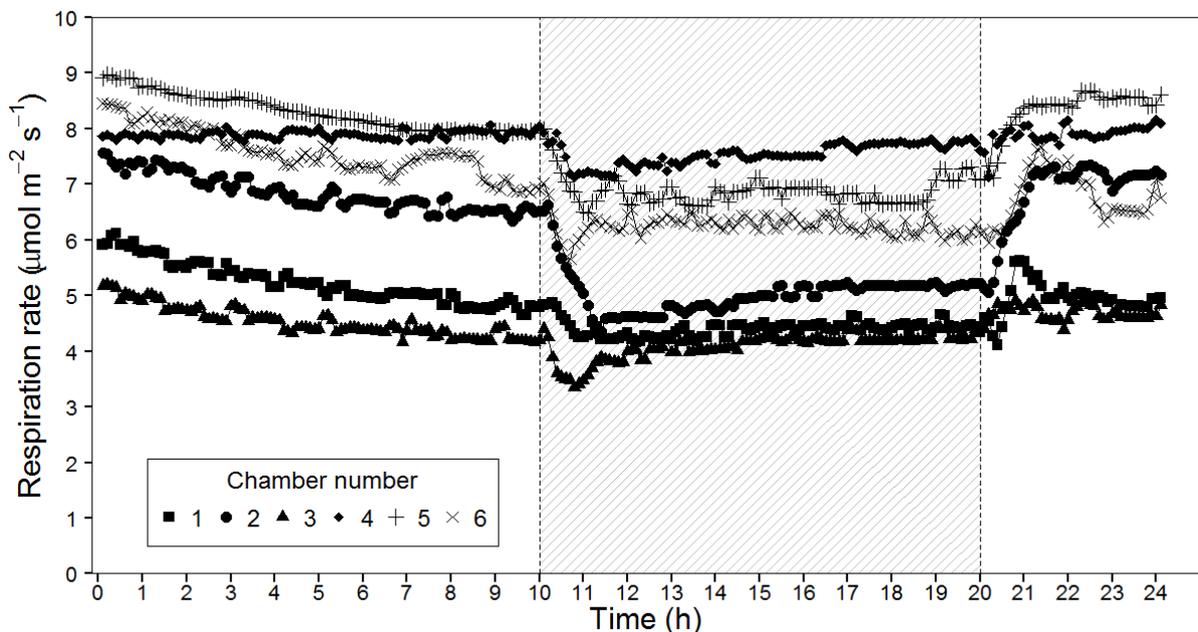


Figure 4.2: Soil respiration rates from 6 pots inside a growth chamber, sampled every 6 minutes for 24 hours. The hours of light were from 0 – 10 hours and 20 – 24 hours, shaded area indicates lights-off period.

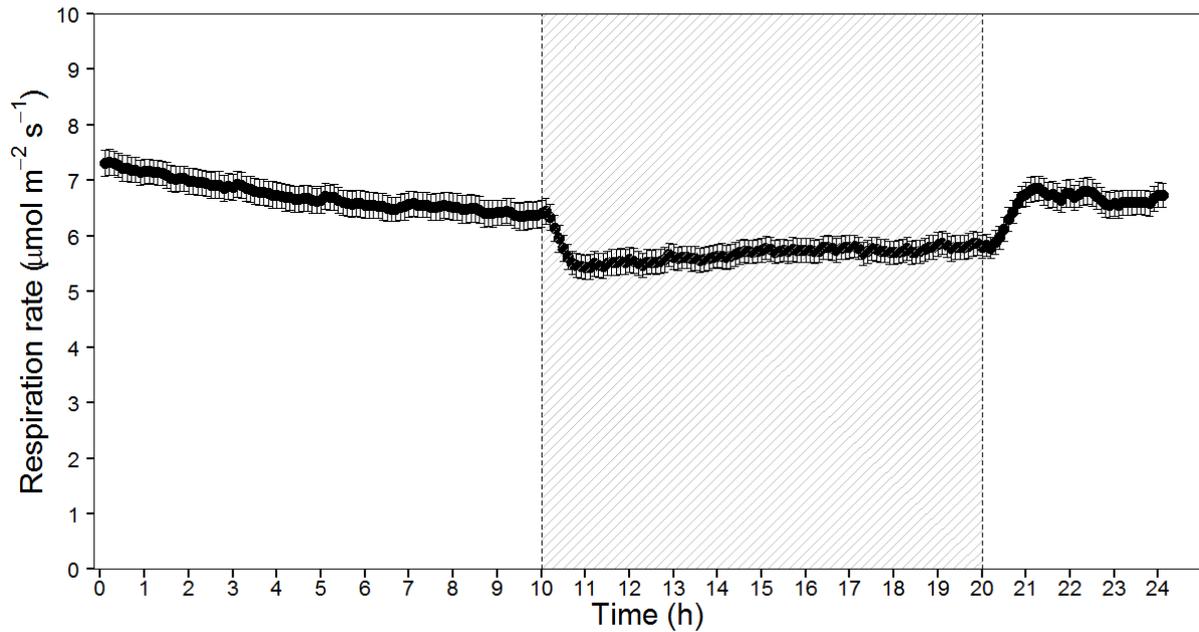


Figure 4.3: Average respiration rate ($n=6$) \pm SEM of all samples over 24 hours. Each measurement was made 6 min apart. The hours of light were from 0 – 10 hours and 20 – 24 hours, shaded area indicates lights-off period.

As the day progresses there was a reduction in the respiration rate in all the samples (Figure 4.3). There is a significant reduction in respiration rates of all the samples at the onset of night-time. Interestingly, even though the soil temperature was falling, there is a slight increase in respiration in all the pots as the night progresses (Figure 4.2 and 4.3). When the lights came back on there is a sharp rise in the soil respiration rates of $1.5 \mu\text{mol m}^{-2} \text{s}^{-1}$, then stabilising again. (Figure 4.2 and 4.3). While these findings are similar to other studies (Höglin *et al.*, 2011), it also shows there is a considerable amount of respiration occurring by soil microbes when there is a significant reduction in input from the plants. It can be confidently said that the SRISS can faithfully record changes in the efflux rate of the soil samples over prolonged periods of time. While this experiment was only run over 24-hours, it suggests that the SRISS will run over significantly longer periods of time (weeks). At the start of recording for this experiment the respiration rates were as high as $9 \mu\text{mol m}^{-2} \text{s}^{-1}$, however they steadily reduced in respiration rate as the day progressed. The respiration rates did not recover to original levels when the lights came back on after the night time period. There are a number of possibilities for this, however the most likely is there was a watering event approximately 24-hours before the start of the experiment and coupled with the opening of the cabinets to place the chambers on the soil samples, this may have caused this spike, however the growth cabinet doors

remained closed for the remainder of the experiment. Later experiments confirmed this was a spike due to the initial starting conditions.

4.3.3 $\delta^{13}\text{C}$ Analysis

As plants actively discriminate against ^{13}C it was predicted that the input from the plants to soil respiration would be ^{13}C depleted thus resulting in a depleted $\delta^{13}\text{C}$ signature during the day and an enriched $\delta^{13}\text{C}$ signature during the night. This however was not the case (Figure 4.4).

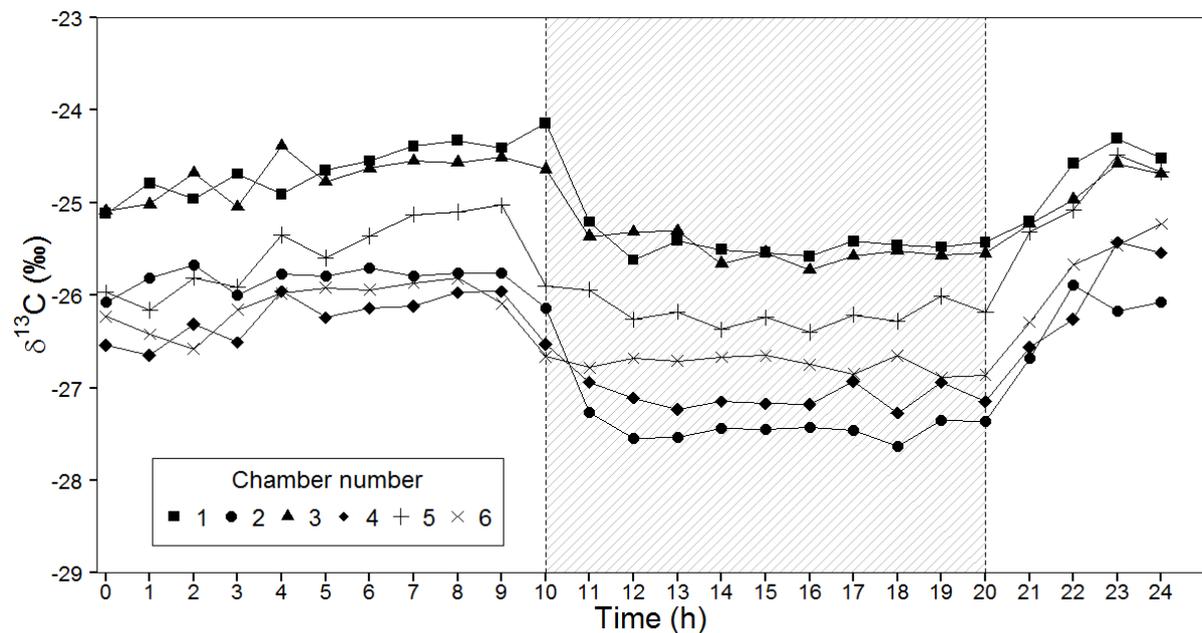


Figure 4.4: $\delta^{13}\text{C}$ (‰) measurement over the 24 hours. The hours of light were from 0 – 10 hours and 20 – 24 hours, shaded area indicates night-time (lights-off) period.

The $\delta^{13}\text{C}$ signature of total soil respiration decreased as the night period started, indicating a shift towards more autotrophic respiration (Figure 4.4), there was also a decrease in the ambient $\delta^{13}\text{C}$ signature inside the cabinets of approx. 4 ‰ at the same time (Figure 4.5).

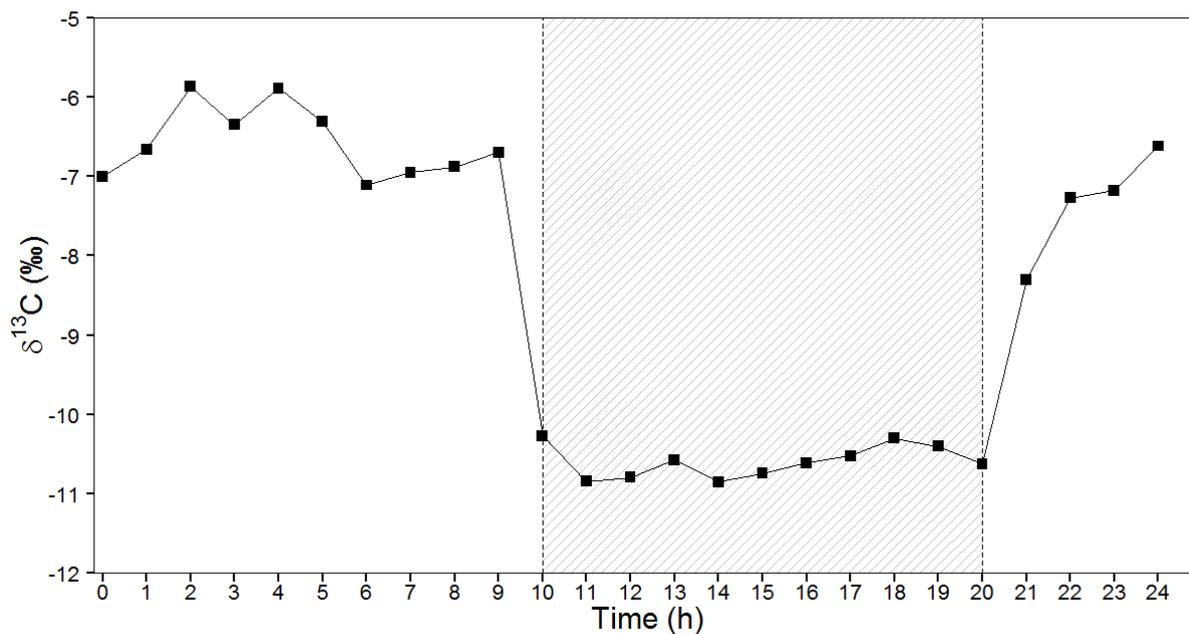


Figure 4.5: $\delta^{13}\text{C}$ (‰) cabinet measurement over the 24 hours. The hours of light were from 0 – 10 hours and 20 – 24 hours, shaded area indicates night-time (lights-off) period.

Subsequent testing of the cabinets to test whether the dramatic change in the $\delta^{13}\text{C}$ of the ambient air in the cabinet was due to cabinet influences or influences by the atmosphere directly outside the cabinet resulted in negative results indicating that any changes in the $\delta^{13}\text{C}$ of the ambient air inside the cabinet were caused by the samples and not the cabinet or outside environment. These test were performed by measuring the $\delta^{13}\text{C}$ of the ambient air inside the cabinet under the same conditions as the experiment in a clean cabinet with no sample pots inside.

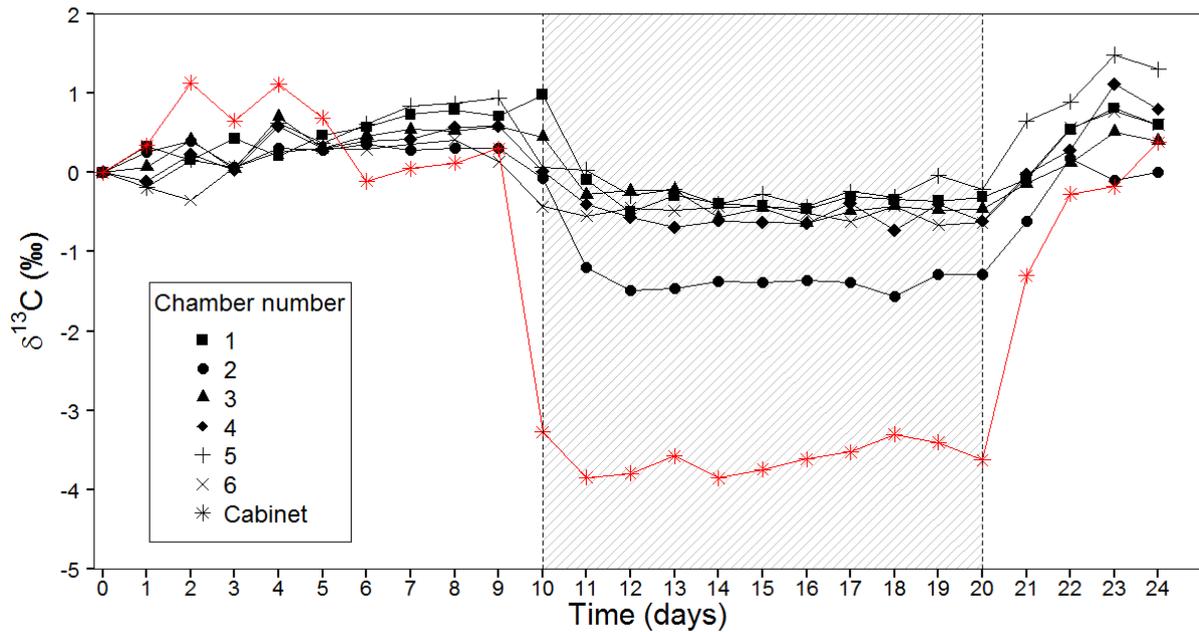


Figure 4.6: Normalised $\delta^{13}\text{C}$ data. Cabinet (ambient) has been shown in red for clarity. The hours of light were from 0 – 10 hours and 20 – 24 hours, shaded area indicates lights-off period.

Several studies have shown there can be a very small time delay between CO_2 entering a plant through leaves and the time it takes to be transported and exuded through the roots and respired via R_A (about 30 min in some grasses) (Barthel *et al.*, 2014; Gavrichkova *et al.*, 2011; Knohl *et al.*, 2005). This shows that there is the possibility of a direct feedback cycle between the $\delta^{13}\text{C}$ of the CO_2 in the ambient air growth cabinet and the $\delta^{13}\text{C}$ of R_S . This situation might be unique to the closed environment of growth cabinets, however similar effects have been recorded in studies involving measuring $\delta^{13}\text{C}$ fluctuations in forest canopies and its effect on the $\delta^{13}\text{C}$ of the respired CO_2 from the surrounding soil (Comstedt *et al.*, 2006; Knohl *et al.*, 2005; McDowell *et al.*, 2004a). During the day there is a build-up of ^{13}C in leaves which is then used as a substrate for respiration at night (Ghashghaie & Badeck, 2014; Klumpp *et al.*, 2005; Werner & Gessler, 2011). These results are also similar to studies involving Eucalyptus trees which have found the $\delta^{13}\text{C}$ of R_S to be proportional to that of the $\delta^{13}\text{C}$ surrounding air used for respiration and a recorded reduction in the $\delta^{13}\text{C}$ of R_S during night time respiration. The exact cause of this was not defined but it was suggested that the internal pathways of the tree could be sequestering the ^{12}C during night time as there was less input from leaves (Grossiord *et al.*, 2012). While that study was based on Eucalyptus trees, the same mechanism could apply to pasture plants.

A photosynthate analysis could be used here to confirm the origin of these effects. Also by partitioning end-members of the samples and determining the proportion of R_A and R_H would

be beneficial in elucidating these results. To confirm these suggestions a $\delta^{13}\text{C}$ analysis on the soil, roots and leaves of the plants used in this experiment needs to be conducted.

4.3.4 Conclusion

This experiment has provided valuable information about the response of the soil-pasture system. It has shown that while *L. perenne* can be influenced by the isotopic signatures of the immediate outside environment, however the scope of the change is far smaller proportionally to that experienced by the environment suggesting *L. perenne* also has some resilience against outside influences shown by the soil respired $\delta^{13}\text{C}$ signature only changing by 1 ‰ compared with the 4 ‰ recorded in the cabinet (Figure 4.6). It can also be noted, the 1 ‰ reduction at night-time cannot be attributed to a change in soil temperature.

As a test for the SRISS, this work was very successful. The system can continuously measure changes in the $\delta^{13}\text{C}$ signature of all 6 chambers simultaneously, as well as changes in the respiration rates. The system can also faithfully record these changes even when there are large changes in the surrounding atmosphere.

Chapter 5 Impact of reduced photosynthesis on soil CO₂ efflux and $\delta^{13}\text{C}$ of R_s

5.1 Introduction

New Zealand's climate is complex and varies from warm subtropical in the far north to cool temperate climates in the far south. It is not uncommon for there to be prolonged cloudy periods with low light, where plants might not experience direct sunlight for up to two weeks at a time. With two thirds of the terrestrial carbon pool stored in soil (Amundson, 2001) it is important for us to understand if prolonged periods of low light have an impact on the carbon source for respiration. Partitioning and quantifying soil respiration into heterotrophic and autotrophic components will improve our understanding of how these systems react when subjected to prolonged periods of low light. Understanding the factors which can cause changes to the behaviour of the soil system is extremely important in understanding how soil may contribute to atmospheric CO₂ partial pressure and global climate change in the future (Lal, 2009; Schipper *et al.*, 2010).

There have been a number of studies describing the effects of low light levels and thus photosynthesis with all showing a strong reduction in soil respiration as available light for photosynthesis was decreased (Höglin *et al.*, 2011; Israeli *et al.*, 1996; McDowell *et al.*, 2004a; McDowell *et al.*, 2004b; Risch & Frank, 2010), however there is little information on the effect of low light levels on the $\delta^{13}\text{C}$ signature of respired CO₂ from pastures.

Carbon accumulation in soil helps offset increased atmospheric concentrations of CO₂ in the atmosphere. Soil respired carbon originating from recent photosynthate input into the soil has no net effect on atmospheric CO₂ levels, however soil respired carbon originating from soil organic matter (SOM) adds to the atmospheric CO₂ load and thus has a positive feedback effect on global warming (Melillo *et al.*, 2002). Many factors can influence the rate and type of carbon input into soil, including temperature (Melillo *et al.*, 2002), moisture (Millard *et al.*, 2008), addition of nutrients (for example in the form of fertilisers) and sunlight hours (Davidson & Janssens, 2006; Kelliher *et al.*, 2005; Uchida *et al.*, 2010a; Uchida *et al.*, 2010b). This thesis specifically looks at the effect of new photosynthate from short-term day/night cycles, and long term (12 days) light starvation on R_s and partitioning of soil respired CO₂ from roots and SOM.

By growing pasture plants under ideal conditions in a growth cabinet and applying a 90% reduction in PAR for 2 weeks it was hoped to ascertain the effects of low light (and altered

carbon input) on both respiration and partitioning it into autotrophic and heterotrophic sources. It was hypothesised that there would be a shift towards more heterotrophic dominated soil respiration, due to a significant reduction in photosynthesis (Höglind *et al.*, 2011) and a resulting reduction in root exudates and autotrophic respiration rates.

The reduction in short and long wave radiation at the soil surface caused by persistent cloud cover, leads to a reduction in soil temperatures, which could potentially counteract the effects on the $\delta^{13}\text{C}$ of soil respired CO_2 caused by reduced photosynthesis. However if the soil respiration is carbon limited, it was still expected to see a shift in the $\delta^{13}\text{C}$ signature, as the reduction in autotrophic respiration will be proportionally more pronounced. By using the equation derived by Lloyd and Taylor (1994) (equation 2) the respiration rates of the samples at different temperatures can be predicted, provided the current temperature and corresponding respiration rate are known.

$$R = R_{10}e^{308.56\left(\frac{1}{56.02} - \frac{1}{T-227.13}\right)} \quad (\text{Equation 2})$$

Where R is soil respiration rate ($\mu\text{mol m}^{-2} \text{s}^{-1}$), R_{10} is the soil respiration rate at 10 °C and T is the absolute soil temperature (K). This formula can predict the respiration rate of a soil sample at a different temperature under well watered conditions.

Gross ecosystem productivity (GEP), which can also be referred to as gross primary productivity is an indicator of how much photosynthesis is taking place in a system under varying conditions, in this case different levels of photosynthetically active radiation (PAR, 400-700 nm) (Beer *et al.*, 2010). Measuring the GEP of the *L. perenne* under different levels of PAR, the difference in photosynthetic input into the soil can be determined at different levels of light (Gilmanov *et al.*, 2003). This will help ascertain the source of reductions in soil respiration.

Measurements were taken in both night and day conditions. As there were reductions in both the respiration rates and the $\delta^{13}\text{C}$ of the soil respired CO_2 from the samples pots used (Chapter 4) it was important to take measurements during both these periods. The most effective times to do measurements was within two hours of sunrise for night time measurements and after 1 hour of sunrise for day time measurements, these times were determined as they were when the respiration rates and $\delta^{13}\text{C}$ of the soil respired CO_2 were most stable for measurement (Chapter 4).

This experiment also gave us the opportunity to further test the SRISS over a prolonged period of time, and under different environmental conditions. Also, newly developed automation routines were tested under changing external CO₂ conditions. The experiment thus functioned as a useful test of whether the SRISS was ready for field deployment.

5.2 Methods

5.2.1 Soil pasture samples

The same 12 intact soil-pasture pot samples from the previous experiment were used. The pots were ordered by biomass and sequentially allocated to the light and shaded treatment groups to ensure both treatments had the same variance. The light treatment was the control. The light treatment pots received an average of 600 $\mu\text{mol m}^{-2} \text{s}^{-1}$ PAR and an average of 60 $\mu\text{mol m}^{-2} \text{s}^{-1}$ PAR under the shaded treatment for fifteen hours a day at 80% relative humidity. The position of sample pots was randomised daily to ensure even exposure to light. Day time temperature was 17 °C and night time was 21 °C. This temperature regime offset the daytime thermal soil warming and kept the soil temperature, at 50 mm below the surface, at a constant 21 ± 1 °C. The plants were watered on days 2, 6 and 10.

5.2.2 Simulation of cloud cover

In order to simulate prolonged cloud cover, a 750 mm tall frame was constructed to cover one half of the growth cabinet (Figure 5.1). The frame was covered with 2 layers of shade cloth on the top part and 1 layer on the sides to reduce PAR at the top of the canopy to 10% of the light treatment (approximately 60 $\mu\text{mol m}^{-2} \text{s}^{-1}$). The open mesh shade cloth allowed free airflow from below to stop the build-up of CO₂. The mesh had an opening in the front, to allow easy access for placing the experimental chambers. At this stage all pots had been in the same diurnal cycle for approximately 12 weeks.



Figure 5.1: Shade cloth construction used to simulate shaded cloud cover.

5.2.3 Measurement procedure

After reviewing the average $\delta^{13}\text{C}$ signature of soil respired CO_2 (see Figure 4.5) the most stable time to take measurements of CO_2 samples was within 2 hours before sunrise and 1-3 hours after sunrise. With 12 samples (6 shade, 6 light) and only 6 chambers, the measurements were done in two batches, each 40 min apart, consisting of 3 pots from the shade treatment and 3 from the light treatment group. Each day all pots were randomly allocated to either the first or second batch of measurements. Sunrise occurred at 10 AM each day, thus the night-time measurement sequence was started at 8 AM and day time measurements took place between 11AM and 1PM.

Chambers were placed on the pots as quickly as possible to minimise the time the growth cabinet was open, the chambers were also checked to ensure there was an airtight seal around the base. Each chamber was flushed for 1 min with CO_2 free air at a rate of 200 mL min^{-1} , to ensure the CO_2 concentration inside the chamber was lower than 20 ppm. After flushing, the chambers were automatically controlled to a CO_2 set point 20 ppm above ambient and left to stabilise. The ambient cabinet CO_2 concentration was determined with a hand-held calibrated CO_2 sensor (GMT220 Series CO_2 transmitter, Vaisala Oyj, Helsinki, Finland) placed at 1000 mm above canopy height in the middle of the two treatments. Once the CO_2 concentration had stabilised inside the chambers, respiration rates were recorded. Teldar[®] bags were then attached to the gas outlets for isotopic gas sampling and removed for measurement as soon as ~400 mL of gas was collected. The $\delta^{13}\text{C}$ signature was measured using a tuneable diode laser (TGA100A,

CSI, Logan, UT, USA) as described in Chapter 4.2.2. Once the first batch of 6 pots (3 shade treatment, 3 light treatment) was completed, the chambers were switched over to the remaining pots and the same procedure was repeated. Each pot was measured once during the night time period and once during the daytime period. At the time the Teldar[®] bags were attached to the SRISS temperature measurements were made in 6 pots at a depth of 50 mm, 3 in the shade treatment and 3 in the control treatment pots. After each measurement, leaf samples from *L. perenne* plants were collected from each pot (approx. 3 cm²) and immediately placed in a separate labelled envelope and dried at 80°C for 1 week.

5.2.4 End-member analysis

At three stages during the experiment, soil and root cores were taken from each sample pot for end-member analysis. These were taken before the first measurements on day 1, and after the measurements on days 6 and 12. On days 1 and 6 a small core of 20 mm diameter and 60 mm deep was taken from just outside the measurement ring from all pots. The soil was separated from the roots and both components were placed in separate sealed incubation bags (Tedlar[®]). The bags were flushed with CO₂-free air and the $\delta^{13}\text{C}$ signature of the respired CO₂ was measured following the protocol of Zakharova (2014). On day 12 the same procedure was followed, however the measurement collar was removed and a larger core (80 mm diameter) was taken from the centre of each pot. After incubation all soil and root samples were dried at 80 °C for one week, and then stored for further analysis if required.

5.3 Results and Discussion

5.3.1 Temperature

Soil temperatures remained constant throughout the experiment. Day-time temperatures at 50 mm were ~21.5 °C in the light treatment and ~19.5 °C in the shaded treatment respectively (Figure 5.2). The night-time soil temperatures reduced by ~0.5 °C (Figure 5.3), with the exception of the first night-time shaded treatment measurement, this measurement was expected to be higher as the soil had been subjected to full light the day before.

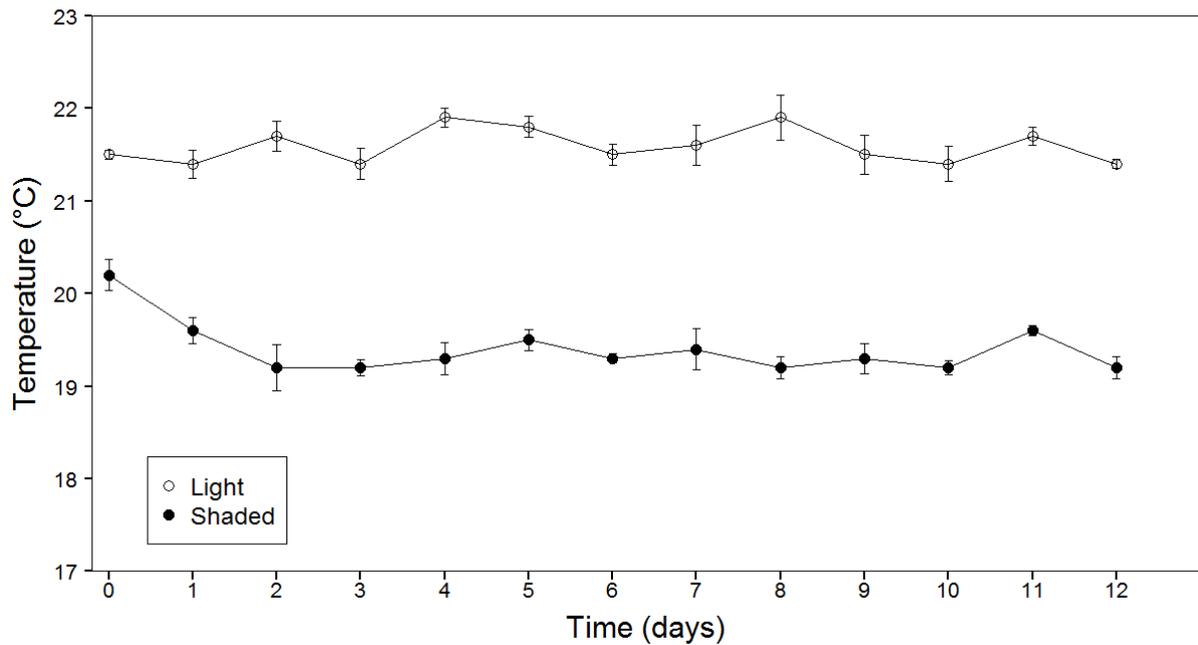


Figure 5.2: Day-time soil temperature recordings. Temperature was measured at 50 mm into the soil in representative pots. Results are the mean (n=3) ± SEM.

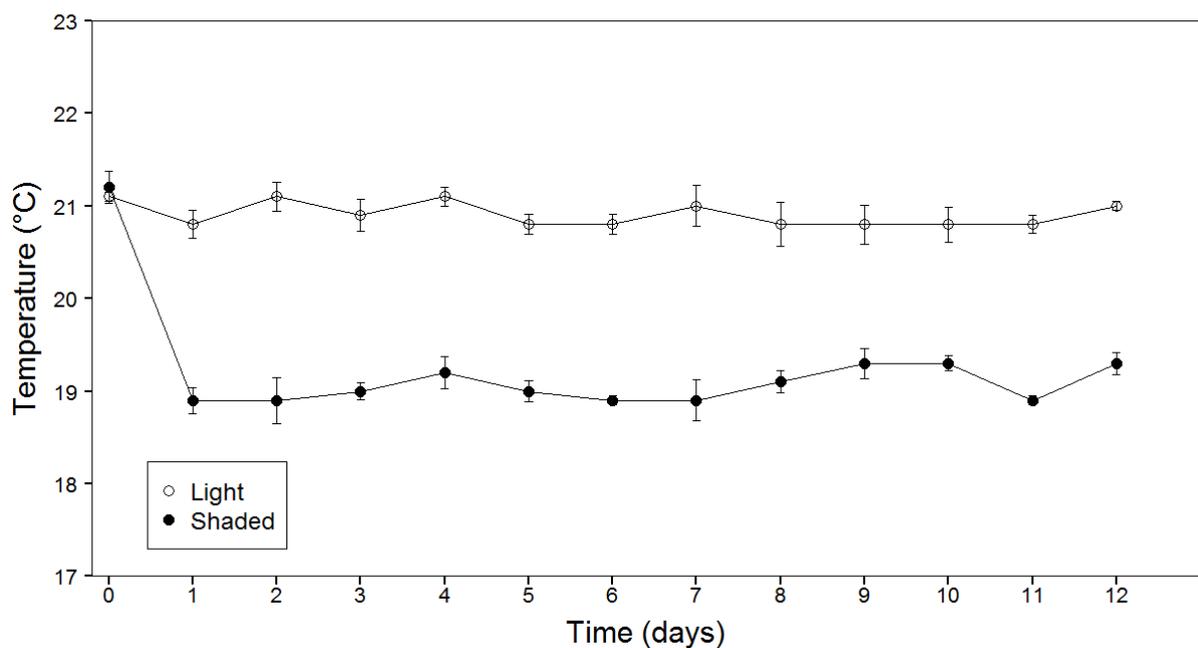


Figure 5.3: Night-time soil temperature recordings. Temperature was measured at 50 mm into the soil in representative pots. Results are the mean (n=3) ± SEM.

5.3.2 Soil respiration rates

Throughout this experiment the respiration rates of the light treatment pots remained consistently around $9 \mu\text{mol m}^{-2} \text{s}^{-1}$ during the day-time and $6.5 \mu\text{mol m}^{-2} \text{s}^{-1}$ at night (Figure 5.4, 5.5, and 5.6). However there was a decrease in the soil respiration from the shaded treatment

as the experiment proceeded, both during the day and at night. After 12 days both the night-time and day time respiration rates were about $3 \mu\text{mol m}^{-2} \text{s}^{-1}$ less in the shade treatment compared to the light treatment (Figure 5.6). Both these differences are statistically significant showing that the differing levels of PAR had an effect on the respiration rates of the shaded treatment samples ($p=0.001$ for day-time and $p=0.015$ for night time respectively). p -values were calculated using a repeated measures ANOVA to test if there was a significant difference in the recorded values as time progressed using R (R-Core-Team, 2013). However this is not only caused by the reduction of PAR, as there was also a soil temperature difference of $\sim 2 \text{ }^\circ\text{C}$ at 50 mm between the light and the shaded treatment. This would have also contributed to the reduction in R_s (Graham *et al.*, 2012; Lloyd & Taylor, 1994; Ubierna *et al.*, 2013). If the reduction in soil respiration was caused by reduction in soil temperature, it should have been most obvious on the first day of the experiment when the temp dropped by $2 \text{ }^\circ\text{C}$. Since the soil respiration rate slowly fell over the 12 days, it is more consistent with a long-term decrease in available substrate for the roots and microbes to respire.

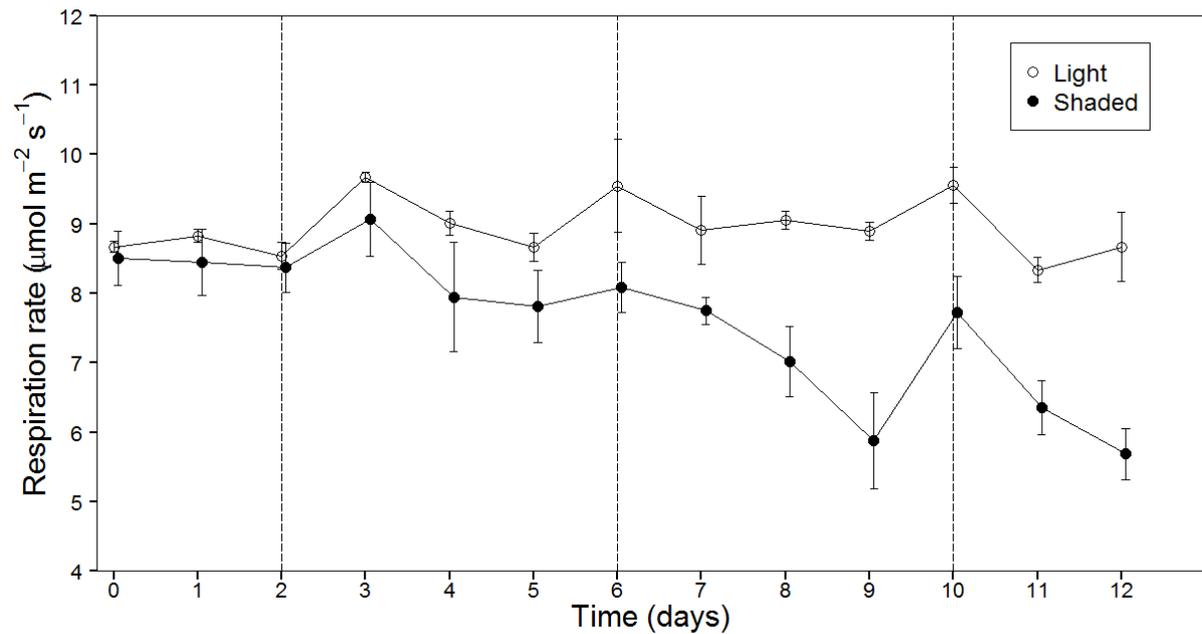


Figure 5.4: Soil respiration rates for day-time respired CO₂, all points are averages ($n=6$) \pm SEM. Shaded measurements have been shifted by 0.05 for error bar clarity. Vertical dotted lines indicate watering events.

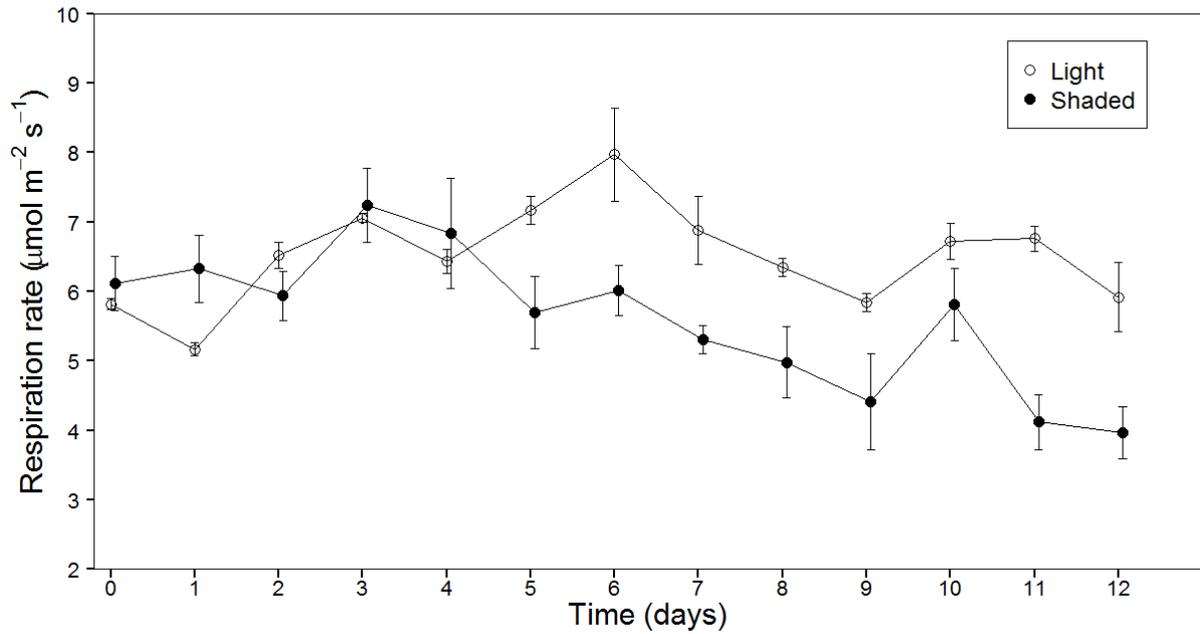


Figure 5.5: Soil respiration rates for night-time respired CO₂, all points are averages (n=6) ± SEM. Shaded measurements have been shifted by 0.05 for error bar clarity.

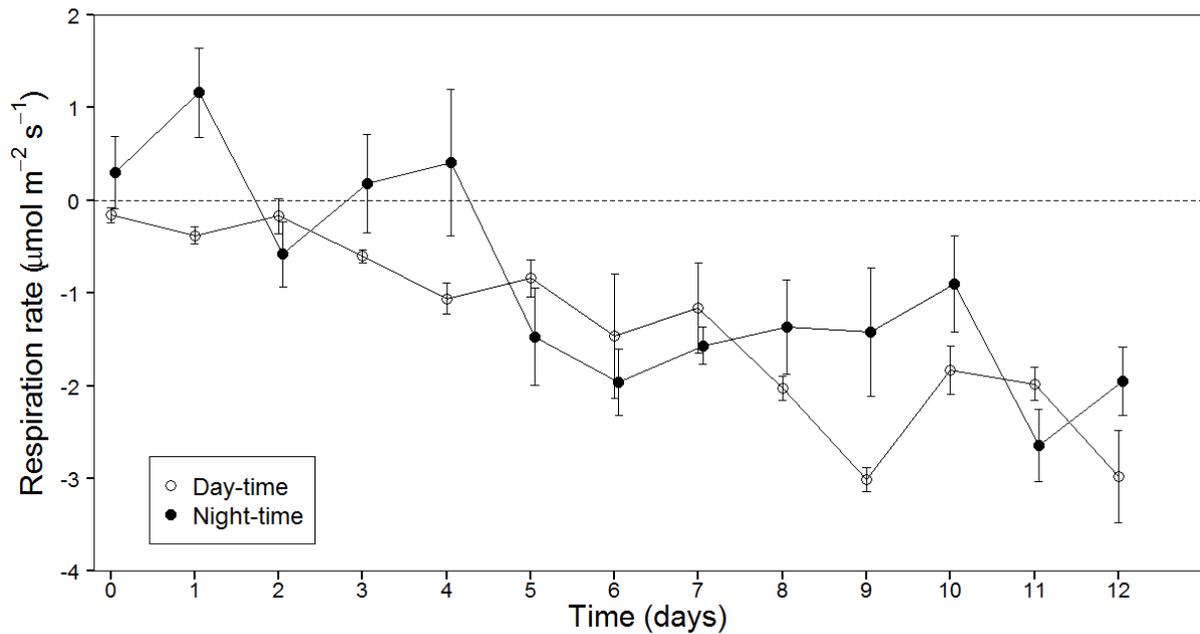


Figure 5.6: Respiration rate difference (shade – light) of both day and night respired CO₂, all points are averages (n=6) ± SEM. Night-time measurements have been shifted by 0.05 for error bar clarity.

On days 2, 6 and 10 there was a slight rise in respiration rates for both treatments (Figure 5.4, 5.5) which were associated with watering events. Even though respiration rates were steadily reducing there was still an increase in respiration in the treatment samples from watering events. This could be attributed to either an increase in respiration or a physical effect of water forcing out CO₂ (Liu *et al.*, 2002), or a combination of both. The recorded overall reduction in respiration rate is most likely attributed to a combination of lower photosynthesis rates due to

the reduction in PAR and the recorded temperature reduction (2 °C) in the shade treatment pots causing less microbial activity within the SOM (Graham *et al.*, 2012; Lloyd & Taylor, 1994; Ubierna *et al.*, 2013).

5.3.3 Respiration analysis

By using a light response curve of light vs Gross ecosystem productivity (GEP) as being a measurement of photosynthetic activity, and the temperature response equation (equation 2) by Lloyd and Taylor (1994) the changes in respiration rates recorded can be attributed to both the change in temperature and the long-term decrease available photosynthate. The R_{10} value was calculated to be $3.48 \mu\text{mol m}^{-2} \text{s}^{-1}$.

During the first days of the experiment the changes in respiration are most likely attributed to the change in soil temperature (Figures 5.7 and 5.8). During days 5 and 6 for day time respiration and after day 5 for night time respiration, there is an indication that the decrease of PAR is causing an effect on the plants resulting in a reduction of R_s .

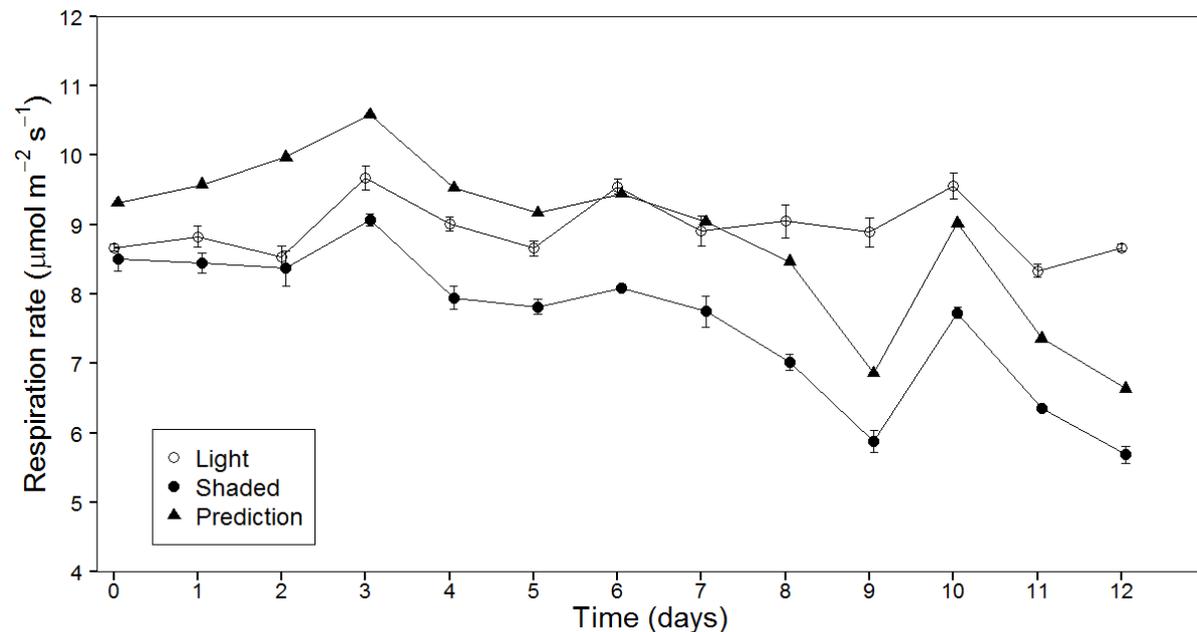


Figure 5.7: Soil respiration rates for day-time respired CO_2 , all points are averages ($n=6$) \pm SEM. Triangle symbols are the predicted soil respiration rates if the shaded soil temperature was raised to the average light treatment soil temperatures for the corresponding day. Predictions were calculated with equation (2). Shaded measurements have been shifted by 0.05 for error bar clarity.

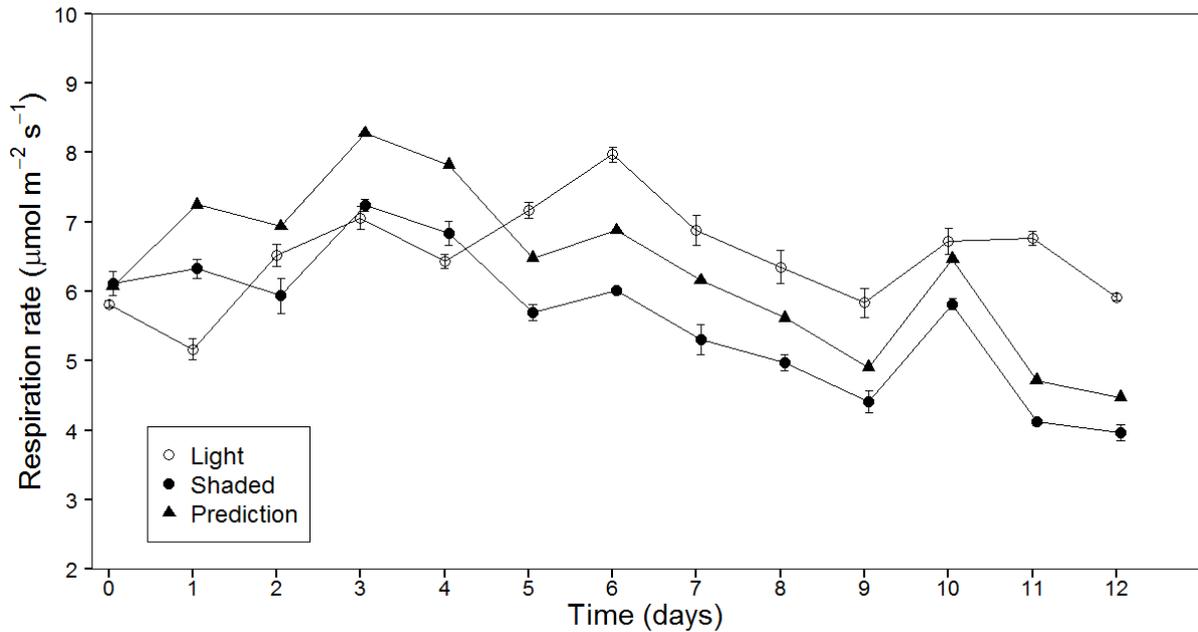


Figure 5.8: Soil respiration rates for night-time respired CO₂, all points are averages (n=6) ± SEM. Triangle symbols are the predicted soil respiration rates if the shaded soil temperature was raised to the light soil temperatures. Predictions were calculated with equation (2). Shaded measurements have been shifted by 0.05 for error bar clarity.

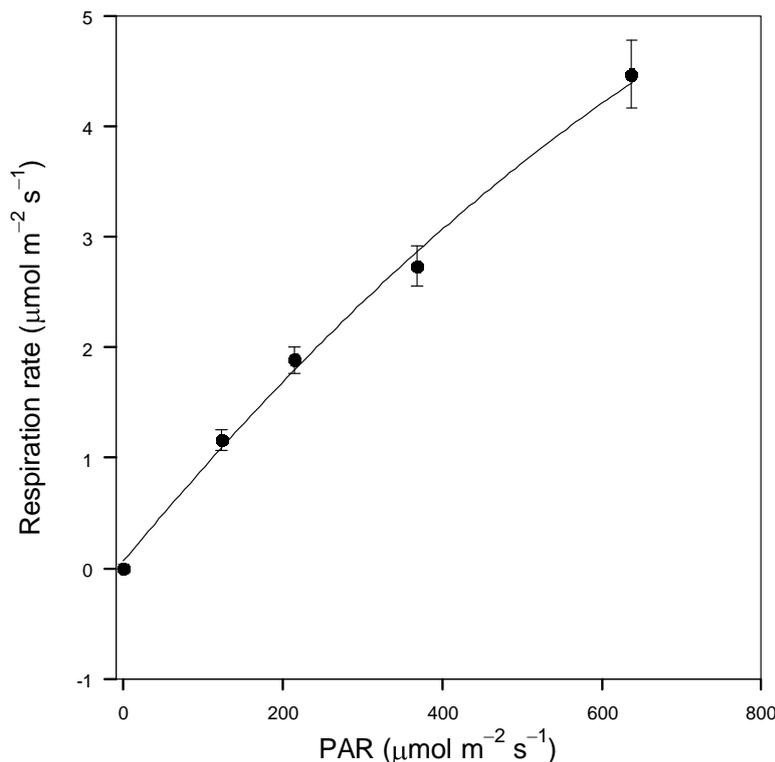


Figure 5.9: Gross Ecosystem Production (GEP) measured at different photosynthetic available radiation (PAR) levels. Values are averages (n=30) ± SEM.

Measurements of GEP under varying levels of PAR carried out in the same lab using *L. perenne* grown under similar conditions to this experiment, show there is a substantial reduction in GEP as PAR decreases (G. Moinet, personal communication, April 29, 2014) (Figure 5.9).

This reduction in GEP as PAR decreases is consistent with other studies using different plant species (Höglin *et al.*, 2011; Israeli *et al.*, 1996). This indicates that the most likely

causes of the decrease in respiration is due to a combination of two factors, the decrease in soil

temperature and the reduction in photosynthesis leading to a reduction in heterotrophic and autotrophic respiration.

5.3.4 $\delta^{13}\text{C}$ measurements

In contrast with the decrease in soil respiration over the 12 days of light reduction, there was no consistent or significant change in the $\delta^{13}\text{C}$ of R_s in both the light and shaded light treatments (Figure 5.10, 5.11 and 5.12).

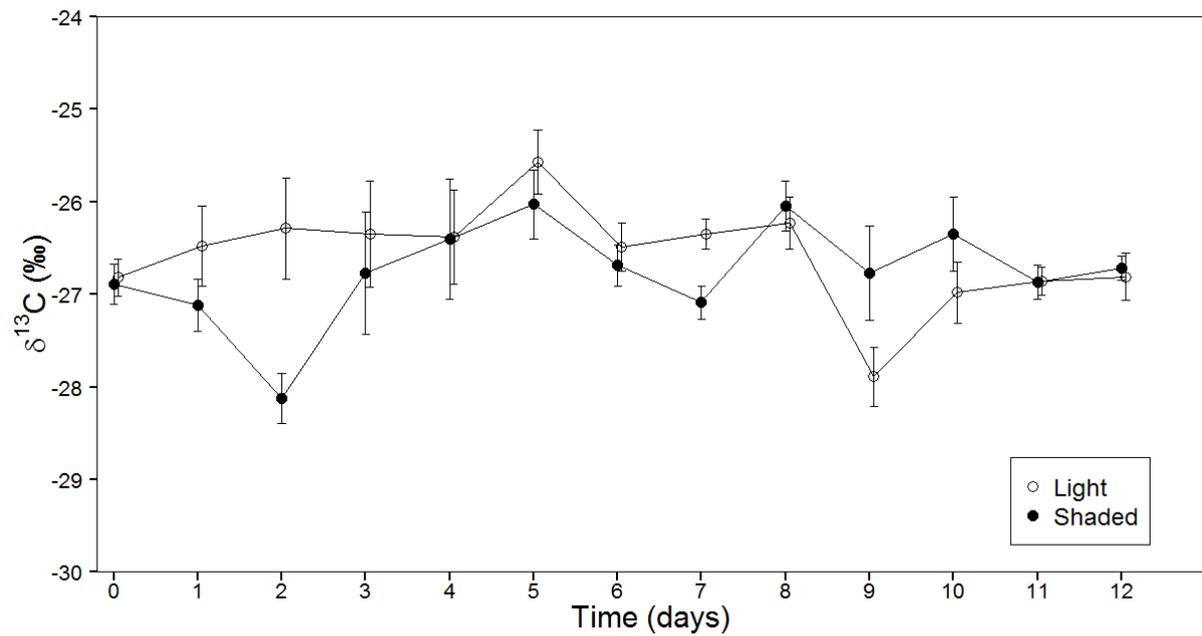


Figure 5.10: Day-time $\delta^{13}\text{C}$ of soil respired CO_2 from both shade and light treatments. All measurements are averages ($n=6$) \pm SEM. Light treatment measurements have been shifted by 0.05 for error bar clarity.

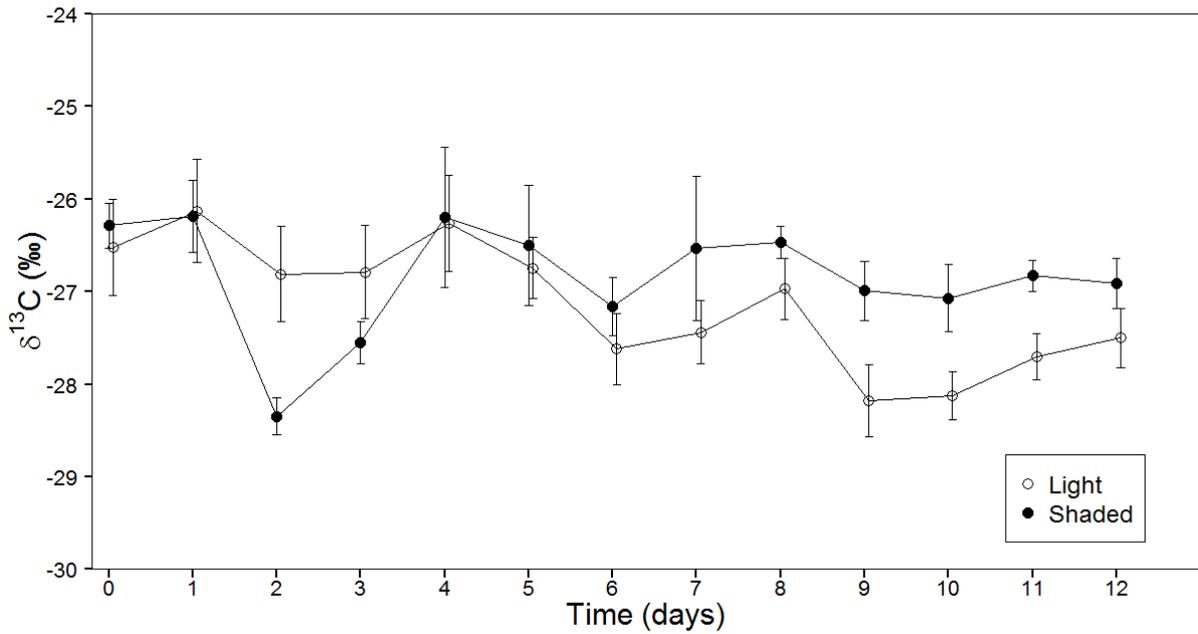


Figure 5.11: $\delta^{13}\text{C}$ measurements of night-time respired CO_2 from both shaded and light pots. All measurements are averages ($n=6$) \pm SEM. Light treatment measurements have been shifted by 0.05 for error bar clarity.

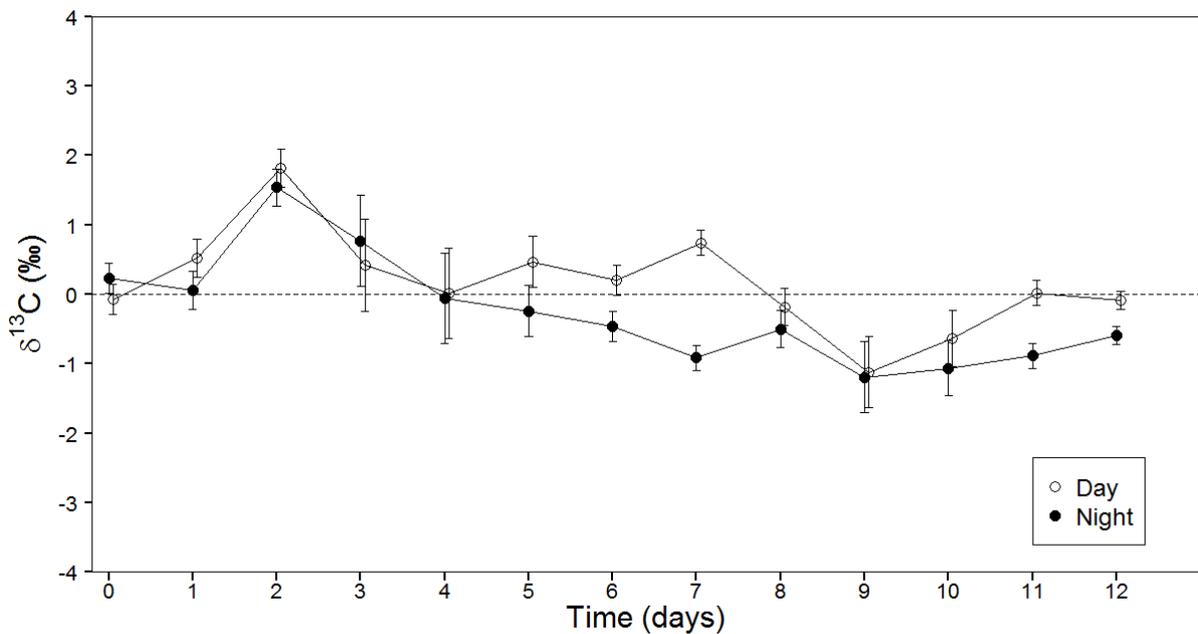


Figure 5.12: Difference (shade - light) of $\delta^{13}\text{C}$ of soil respired CO_2 between light and shaded treatments. All measurements are averages ($n=6$) \pm SEM. Day measurements have been shifted by 0.05 for error bar clarity.

Differences between day-time and night-time $\delta^{13}\text{C}$ were small ($< 2\%$) $p=0.40$ for day-time and $p=0.26$ for night-time respectively. p -values were calculated using a repeated measures ANOVA to test if there was a significant difference in the recorded values as the experiment progressed.

5.3.5 Root and soil end members

The root and soil end members were measured on days 1, 6 and 12 (Table 5.1) on all 12 pots. The end members remained relatively consistent and did not change significantly over the course of the experiment. On average the SOM respired CO₂ was 4 ‰ enriched compared to the root respired CO₂. Since the $\delta^{13}\text{C}$ of soil respired CO₂ remained constant in the treatment suggests that even with a prolonged decrease in light, this did not translate to a change in the $\delta^{13}\text{C}$ of the root respired CO₂. This suggests that *L. perenne*, which has a short time lag between the uptake of CO₂ and its respiration by the roots (Barthel *et al.*, 2014) must have a large store of carbohydrates to use as a source for root respiration.

Table 5.1: Average end member measurement of respired $\delta^{13}\text{C}$ CO₂ for shaded and light treatments (n=6). The average value was calculated from all samples taken (n=18).

Treatment Component Day	Shaded Roots		Light (control) Roots		Shaded Soil		Light (control) Soil	
	$\delta^{13}\text{C}$	SEM	$\delta^{13}\text{C}$	SEM	$\delta^{13}\text{C}$	SEM	$\delta^{13}\text{C}$	SEM
1	-29.18	0.23	-28.76	0.33	-24.16	0.28	-24.38	0.15
6	-28.97	0.11	-28.85	0.28	-24.26	0.20	-24.11	0.19
12	-28.04	0.20	-28.94	0.20	-24.25	0.27	-23.97	0.18
Average	-28.73	0.11	-28.85	0.13	-24.22	0.13	-24.15	0.12

5.3.6 $\delta^{13}\text{C}$ Analysis

A difference of 4 ‰ in the respired CO₂ between roots and soil end members has been shown to be enough to be able to partition the respired CO₂ into autotrophic and heterotrophic components (Millard *et al.*, 2010). Measurements were converted into a percentage of heterotrophic respiration where 100 % was all heterotrophic respiration and 0 % was all autotrophic respiration.

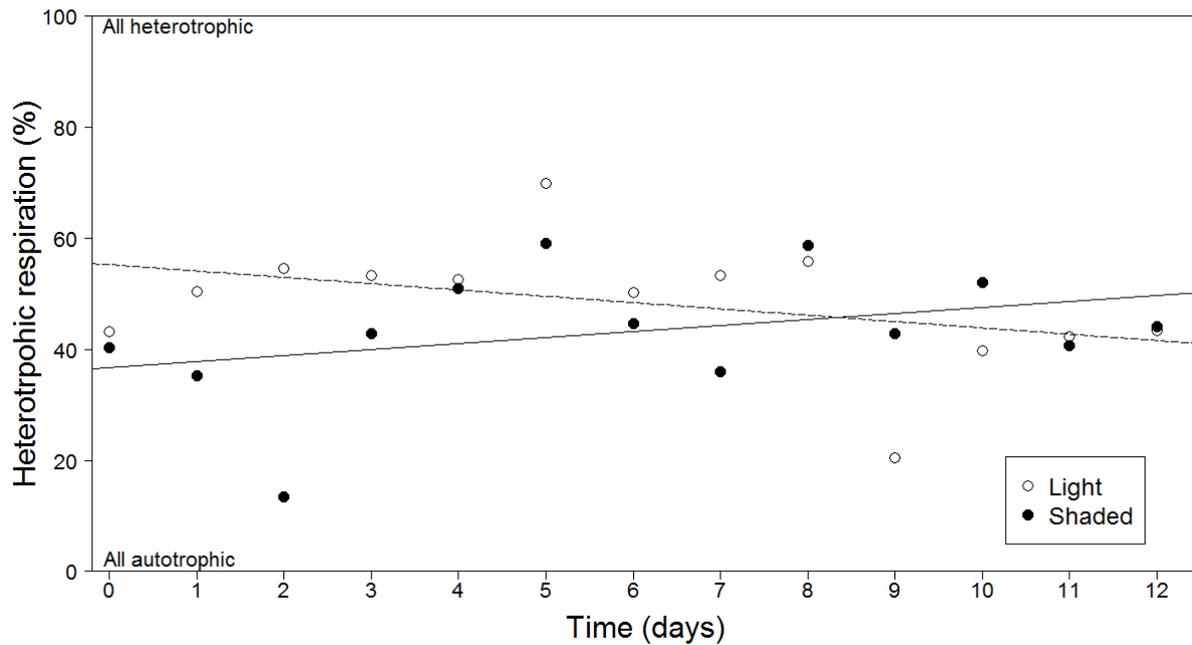


Figure 5.13: Partition of day-time $\delta^{13}\text{C}$. Lines are linear regression. Dotted line represents light with solid line representing shaded.

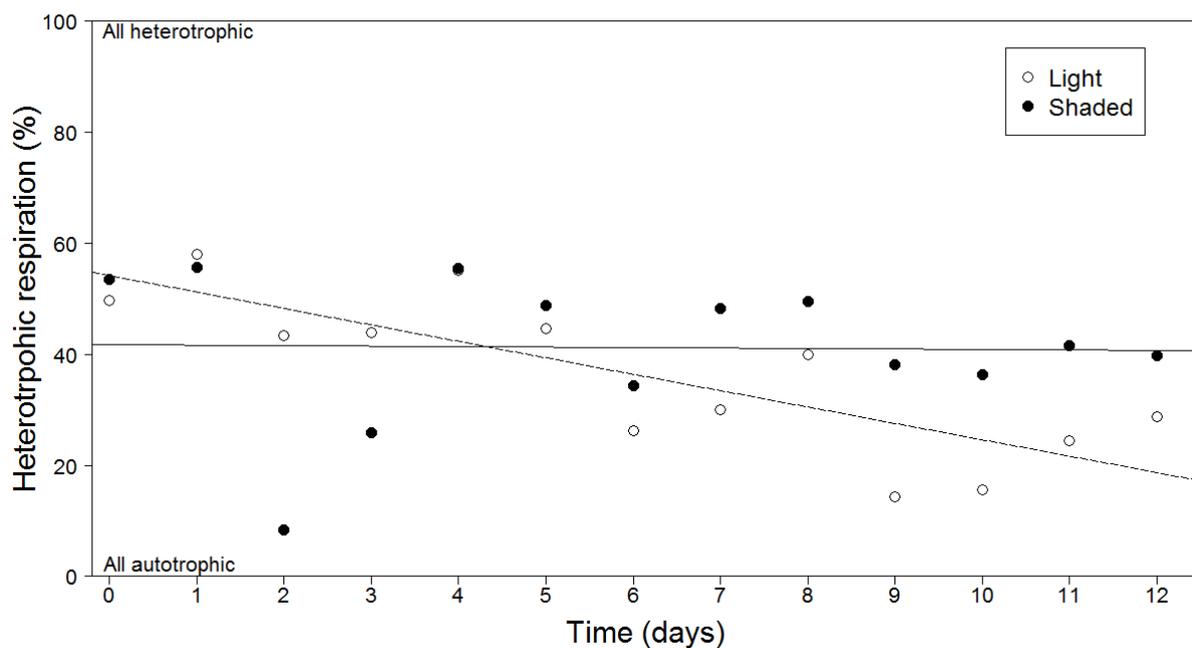


Figure 5.14: Partition of night-time $\delta^{13}\text{C}$. Lines are linear regression. Dotted line represents light with solid line representing shaded.

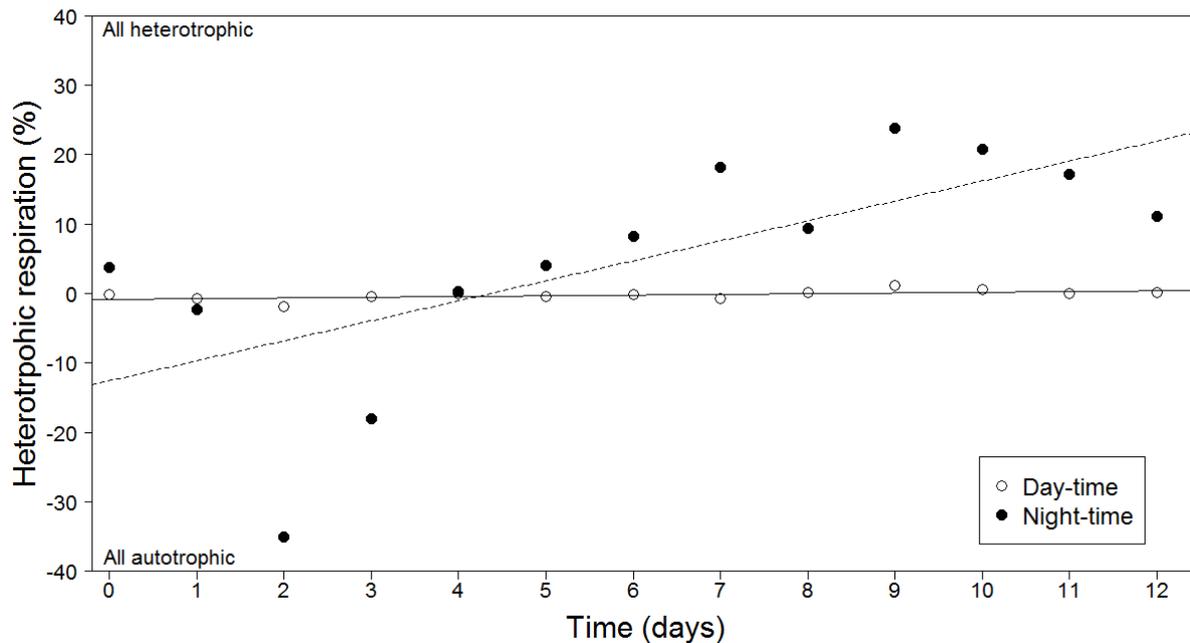


Figure 5.15: Difference between light and shaded treatments (shaded – light) for partitioning of $\delta^{13}\text{C}$. Lines are linear regression. Dotted line represents night with solid line representing day.

All pots were successfully partitioned, so that the soil respired $\delta^{13}\text{C}$ lay between the two end members. While the partitioning was successful, there appear to be some anomalies in the night-time results however there is an upward trend towards R_H dominance, whereas the day-time measurements remain fairly consistent throughout the experiment at an average of 50 % for heterotrophic and autotrophic influence, this is lower compared to other studies which have averaged ~60 % heterotrophic dominance (Millard *et al.*, 2010), this difference is possibly due to the soil-pasture pots being in the growth cabinets for a significant length of time and thus coming to a new equilibrium. There is no significant difference for the $\delta^{13}\text{C}$ of soil respired CO_2 between the light and shaded treatments. However this is not backed up in the partitioning in the night-time data. It appears there is a significant shift towards heterotrophic dominated soil respiration in the night time measurements, whilst there is no significant change in the day-time partitioning.

5.3.7 Conclusion

There was a significant reduction in the soil respiration rates of the pots under the shade treatment. Along with the reduction in PAR there was also a reduction in soil temperature of ~2 °C at 50 mm for the shaded treatment pots. Both of these factors were major influences in the observed reduction in R_S . By using the equation derived by Lloyd and Taylor (1994) it is possible to determine what the respiration rates of the shaded treatment pots would have been

if their soil temperature was consistent with those recorded for the light treatment. For the first 5 days of measurements the most likely cause for the reduction in R_S is predominantly that of the temperature effect. However after day 5 temperature alone cannot be responsible for the reduction in R_S (Figures 5.7 and 5.8). By recording the GEP of *L. perenne* under different PAR levels there is a significant difference in photosynthetic activity between 600 and 60 (Figure 5.9). The most likely explanation for the reduction in R_S at day 12 is a combination of both temperature effect and the reduction in available photosynthate.

There was no significant divergence in the recorded $\delta^{13}C$ of R_S indicating there was no change in the contributions in the proportions of R_H and R_A to R_S (Figures 5.10, 5.11 and 5.12). Both the light and shade treatments were able to be partitioned into their R_H and R_A components successfully. The night-time partitioning data shows there is a shift towards more R_H dominated soil respiration the longer the plants were in the shade (Figure 5.14). The day time partitioning shows a consistently even distribution of both R_H and R_A . The most likely possibility for this is the reduction in temperature and consequently the reduction R_H is offsetting any partitioning difference, they have reached a new equilibrium. However the night-time partitioning shows a shift towards R_H dominance the longer the pots are in the shaded treatment. This would most likely be attributed to less photosynthate exudate being available for microbial respiration and also for growth and maintenance of the roots, initially during the first days of the experiment there was enough exudate to sustain a significant amount of R_A but as the pots were subjected to more shaded days this excess was used up and as a result R_A continued to diminish. Since this shift in respiration proportion wasn't noticed immediately and took several days to show it suggests the common pasture species *L. perenne* has a sufficient amount of stored carbohydrate so that it can survive prolonged periods of reduced light with only a slight reduction in respiration rates and only a small increase in the $\delta^{13}C$ of the respired CO_2 (Figure 5.9, Table 5.1).

To confirm these observations a carbohydrate analysis of plant leaves that were collected during the experiment could be also carried out. It would also be highly beneficial to determine the $\delta^{13}C$ signature of bulk soil, root and leaf samples collected at the start and end of the treatment to determine the influence of the treatments on the $\delta^{13}C$ of the newly fixed carbon.

In the field there is significant variability in hourly soil temperature and daily moisture levels as well as photosynthate inputs into the soil. Further studies should be undertaken in the field in order to determine other possible influences to soil respiration and $\delta^{13}C$ and how *L. perenne*

responds to these influences, possible influences are greater variation in temperature, significantly more water application and animal factors such as excrement and trampling.

The SRISS performed well - it was able to automatically take respiration measurements accurately over a prolonged period of time, and kept the environment inside the SRISS efflux chambers at a constant and controlled CO₂ concentration, even under changing external conditions. It can now also be used for longer term experiments, where changes beyond two weeks are investigated.

Chapter 6 Conclusion

6.1: Overview of findings

6.1.1: SRISS Overview

The new, improved soil respiration isotope sampling system (SRISS) was successfully built over the course of four months in 2013. Firstly, a previous model had to be deconstructed, with most components salvaged for the new SRISS. A new housing was built to accommodate the extra components. While the original SRISS was a four chamber system, the new SRISS was extended to a six chamber system to provide greater replication. Once the system was built and wired up, all the individual components were calibrated and tested, and finally the whole system was tested.

There are many individual components in the SRISS and any one of them can be subject to small leaks and major errors in the determination of the $\delta^{13}\text{C}$ of the respired soil CO_2 . Since there is a large difference in the $\delta^{13}\text{C}$ of atmospheric (typically $\sim 9\text{‰}$) and the soil respired CO_2 ($\sim 27\text{‰}$), any small leak can have detrimental effects on the determination of the true values of the respired $\delta^{13}\text{C}$ and the efflux rates. During the testing phase, there were a number of issues with the mass flow controllers that came to light that needed to be fixed to ensure accurate measurements of efflux and $\delta^{13}\text{C}$. The main problems stemmed from the oscillating frequency of the pumps. This created several problems for the MFCs and coupled with the original positioning of the pumps after the mass flow controllers, which created a negative pressure area prone to contamination. These issues were overcome by rearranging the pneumatic components of the SRISS and placing so that the pumps pushed before the MFCs, creating a positive pressure system and thus eliminating outside air from entering the system. The other solution was to replace the 0.2 L min^{-1} MFC's with 2 L min^{-1} MFCs, as these were able to handle the oscillating air from the pumps more effectively. The final part of the solution was to install inline filters to buffer the air flow before it reached the MFCs. The combination of these changes overcame the issues and produced a reliable SRISS.

The SRISS was tested using bag incubations to obtain $\delta^{13}\text{C}$ values of soil respired CO_2 (Zakharova *et al.*, 2014) and comparing these to the same soil samples with $\delta^{13}\text{C}$ values collected using the SRISS. The results from these tests ($R^2=0.96$, Figure 2.4) proved that the SRISS could reliably measure soil respired CO_2 and that there was no contamination as air flowed through the SRISS. The SRISS performed well in all tests.

6.1.2: 24-hour experiment overview

The soil the respiration rate of all pots decreased after the transition from day to night. Respiration rates varied from 4 to 9 $\mu\text{mol m}^{-2} \text{s}^{-1}$ (Figure 4.2) and on average respiration rates decreased by 2 $\mu\text{mol m}^{-2} \text{s}^{-1}$ during the dark period (Figure 4.3). Surprisingly, the respiration rates did not recover when the light came back on. This can be attributed to two reasons: either the watering event that occurred 24 hours before the experiment started, or the opening of the growth cabinet to place the chambers on the soil samples at the start of the experiment. Even though the watering event, occurred 24 hours earlier, it could have still influenced the respiration rates by changing the available oxygen in the soil. Later experiments showed that the most likely reason for the increase in respiration was from the opening of the growth cabinet at the start of the experiment. Therefore a change in the experimental protocol was designed to mitigate this problem in future experiments. Not with standing these small problems, the SRISS was able to successfully measure the respiration rates of the soil samples over the entire 24 hour period.

Contrary to my hypothesis, the daytime $\delta^{13}\text{C}$ of the soil respired CO_2 was enriched ($\sim 1\text{-}1.5\text{ ‰}$ enrichment) compared to night-time $\delta^{13}\text{C}$ (Figure 4.4). At night the $\delta^{13}\text{C}$ respired CO_2 of all the pots was more depleted in ^{13}C (Figure 4.4). At the same time the ambient air in the growth cabinet became more depleted in ^{13}C (Figure 4.5). However the change in the growth cabinet was significantly more pronounced than what was recorded from the soil pasture samples (Figure 4.6). Testing of the cabinet growth eliminated the growth cabinet or its outside environment as the cause of the change, leaving the only source of the change to be the soil pasture pots. One possibility is during the day there is a build-up of ^{13}C in leaves which is then used as a substrate for respiration at night (Ghashghaie & Badeck, 2014; Klumpp *et al.*, 2005; Werner & Gessler, 2011). Similar examples of this effect have been found in forests (Comstedt *et al.*, 2006; Knohl *et al.*, 2005; McDowell *et al.*, 2004b). Further testing is required to explore this effect more fully.

This experiment showed that the SRISS was able to continuously and simultaneously measure changes in the $\delta^{13}\text{C}$ signature and respiration rates of all six chamber. It also provided information for the best time to take measurements for the shade experiment. The most effective times to do measurements was within 2 hours of sunrise for night time measurements and after 1 hour of sunrise, these times were determined as they were when the respiration rates and $\delta^{13}\text{C}$ of the soil respired CO_2 were most stable for measurement

6.1.3: Impact of reduced photosynthesis on soil CO₂ efflux and δ¹³C of R_s overview.

During the day, R_S rates of the shaded treatment pasture pots decreased over twelve days while the control samples under the light treatment remained steady (Figure 5.4). The same was true for night time respiration measurements for both treatments (Figure 5.5). This was consistent with the hypothesis that due to a reduction in PAR there would be less photosynthate entering the soil leading to a reduction in autotrophic respiration. A reduction in PAR, however, was not the only explanation for the reduction in soil respiration. As a by-product of the shading treatment, soil temperature was reduced by approximately 2 °C (Figures 5.2, 5.3). This also affected the soil respiration rates. By using the Lloyd and Taylor (1994) equation, it was possible to adjust the respiration rates as if these samples were the same temperature as those of the light treatment sample. This showed that during the first 5 days of the experiment, the reduction in respiration rates measured was most likely caused by the difference in temperature (Figures 5.7 and 5.8), however after the 5th day this was not the only cause. By measuring GEP of the same grass species at different levels of PAR (Figure 5.9) the rest of this reduction can be attributed to the reduction in available photosynthate available to the roots and soil microbes.

The soil respired δ¹³C remained consistent throughout the experiment with no significant change in either treatment over 12 days (Figures 5.10, 5.11, 5.12). This was unexpected as it was hypothesised that a reduction in PAR would reduce photosynthate exudate entering the soil which would lead to a reduction in R_A and a more pronounced change in δ¹³C of soil respired CO₂. These results however would suggest that the reduction in R_A from less photosynthate entering the soil was proportionally equal to the reduction in R_H due to the lower temperature experienced under the shade treatment.

δ¹³C of end members from all the samples in both treatments were calculated on days 1, 6 and 12 of the experiment (Table 5.1). The end members remained consistent for the duration of the experiment. On average the soil respired δ¹³C end member was 4 ‰ enriched compared to the root respired δ¹³C end member.

With an average difference of 4 ‰ between the end members, it was possible to partition the soil respiration into their heterotrophic and autotrophic components (Millard *et al.*, 2010). While there was no indication of a shift between R_H and R_A from the δ¹³C results there was an indication there was a shift towards more heterotrophic dominated respiration from the partitioning data (Figures 5.15). It would be beneficial to run this experiment over a longer

time (for example six weeks) in order to determine whether this is part of an oscillating pattern or a significant change in the respiration dominance.

6.2: Significance of findings

These results demonstrate that the SRISS was able to make both respiration measurements from 6 chambers and provide a technique to faithfully sample soil respired CO₂ that could be used for δ¹³C analysis.

There is a definite change in the δ¹³C of soil respired CO₂ over a diurnal period. *L. perenne* can be influenced by the isotopic signatures of the immediate outside environment, however the scope of the change is in far smaller (proportionally) to that experienced by the environment. This suggests *L. perenne* has some resilience against outside influence. This is important in respect to global climate change as it shows that *L. perenne* is reactive to its immediate environment, indicating any detrimental changes in the global climate could be reciprocated by *L. perenne* exacerbating the problem.

L. perenne appears to store a significant amount of carbohydrate, enabling it to survive prolonged periods of low PAR with little to no change in the δ¹³C of soil respired CO₂. While there was a reduction in the rate of soil respiration, the effect of the lower temperature and low PAR cause the R_H and R_A to be reduced decrease by proportionally similar amounts, keeping the δ¹³C of soil respired CO₂ unchanged for a limited time.

Repeating the shade experiment over a longer period time, for example 60 days or more, would provide a more accurate picture to what effects a reduction in PAR has on soil respiration and the δ¹³C of soil respired CO₂. While these small, subtle changes that can be measured under constant conditions in a growth cabinet, however may be overwhelmed in the field due to significant diurnal changes in the environment, such as temperature, PAR and relative humidity. Also other short-term changes in soil moisture, livestock trampling, and grazing may negate these subtle effects.

6.3: Future applications

The SRISS has already been used again in the growth cabinets by a University of Canterbury PhD student (G. Moinet). It is being used to determine the effects of grazing and fertilisation

on root and SOM respiration. In the immediate future the SRISS will be used in a grazed pasture to measure the partitioning of soil respiration under a dairy regime. This will require more robust housing and sufficient water-proofing. It is envisaged to install a full chemical CO₂ scrubber to eliminate the need for a heavy CO₂-free air bottles. With a weatherproof housing, the SRISS has the potential to be used in a large range of applications from rain forests to pasture and including extreme conditions found in the arctic and hot arid regions.

A consensus on the best method to collect soil respired CO₂ for isotope analysis is progressing, but a definitive method has not been arrived at yet. There is one commercial system available (Picarro) but it has not been tested by the science community. In the meantime scientists want to use CO₂ isotope techniques to determine the effect of increased CO₂ concentrations have on below ground processes, and especially the effect of changes in temperature on soil respiration – to track carbon molecules below ground. This 6 chamber SRISS could, in the future, be connected to a Picarro ring-down cavity laser and produce online, instantaneous measurements of the $\delta^{13}\text{C}$ of soil respired CO₂ from potentially anywhere on Earth. This would increase the temporal resolution by an order of magnitude and help disentangle the effects of multiple environmental factors all changing at the same time.

References

- Amundson, R. (2001). The carbon budget in soils. *Annual Review of Earth and Planetary Sciences*, 29, 535-562.
- Barbour, M. M. (2007). Stable oxygen isotope composition of plant tissue: A review. *Functional Plant Biology*, 34(2), 83-94.
- Barbour, M. M., Hunt, J. E., Kodama, N., Laubach, J., McSeveny, T. M., Rogers, G. N., Tcherkez, G., & Wingate, L. (2011). Rapid changes in $\delta^{13}\text{C}$ of ecosystem-respired CO_2 after sunset are consistent with transient ^{13}C enrichment of leaf respired CO_2 . *New Phytologist*, 190(4), 990-1002.
- Barthel, M., Cieraad, E., Zakharova, A., & Hunt, J. E. (2014). Sudden cold temperature delays plant carbon transport and shifts allocation from growth to respiratory demand. *Biogeosciences*, 11(5), 1425-1433.
- Beer, C., Reichstein, M., Tomelleri, E., Ciais, P., Jung, M., Carvalhais, N., Rödenbeck, C., Arain, M. A., Baldocchi, D., Bonan, G. B., Bondeau, A., Cescatti, A., Lasslop, G., Lindroth, A., Lomas, M., Luyssaert, S., Margolis, H., Oleson, K. W., Rouspard, O., Veenendaal, E., Viovy, N., Williams, C., Woodward, F. I., & Papale, D. (2010). Terrestrial gross carbon dioxide uptake: Global distribution and covariation with climate. *Science*, 329(5993), 834-838.
- Bergeron, O., Margolis, H. A., Black, T. A., Coursolle, C., Dunn, A. L., Barr, A. G., & Wofsy, S. C. (2007). Comparison of carbon dioxide fluxes over three boreal black spruce forests in Canada. *Global Change Biology*, 13(1), 89-107.
- Bernacchi, C. J., Singaas, E. L., Pimentel, C., Portis Jr, A. R., & Long, S. P. (2001). Improved temperature response functions for models of Rubisco-limited photosynthesis. *Plant, Cell and Environment*, 24(2), 253-259.
- Bond-Lamberty, B., & Thomson, A. (2010). Temperature-associated increases in the global soil respiration record. *Nature*, 464(7288), 579-582.
- Boone, R. D., Nadelhoffer, K. J., Canary, J. D., & Kaye, J. P. (1998). Roots exert a strong influence on the temperature sensitivity of soil respiration. *Nature*, 396(6711), 570-572.
- Bowden, R. D., Davidson, E., Savage, K., Arabia, C., & Steudler, P. (2004). Chronic nitrogen additions reduce total soil respiration and microbial respiration in temperate forest soils at the Harvard Forest. *Forest Ecology and Management*, 196(1), 43-56.
- Bowling, D. R., Pataki, D. E., & Randerson, J. T. (2008). Carbon isotopes in terrestrial ecosystem pools and CO_2 fluxes. *New Phytologist*, 178(1), 24-40.
- Bowling, D. R., Sargent, S. D., Tanner, B. D., & Ehleringer, J. R. (2003). Tunable diode laser absorption spectroscopy for stable isotope studies of ecosystem-atmosphere CO_2 exchange. *Agricultural and Forest Meteorology*, 118(1-2), 1-19.
- Brugnoli, E., & Farquhar, G. D. (2000). Photosynthetic Fractionation of Carbon Isotopes. In R. C. Leegood, T. D. Sharkey & S. von Caemmerer (Eds.), *Photosynthesis: Physiology and Metabolism* (pp. 399-343). The Netherlands: Kluwer Academic Publishers.
- Cernusak, L. A., Ubierna, N., Winter, K., Holtum, J. A. M., Marshall, J. D., & Farquhar, G. D. (2013). Environmental and physiological determinants of carbon isotope discrimination in terrestrial plants. *New Phytologist*, 200(4), 950-965.
- Ciais, P., Sabine, C., Bala, G., Bopp, L., Brovkin, V., Canadell, J., Chhabra, A., DeFries, R., Galloway, J., Heimann, M., Jones, C., Le Quéré, C., Myneni, R. B., S., P., & P., T. (2013).

Carbon and Other Biogeochemical Cycles. In T. F. Stocker, D. Qin, G.-K. Plattner, M. Tignor, S. K. Allen, J. Boschung, A. Nauels, Y. Xia, V. Bex & P. M. Midgley (Eds.), *Climate Change 2013: The Physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change*. Cambridge, United Kingdom and New York, NY, USA.: Cambridge University Press.

Comont, D., Martinez Abaigar, J., Albert, A., Aphalo, P., Causton, D. R., Figueroa, F. L., Gaberscik, A., Llorens, L., Hauser, M. T., Jansen, M. A. K., Kardefelt, M., de la Coba Luque, P., Neubert, S., Núñez-Olivera, E., Olsen, J., Robson, M., Schreiner, M., Sommaruga, R., Strid, A., Torre, S., Turunen, M., Veljovic-Jovanovic, S., Verdaguer, D., Vidovic, M., Wagner, J., Winkler, J. B., Zipoli, G., & Gwynn-Jones, D. (2012). UV responses of *Lolium perenne* raised along a latitudinal gradient across Europe: A filtration study. *Physiologia Plantarum*, *145*(4), 604-618.

Comstedt, D., Boström, B., Marshall, J. D., Holm, A., Slaney, M., Linder, S., & Ekblad, A. (2006). Effects of elevated atmospheric carbon dioxide and temperature on soil respiration in a boreal forest using $\delta^{13}\text{C}$ as a labeling tool. *Ecosystems*, *9*(8), 1266-1277.

DairyNZ. (2012). New Zealand Dairy Statistics 2011-2012. www.dairynz.co.nz: DairyNZ.

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

Davidson, E. A., Verchot, L. V., Henrique Cattânio, J., Ackerman, I. L., & Carvalho, J. E. M. (2000). Effects of soil water content on soil respiration in forests and cattle pastures of eastern Amazonia. *Biogeochemistry*, *48*(1), 53-69.

Dynes, R. A., Burggraaf, V. T., Goulter, C. G., & Dalley, D. E. (2010). Canterbury farming: production, processing and farming systems. *Canterbury farming: production, processing and farming systems*, 72, I-VIII.

Fang, C., & Moncrieff, J. B. (1998). An open-top chamber for measuring soil respiration and the influence of pressure difference on CO₂ efflux measurement. *Functional Ecology*, *12*(2), 319-325.

Fontaine, S., Mariotti, A., & Abbadie, L. (2003). The priming effect of organic matter: A question of microbial competition? *Soil Biology and Biochemistry*, *35*(6), 837-843.

Gannes, L. Z., Del Rio, C. M., & Koch, P. (1998). Natural abundance variations in stable isotopes and their potential uses in animal physiological ecology. *Comparative Biochemistry and Physiology - A Molecular and Integrative Physiology*, *119*(3), 725-737.

Gavrichkova, O., Proietti, S., Moscatello, S., Portarena, S., Battistelli, A., Matteucci, G., & Brugnoli, E. (2011). Short-term natural $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ variations in pools and fluxes in a beech forest: The transfer of isotopic signal from recent photosynthates to soil respired CO₂. *Biogeosciences*, *8*(10), 2833-2846.

Ghashghaie, J., & Badeck, F. W. (2014). Opposite carbon isotope discrimination during dark respiration in leaves versus roots - a review. *New Phytologist*, *201*(3), 751-769.

Gilmanov, T. G., Verma, S. B., Sims, P. L., Meyers, T. P., Bradford, J. A., Burba, G. G., & Suyker, A. E. (2003). Gross primary production and light response parameters of four Southern Plains ecosystems estimated using long-term CO₂-flux tower measurements. *Global Biogeochemical Cycles*, *17*(2), 40-41.

- Goulden, M. L., Munger, J. W., Song-Miao, F., Daube, B. C., & Wofsy, S. C. (1996). Measurements of carbon sequestration by long-term eddy covariance: methods and a critical evaluation of accuracy. *Global Change Biology*, 2(3), 169-182.
- Graham, S. L., Millard, P., Hunt, J. E., Rogers, G. N., & Whitehead, D. (2012). Roots affect the response of heterotrophic soil respiration to temperature in tussock grass microcosms. *Annals of Botany*, 110(2), 253-258.
- Grossiord, C., Mareschal, L., & Epron, D. (2012). Transpiration alters the contribution of autotrophic and heterotrophic components of soil CO₂ efflux. *New Phytologist*, 194(3), 647-653.
- Högberg, P. (1997). Tansley review no. 95 natural abundance in soil-plant systems. *New Phytologist*, 137(2), 179-203.
- Höglind, M., Hanslin, H. M., & Mortensen, L. M. (2011). Photosynthesis of *Lolium perenne* L. at low temperatures under low irradiances. *Environmental and Experimental Botany*, 70(2-3), 297-304.
- Israeli, Y., Schwartz, A., Plaut, Z., & Yakir, D. (1996). Effects of light regime on $\delta^{13}\text{C}$, photosynthesis and yield of field-grown banana (*Musa* sp., Musaceae). *Plant, Cell and Environment*, 19(2), 225-230.
- Janssens, I. A., Dieleman, W., Luyssaert, S., Subke, J. A., Reichstein, M., Ceulemans, R., Ciais, P., Dolman, A. J., Grace, J., Matteucci, G., Papale, D., Piao, S. L., Schulze, E. D., Tang, J., & Law, B. E. (2010). Reduction of forest soil respiration in response to nitrogen deposition. *Nature Geoscience*, 3(5), 315-322.
- Janzen, H. H. (2006). The soil carbon dilemma: Shall we hoard it or use it? *Soil Biology and Biochemistry*, 38(3), 419-424.
- Kelliher, F. M., Barbour, M. M., & Hunt, J. E. (2005). Sucrose application, soil microbial respiration and evolved carbon dioxide isotope enrichment under contrasting land uses. *Plant and Soil*, 268(1), 233-242.
- Kindler, R., Siemens, J., Kaiser, K., Walmsley, D. C., Bernhofer, C., Buchmann, N., Cellier, P., Eugster, W., Gleixner, G., Grunwald, T., Heim, A., Ibrom, A., Jones, S. K., Jones, M., Klumpp, K., Kutsch, W., Larsen, K. S., Lehuger, S., Loubet, B., McKenzie, R., Moors, E., Osborne, B., Pilegaard, K., Reibmann, C., Saunders, M., Schmidt, M. W. I., Schruppf, M., Seyfferth, J., Skiba, U., Soussana, J., Sutton, M. A., Tefs, C., Vowinckel, B., Zeeman, M. J., & Kaupenjohann, M. (2011). Dissolved carbon leaching from soil is a crucial component of the net ecosystem carbon balance. *Global Change Biology*, 17(2), 1167-1185.
- Kirschbaum, M. U. F., Saggar, S., Tate, K. R., Giltrap, D. L., Ausseil, A. G. E., Greenhalgh, S., & Whitehead, D. (2012). Comprehensive evaluation of the climate-change implications of shifting land use between forest and grassland: New Zealand as a case study. *Agriculture, Ecosystems and Environment*, 150, 123-138.
- Klumpp, K., Schäufele, R., Lötscher, M., Lattanzi, F. A., Feneis, W., & Schnyder, H. (2005). C-isotope composition of CO₂ respired by shoots and roots: Fractionation during dark respiration? *Plant, Cell and Environment*, 28(2), 241-250.
- Knohl, A., Werner, R. A., Brand, W. A., & Buchmann, N. (2005). Short-term variations in $\delta^{13}\text{C}$ of ecosystem respiration reveals link between assimilation and respiration in a deciduous forest. *Oecologia*, 142(1), 70-82.

- Kuzyakov, Y. (2006). Sources of CO₂ efflux from soil and review of partitioning methods. *Soil Biology and Biochemistry*, 38(3), 425-448.
- Lal, R. (2009). Challenges and opportunities in soil organic matter research. *European Journal of Soil Science*, 60(2), 158-169.
- Lambie, S. M. (2012). *Soil carbon loss under pasture and pine: Responses to urine addition*. (Doctor of Philosophy), The University of Waikato.
- Le Dantec, V., Epron, D., & Dufrêne, E. (1999). Soil CO₂ efflux in a beech forest: Comparison of two closed dynamic systems. *Plant and Soil*, 214(1-2), 125-132.
- Lee, K. H., & Jose, S. (2003). Soil respiration, fine root production, and microbial biomass in cottonwood and loblolly pine plantations along a nitrogen fertilization gradient. *Forest Ecology and Management*, 185(3), 263-273.
- Litton, C. M., Raich, J. W., & Ryan, M. G. (2007). Carbon allocation in forest ecosystems. *Global Change Biology*, 13(10), 2089-2109.
- Liu, X., Wan, S., Su, B., Hui, D., & Luo, Y. (2002). Response of soil CO₂ efflux to water manipulation in a tallgrass prairie ecosystem. *Plant and Soil*, 240(2), 213-223.
- Lloyd, J., & Taylor, J. A. (1994). On the temperature dependence of soil respiration. *Functional Ecology*, 8(3), 315-323.
- Lorenz, K., Preston, C. M., & Kandeler, E. (2006). Soil organic matter in urban soils: Estimation of elemental carbon by thermal oxidation and characterization of organic matter by solid-state ¹³C nuclear magnetic resonance (NMR) spectroscopy. *Geoderma*, 130(3-4), 312-323.
- Lötscher, M., & Gayler, S. (2005). Contribution of current photosynthates to root respiration of non-nodulated *Medicago sativa*: Effects of light and nitrogen supply. *Plant Biology*, 7(6), 601-610.
- McDowell, N. G., Bowling, D. R., Bond, B. J., Irvine, J., Law, B. E., Anthony, P., & Ehleringer, J. R. (2004a). Response of the carbon isotopic content of ecosystem, leaf, and soil respiration to meteorological and physiological driving factors in a *Pinus ponderosa* ecosystem. *Global Biogeochemical Cycles*, 18(1), GB1013 1011-1012.
- McDowell, N. G., Bowling, D. R., Schauer, A., Irvine, J., Bond, B. J., Law, B. E., & Ehleringer, J. R. (2004b). Associations between carbon isotope ratios of ecosystem respiration, water availability and canopy conductance. *Global Change Biology*, 10(10), 1767-1784.
- Melillo, J. M., Steudler, P. A., Aber, J. D., Newkirk, K., Lux, H., Bowles, F. P., Catricala, C., Magill, A., Ahrens, T., & Morrisseau, S. (2002). Soil warming and carbon-cycle feedbacks to the climate system. *Science*, 298(5601), 2173-2176.
- Midwood, A. J., & Millard, P. (2011). Challenges in measuring the $\delta^{13}\text{C}$ of the soil surface CO₂ efflux. *Rapid Communications in Mass Spectrometry*, 25(1), 232-242.
- Millard, P., Midwood, A. J., Hunt, J. E., Barbour, M. M., & Whitehead, D. (2010). Quantifying the contribution of soil organic matter turnover to forest soil respiration, using natural abundance $\delta^{13}\text{C}$. *Soil Biology and Biochemistry*, 42(6), 935-943.
- Millard, P., Midwood, A. J., Hunt, J. E., Whitehead, D., & Boutton, T. W. (2008). Partitioning soil surface CO₂ efflux into autotrophic and heterotrophic components, using natural gradients in soil $\delta^{13}\text{C}$ in an undisturbed savannah soil. *Soil Biology and Biochemistry*, 40(7), 1575-1582.

- Nier, A. O., & Gulbransen, E. A. (1939). Variations in the relative abundance of the carbon isotopes. *Journal of the American Chemical Society*, 61(3), 697-698.
- Post, W. M., & Kwon, K. C. (2000). Soil carbon sequestration and land-use change: Processes and potential. *Global Change Biology*, 6(3), 317-327.
- Pregitzer, K. S., Zak, D. R., Maziasz, J., DeForest, J., Curtis, P. S., & Lussenhop, J. (2000). Interactive effects of atmospheric CO₂ and soil-N availability on fine roots of *Populus tremuloides*. *Ecological Applications*, 10(1), 18-33.
- R-Core-Team. (2013). R: A language and environment for statistical computing. Vienna, Austria.: R Foundation for Statistical Computing Retrieved from <http://www.R-project.org/>
- Ramirez, K. S., Craine, J. M., & Fierer, N. (2010). Nitrogen fertilization inhibits soil microbial respiration regardless of the form of nitrogen applied. *Soil Biology and Biochemistry*, 42(12), 2336-2338.
- Risch, A. C., & Frank, D. A. (2010). Diurnal and seasonal patterns in ecosystem CO₂ fluxes and their controls in a temperate grassland. *Rangeland Ecology and Management*, 63(1), 62-71.
- Risk, D., Nickerson, N., Creelman, C., McArthur, G., & Owens, J. (2011). Forced Diffusion soil flux: A new technique for continuous monitoring of soil gas efflux. *Agricultural and Forest Meteorology*, 151(12), 1622-1631.
- Risk, D., Relia, C., Nickerson, N., & Van Pelt, A. (2009). *Continuous, in-situ isotopic CO₂ measurements of soil respiration using a WS-CRDS-based analyzer*.
- Rochette, P., Ellert, B., Gregorich, E. G., Desjardins, R. L., Pattey, E., Lessard, R., & Johnson, B. G. (1997). Description of a dynamic closed chamber for measuring soil respiration and its comparison with other techniques. *Canadian Journal of Soil Science*, 77(2), 195-203.
- Ryan, M. G. (1991). Effects of climate change on plant respiration. *Ecological Applications*, 1(2), 157-167.
- Ryan, M. G., & Law, B. E. (2005). Interpreting, measuring, and modeling soil respiration. *Biogeochemistry*, 73(1), 3-27.
- Saarinen, T., Lundell, R., & Hänninen, H. (2011). Recovery of photosynthetic capacity in *Vaccinium vitis-idaea* during mild spells in winter. *Plant Ecology*, 212(9), 1429-1440.
- Sage, R. F., & Kubien, D. S. (2007). The temperature response of C₃ and C₄ photosynthesis. *Plant, Cell and Environment*, 30(9), 1086-1106.
- Schaeffer, S. M., Anderson, D. E., Burns, S. P., Monson, R. K., Sun, J., & Bowling, D. R. (2008). Canopy structure and atmospheric flows in relation to the $\delta^{13}\text{C}$ of respired CO₂ in a subalpine coniferous forest. *Agricultural and Forest Meteorology*, 148(4), 592-605.
- Schipper, L. A., Parfitt, R. L., Ross, C., Baisden, W. T., Claydon, J. J., & Fraser, S. (2010). Gains and losses in C and N stocks of New Zealand pasture soils depend on land use. *Agriculture, Ecosystems and Environment*, 139(4), 611-617.
- Sejian, V., Lal, R., Lakritz, J., & Ezeji, T. (2011). Measurement and prediction of enteric methane emission. *International Journal of Biometeorology*, 55(1), 1-16.
- Smith, P., Fang, C., Dawson, J. J. C., & Moncrieff, J. B. (2008) Impact of Global Warming on Soil Organic Carbon. *Vol. 97* (pp. 1-43).
- Stevenson, I. L. (1956). Some observations on the microbial activity in remoistened air-dried soils. *Plant and Soil*, 8(2), 170-182.

- Tivet, F., De Moraes Sá, J. C., Borszowski, P. R., Letourmy, P., Briedis, C., Ferreira, A. O., Dos Santos, J. B., & Inagaki, T. M. (2012). Soil carbon inventory by wet oxidation and dry combustion methods: Effects of land use, soil texture gradients, and sampling depth on the linear model of C-Equivalent correction factor. *Soil Science Society of America Journal*, 76(3), 1048-1059.
- Ubierna, N., Sun, W., Kramer, D. M., & Cousins, A. B. (2013). The efficiency of C4 photosynthesis under low light conditions in *Zea mays*, *Miscanthus x giganteus* and *Flaveria bidentis*. *Plant, Cell and Environment*, 36(2), 365-381.
- Uchida, Y., Clough, T. J., Kelliher, F. M., & Sherlock, R. R. (2010a). Soil microbial respiration responses to changing temperature and substrate availability in fertile grassland. *Australian Journal of Soil Research*, 48(5), 395-403.
- Uchida, Y., Hunt, J. E., Barbour, M. M., Clough, T. J., Kelliher, F. M., & Sherlock, R. R. (2010b). Soil properties and presence of plants affect the temperature sensitivity of carbon dioxide production by soils. *Plant and Soil*, 337(1), 375-387.
- Vargas, R., & Allen, M. F. (2008). Environmental controls and the influence of vegetation type, fine roots and rhizomorphs on diel and seasonal variation in soil respiration. *New Phytologist*, 179(2), 460-471.
- Wan, S., Norby, R. J., Ledford, J., & Weltzin, J. F. (2007). Responses of soil respiration to elevated CO₂, air warming, and changing soil water availability in a model old-field grassland. *Global Change Biology*, 13(11), 2411-2424.
- Werner, C., & Gessler, A. (2011). Diel variations in the carbon isotope composition of respired CO₂ and associated carbon sources: A review of dynamics and mechanisms. *Biogeosciences*, 8(9), 2437-2459.
- Wickman, F. E. (1952). Variations in the relative abundance of the carbon isotopes in plants. *Geochimica et Cosmochimica Acta*, 2(4), 243-254.
- Wieringa, K. T., & Kerkhof Mogot, M. F. (1957). An apparatus for the determination of the respiration process in soil samples. *Plant and Soil*, 8(4), 395-396.
- Wright, A. L., & Hons, F. M. (2005). Tillage impacts on soil aggregation and carbon and nitrogen sequestration under wheat cropping sequences. *Soil and Tillage Research*, 84(1), 67-75.
- Zakharova, A., Midwood, A. J., Hunt, J. E., Graham, S. L., Artz, R. R. E., Turnbull, M. H., Whitehead, D., & Millard, P. (2014). Loss of labile carbon following soil disturbance determined by measurement of respired $\delta^{13}\text{C}\text{O}_2$. *Soil Biology and Biochemistry*, 68, 125-132.
- Zeng, X., Zhang, W., Shen, H., Cao, J., & Zhao, X. (2014). Soil respiration response in different vegetation types at Mount Taihang, China. *Catena*, 116, 78-85.

'19L MFC In 5 (White)
'20H MFC In 6 (Blue)
'20L MFC In 6 (White)

*****LiCor Gas Analyser*****

'1 +12VDC Power Supply (Red)
'2 Earth (Black)
'7 LiC CO2 (Red)
'8 LiC CO2 (Yellow)
'9 LiC H2O (Green)
'10 LiC H2O (Blue)

*****SDM-CD16AC*****

'1 Pump 1
'2 Pump 2
'3 Pump 3
'4 Pump 4
'5 Pump 5
'6 Pump 6
'7 Valve 1
'8 Valve 2
'9 Valve 3
'10 Valve 4
'11 Valve 5
'12 Valve 6

*****Coms for SDM-AO4, SDM-AO4A, SDM-CD16AC, CR5000*****

'C1 Red
'C2 Yellow
'C3 Green

*****AO_1*****

'1 MFC Out 1
'2 MFC Out 2
'3 MFC Out 3
'4 MFC Out 4

*****AO_2*****

'1 MFC Out 5
'2 MFC Out 6
'3 MFC In 1
'4 MFC In 2

*****AO_3*****

'1 MFC In 3
'2 MFC In 4
'3 MFC In 5
'4 MFC In 6

*****Pins on FMA 5516 0-0.2L/min MFC (12VDC supply)*****

'1 Flow signal common (White)
'2 0-5VDC flow signal output (Blue)
'5 Common power supply (Green)
'8 Remote set point (Yellow)
'13 +12VDC power supply (Red)
'15 Earth (Black)

*****LiCor Gas Analyser*****

'1 +12VDC Power Supply (Red)

'2 Earth (Black)
 '7 LiC CO2 (Red)
 '8 LiC CO2 (Yellow)
 '9 LiC H2O (Green)
 '10 LiC H2O (Blue)

'////////////////////VARIABLES////////////////////////////////////
 SequentialMode

Const L_per_mol = 22.414 ' Molar volume
 Const SurfaceArea = 0.008332 ' m2 Screamer ring, 103 mm diameter

' Data from last average
 Public An_Flow, Slope_ppm_min, Old_Slope, Slope_1

' Variables for thermocouple temperatures
 Public Panel_temp, Soil_temp(6)

'setup sequence
 Public run_setup As Boolean

Public TIME(9)
 ALIAS TIME(1)=YEAR
 ALIAS TIME(2)=MONTH
 Alias TIME(3)=DOM
 ALIAS TIME(4)=HOUR
 ALIAS TIME(5)=MINUTES
 ALIAS TIME(6)=SECONDS
 ALIAS TIME(7)=mSECONDS
 Alias TIME(8)=DAY_OF_WEEK
 Alias TIME(9)=DAY_OF_YEAR

' Variables for MFC
 Public AO_1(4), AO_2(4), AO_3(4) 'Arrays for the 3 x SDMAO4 units used to control
 the MFC

Public MFCIn_SP(6): Units MFCIn_SP = mL_min ' Setpoint flow rates

Public MFCsmp1_SP(6) : Units MFCsmp1_SP = mL_min

Alias MFCIn_SP(1) = InSP_1
 Alias MFCIn_SP(2) = InSP_2
 Alias MFCIn_SP(3) = InSP_3
 Alias MFCIn_SP(4) = InSP_4
 Alias MFCIn_SP(5) = InSP_5
 Alias MFCIn_SP(6) = InSP_6
 Alias MFCsmp1_SP(1) = Smp1SP_1
 Alias MFCsmp1_SP(2) = Smp1SP_2
 Alias MFCsmp1_SP(3) = Smp1SP_3
 Alias MFCsmp1_SP(4) = Smp1SP_4
 Alias MFCsmp1_SP(5) = Smp1SP_5
 Alias MFCsmp1_SP(6) = Smp1SP_6

Public MFCIn_FS(6), MFCsmp1_FS(6)
 Public MFCIn_SPmv(6), MFCsmp1_SPmv(6)
 Public MFCIn_mult(6), MFCsmp1_mult(6)
 Public MFCIn_Offset(6), MFCsmp1_Offset(6)
 Public FlowIn_mLmin(6), FlowIn_mLminraw(6) 'Actual flow rate
 Alias FlowIn_mLmin(1) = In_1

```

Alias FlowIn_mLmin(2) = In_2
Alias FlowIn_mLmin(3) = In_3
Alias FlowIn_mLmin(4) = In_4
Alias FlowIn_mLmin(5) = In_5
Alias FlowIn_mLmin(6) = In_6

Public FlowSmpl_mLmin(6) 'Actual flow rate
Alias FlowSmpl_mLmin(1) = Smpl_1
Alias FlowSmpl_mLmin(2) = Smpl_2
Alias FlowSmpl_mLmin(3) = Smpl_3
Alias FlowSmpl_mLmin(4) = Smpl_4
Alias FlowSmpl_mLmin(5) = Smpl_5
Alias FlowSmpl_mLmin(6) = Smpl_6
Public FlowIn_umols(6)
Public FlowIn_mols(6)

' Variables from LiCor
Public CO2_min, CO2_max, H2O_min, H2O_max, CO2_mult, H2O_mult
Public CO2_umol : units CO2_umol=umol_mol
Public H2O_mmol : Units H2O_mmol=mmol_mol
Public CO2_ppm : Units CO2_ppm=ppm

' Other variables
Public Batt_V, CO2_bottle, CO2_Diff, Chamber, egm_flux, est_flow, DiffInOut, Manual As Boolean, FTime,
flush As Boolean, maxFtime

' Control switch variables
Public Pumps_all_on As Boolean
Public Pumps_all_off As Boolean
Public Switch(16) As Boolean ' Array for SDM16AC/DC
Alias Switch(1) = Pump_1
Alias Switch(2) = Pump_2
Alias Switch(3) = Pump_3
Alias Switch(4) = Pump_4
Alias Switch(5) = Pump_5
Alias Switch(6) = Pump_6
Alias Switch(7) = Chamber_1
Alias Switch(8) = Chamber_2
Alias Switch(9) = Chamber_3
Alias Switch(10) = Chamber_4
Alias Switch(11) = Chamber_5
Alias Switch(12) = Chamber_6

Public CO2_mv , H2O_mv
Public AnChamb, count, MaxCount
Public test1,n,testresp, dontadd As Boolean, oldchamb, delayflag As Boolean
Public Starttime_hour, starttime_minute

'Variables Respiration
Public Resp_raw : Units Resp_raw = umol_m2_s
Public Resp_umolm2s : Units Resp_umolm2s = umol_m2_s
Public Resp_gm2hr : Units Resp_gm2hr = gCO2_m2_hr

%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%Data Tables%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
DataTable (AvgData,True,-1)
CardOut(1,-1)
DataInterval (0,1,Min,10)
Sample(1,oldchamb,FP2)

```

```

    Sample(1,Resp_umolm2s,FP2)
    Average(6,FlowIn_mLmin(),FP2,False)
    Average(6,FlowSmpl_mLmin(),FP2,False)
    Average(6,Soil_temp(),FP2,False)
    Average(1,CO2_Diff,FP2,dontadd)
EndTable

%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%SUB ROUTINES%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%

Sub Initialize
    AnChamb = 1 ' Start with Chamber 1
    CO2_bottle = 0 'CO2 concentration in cylinder (umol/mol)
    Pumps_all_on = true
    Manual = false
    flush = false
    MaxCount = 60 'seconds change to next chamber
    FTime = 0
    maxFTime = 60
    DiffInOut= 20    'mL/min

For n = 1 To 6
    MFCIn_FS(n) = 0.2      'Mass flow controller IN, fullscale Standard Litres per minute
    MFCsmpL_FS(n) = 2.0    'Mass flow controller SAMPLE, fullscale SLPM  '***WARNING*** This has
changed because all MFC_SMPL were replaced with 2L/m MFC's due to malfunction of 0.2L/min MFC;s
    MFCIn_Offset(n) = 0    'MFC IN offset, used for calibration
    MFCsmpL_Offset(n) = 0  'MFC SAMPLE offset, used for calibration
    MFCIn_SP(n) = 190      'mL/min
Next n

'Set LiCor ranges
    CO2_max = 700
    CO2_min = 00
    H2O_max = 80
    H2O_min = 0
'Set GasHound DAC multipliers
    CO2_mult = (CO2_max-CO2_min)/5000' ppm/mV
    H2O_mult = (H2O_max-H2O_min)/5000 ' ppm/mV

    run_setup = false
EndSub

Sub Pumps
    If Pumps_all_on Then
        Pumps_all_off = false
        For n = 1 To 6
            Switch(n)= true
        Next n
    EndIf
    If Pumps_all_off = true Then
        Pumps_all_on = false
        For n = 1 To 6
            Switch(n) = false
        Next n
    EndIf
    SDMCD16AC (Switch(),1,00)
EndSub

Sub flush_all
    Pumps_all_on = false

```

```

Pumps_all_off = true
FTime = FTime + 1
For n = 1 To 6
    MFCIn_SP(n) = 200
Next n
If FTime >= maxFtime Then run_setup = true
EndSub

```

```

Sub Auto_valve
For n = 1 To 6
    If AnChamb = n Then
        If CO2_Diff >= 455 AND MFCIn_SP(n) <= 195 Then MFCIn_SP(n) = MFCIn_SP(n) + 5
        If CO2_Diff <= 445 AND MFCIn_SP(n) >= 30 Then MFCIn_SP(n) = MFCIn_SP(n) - 5
    EndIf
Next n
EndSub

```

```

Sub Set_MFC
'Convert Set Point to MFC control voltage
If NOT Manual AND count = (MaxCount-1) Then Call Auto_valve

    For n = 1 To 6
        MFCsmp1_SP(n) = MFCIn_SP(n) - DiffInOut

        MFCIn_mult(n) = (MFCIn_FS(n)*1000)/5000 'mL/min/mV
        MFCsmp1_mult(n) = (MFCsmp1_FS(n)*1000)/5000 'mL/min/mV

        MFCIn_SPmv(n) = MFCIn_SP(n) * (1/MFCIn_mult(n)) 'SPmV = SP (mL/min)
        MFCsmp1_SPmv(n) = MFCsmp1_SP(n) * (1/MFCsmp1_mult(n))
    Next

```

```

For n = 1 To 4
    AO_1(n) = MFCsmp1_SPmv(n)
    AO_3(n) = MFCIn_SPmv(n+2)
Next n

```

```

AO_2(1) = MFCsmp1_SPmv(5)
AO_2(2) = MFCsmp1_SPmv(6)
AO_2(3) = MFCIn_SPmv(1)
AO_2(4) = MFCIn_SPmv(2)

```

```

SDMAO4 (AO_1(),4,1)
SDMAO4 (AO_2(),4,2)
SDMAO4 (AO_3(),4,3)

```

```
EndSub
```

```

Sub Chamb2An
    count = count + 1
    oldchamb = AnChamb
    If count >= MaxCount Then
        count = 0
        AnChamb = AnChamb + 1
        If AnChamb >= 7 Then AnChamb = 1
        An_Flow = FlowIn_mLmin(AnChamb)
    EndIf
EndSub

```

```
Sub Measure_gas
```

```

CO2_ppm = CO2_mv*((CO2_max-CO2_min)/5000)
CO2_umol = CO2_mv*CO2_mult+CO2_min
H2O_mmol = H2O_mv*H2O_mult+H2O_min
EndSub

Sub ChangeAnal
  If AnChamb = 1 Then
    Switch(7) = true
    Switch(12) = false
  ElseIf AnChamb = 2 Then
    Switch(8) = true
    Switch(7) = false
  ElseIf AnChamb = 3 Then
    Switch(9) = true
    Switch(8) = false
  ElseIf AnChamb = 4 Then
    Switch(10) = true
    Switch(9) = false
  ElseIf AnChamb = 5 Then
    Switch(11) = true
    Switch(10) = false
  ElseIf AnChamb = 6 Then
    Switch(12) = true
    Switch(11) = false
  EndIf
  SDMCD16AC (Switch(),1,00)
EndSub

Sub Calc_Resp
'Molar flow into chamber conversion L/min to mol/min
FlowIn_mols() = FlowIn_mLmin() /1000/60/ L_per_mol 'mol/second
FlowIn_umols() = FlowIn_mLmin()/1000/60/ L_per_mol*1000*1000 'umol/second

' Calc Respiration fluxes (raw and adjusted)
CO2_Diff = CO2_umol - CO2_bottle

Resp_umolm2s = (((CO2_Diff*An_Flow)/10^6) / (0.00008311*283))*(1/SurfaceArea)/60 'mols/m2/s *10^6
= umol/m2/s
Resp_gm2hr = Resp_umolm2s/6.31313
est_flow=((((((egm_flux*6.312)*60)/(1/0.007854))*(0.00008311*273.16)))*1000000)/450
EndSub

Sub CalcSlope
' Calc instant slope of 1min running average change in CO2 conc
Old_Slope = Slope_1
AvgRun (Slope_1,1,CO2_umol,30)
Slope_ppm_min = (Slope_1 - Old_Slope)*60
EndSub

Sub controlave
  If count > 30 Then dontadd = false
  If count <= 30 Then dontadd = true
EndSub

Sub delay_start
  RealTime(TIME)
  If HOUR = Starttime_hour AND MINUTES = starttime_minute AND SECONDS = 0 Then delayflag = true
EndSub

```


'Measure soil temperatures
PanelTemp (Panel_temp,250)
TCDiff (Soil_temp(),6,mV20C,3,TypeT,Panel_temp,True ,0,250,1,0,0)
Battery (Batt_V)

' Measure gasflow through MFC (mL/min)
VoltDiff (FlowIn_mLminraw(1),1,mV5000,15,1,0,_50Hz,MFCIn_mult(1),-MFCIn_Offset(1))
VoltDiff (FlowIn_mLminraw(2),1,mV5000,16,1,0,_50Hz,MFCIn_mult(2),-MFCIn_Offset(2))
VoltDiff (FlowIn_mLminraw(3),1,mV5000,17,1,0,_50Hz,MFCIn_mult(3),-MFCIn_Offset(3))
VoltDiff (FlowIn_mLminraw(4),1,mV5000,18,1,0,_50Hz,MFCIn_mult(4),-MFCIn_Offset(4))
VoltDiff (FlowIn_mLminraw(5),1,mV5000,19,1,0,_50Hz,MFCIn_mult(5),-MFCIn_Offset(5))
VoltDiff (FlowIn_mLminraw(6),1,mV5000,20,1,0,_50Hz,MFCIn_mult(6),-MFCIn_Offset(6))
VoltDiff (FlowSmpl_mLmin(1),1,mV5000,9,1,0,_50Hz,MFCSmpl_mult(1),-MFCSmpl_Offset(1))
VoltDiff (FlowSmpl_mLmin(2),1,mV5000,10,1,0,_50Hz,MFCSmpl_mult(2),-MFCSmpl_Offset(2))
VoltDiff (FlowSmpl_mLmin(3),1,mV5000,11,1,0,_50Hz,MFCSmpl_mult(3),-MFCSmpl_Offset(3))
VoltDiff (FlowSmpl_mLmin(4),1,mV5000,12,1,0,_50Hz,MFCSmpl_mult(4),-MFCSmpl_Offset(4))
VoltDiff (FlowSmpl_mLmin(5),1,mV5000,13,1,0,_50Hz,MFCSmpl_mult(5),-MFCSmpl_Offset(5))
VoltDiff (FlowSmpl_mLmin(6),1,mV5000,14,1,0,_50Hz,MFCSmpl_mult(6),-MFCSmpl_Offset(6))

'Calibration from raw to actual flowrates for gas into chambers
FlowIn_mLmin(1) = FlowIn_mLminraw(1) + 11 '12/08/13 calibrated as 11 under Cr5000 record from Agilent
Tech AMD1000 Universal Gas Flowmeter
FlowIn_mLmin(2) = FlowIn_mLminraw(2) + 10 '12/08/13 calibrated as 10 under Cr5000 record from Agilent
Tech AMD1000 Universal Gas Flowmeter
FlowIn_mLmin(3) = FlowIn_mLminraw(3) + 10 '12/08/13 calibrated as 10 under Cr5000 record from Agilent
Tech AMD1000 Universal Gas Flowmeter
FlowIn_mLmin(4) = FlowIn_mLminraw(4) + 7 '12/08/13 calibrated as 7 under Cr5000 record from Agilent
Tech AMD1000 Universal Gas Flowmeter
FlowIn_mLmin(5) = FlowIn_mLminraw(5) + 0 '12/08/13 No Calibration needed
FlowIn_mLmin(6) = FlowIn_mLminraw(6) + 14 '12/08/13 calibrated as 14 under Cr5000 record from Agilent
Tech AMD1000 Universal Gas Flowmeter

' Measure CO2 and H2O from GasHound
VoltDiff (CO2_mv,1,mV5000,1,True ,0,_50Hz,1,0)
VoltDiff (H2O_mv,1,mV5000,2,True ,0,_50Hz,1,0)

Call Pumps
Call Set_MFC
Call Chamb2An
Call Measure_gas
Call Calc_Resp
Call CalcSlope
Call ChangeAnal

'Call data tables:
Call Controlave
CallTable AvgData ' 1 minute corrected averages

NextScan
Loop
EndProg