Carbon inputs to soil from roots of two contrasting pasture swards

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Abstract

The soil carbon (C) pool is one of the largest pools of C in the terrestrial environment. Soil management strategies to increase soil C are of interest globally to reduce atmospheric CO_2 concentrations which are contributing to climate change. Changes in land use and land management can result in gains, or losses of soil C, and consequently change in atmospheric CO_2 concentrations. Increasing the C inputs to soil by either increasing plant root mass has been suggested as one method to increase soil C.

In New Zealand, pastoral agriculture is dominated by the use of perennial ryegrass and white clover pastures which are typically shallow rooting species. The use of more diverse pastures including additional species such as lucerne, chicory and plantain, may increase root mass or rooting depth. However, there is limited data on root mass and C inputs from roots to soil under ryegrass-clover pasture systems and no data on more diverse pasture swards in New Zealand pasture systems. The objective of this research was to investigate whether more diverse pasture swards had greater root mass and C inputs to soil compared to ryegrass-clover and whether diverse pastures offered scope to increase soil C.

Root mass was measured under a moderately diverse and a ryegrass-clover pasture in an existing plant diversity trial at a research dairy farm (Scott Farm, DairyNZ) containing 6 replicate paddocks of each pasture type. Soil cores were collected seasonally over one year to 300 mm depth and divided into three 100 mm depth sections (0 – 100, 100 – 200, 200 – 300 mm) and root mass measured after soil was washed off. There was greater root mass (0 – 300 mm) under the moderately diverse pasture (5320-9350 kg ha^{-1}) compared to the ryegrass-clover

pasture (3810-5700 kg ha^{-1}) for all seasons. Additionally, there was greater root mass lower in the soil profile (100 – 200, 200 – 300 mm) in the moderately diverse pasture. The increased root mass in the moderately diverse pasture resulted in an estimated additional C input to soil of about 1203 kg C ha^{-1} y^{-1} (0 – 300 mm) but this estimate did not include contributions from root exudates. Root trait measurements of individual plant species also demonstrated a greater diversity of root traits (specific root length, surface area, and diameter) in the moderately diverse pasture, which may be important for the C input and C stabilisation processes in soil.

Root turnover and C input to soil was measured on three replicate paddocks each of moderately diverse and ryegrass-clover pastures, also at Scott Farm. An isotope ($^{13}CO_2$) pulse labelling method was used whereby clear chambers (1 m^2) were placed over pasture and $^{13}CO_2$ taken up following photosynthesis. Labelling was carried out once weekly for a period of five weeks, giving a total of 5 labelling events. Soil cores were collected at regular intervals following isotope labelling for up to 138 days and $\delta^{13}C$ measured in the roots (0 – 100, 100 – 200 mm depth) and soil (0 – 100 mm depth) to calculate root turnover and C input.

There was no difference in root turnover rates between the moderately diverse (298 days) and ryegrass-clover (260 days) pastures, with a combined root turnover rate of 276 days for both pastures. However, large variability in data meant that the ability to detect differences between pasture swards was low. The average C input to soil for both pastures was 58 kg C ha^{-1} d^{-1} over an 88 day period which was greater than other reported studies in New Zealand. A likely cause of this high C input from roots to soil was the severe drought conditions during the study that may have increased root death and C inputs from roots to soil.

While previous work determined similar root turnover and C inputs under both conventional and moderately diverse pastures, a final experiment focussed on whether pasture renewal of a ryegrass-clover pasture could result in increased root turnover, and therefore, greater C input to soil. Root turnover and C input to soil under ryegrass-clover pasture with and without pasture renewal was measured using an isotope pulse labelling method. Pastures (paired plots in 3 replicate paddocks) were labelled with $^{13}CO_2$ daily for five days within clear chambers (1 m^2) before one replicate in each pair was sprayed with herbicide and seed direct drilled. Soil cores were collected (0 – 100, 100 – 200 mm depths) at regular intervals over a 89 day period following isotope labelling and root turnover and C input measured by following the decline in ^{13}C in extracted roots.

Following herbicide application, there was an initial rapid increase in root turnover (17 days) followed by a more similar turnover (524 days) compared to the unsprayed treatment (585 days). The increased root turnover following the use of herbicide resulted in increased C input to the soil in the sprayed treatment (3238 kg C ha^{-1}) compared to the unsprayed ryegrass-clover treatment (1726 kg C ha^{-1}). This suggested that during pasture renewal there is a large input of C. However, the proportion of this C that is stabilised in soil requires further investigation.

This research demonstrated there is potential to increase soil C by using more diverse pastures through increased root mass and rooting depth. This work also provided the first measurements of root mass and C input to soil under moderately diverse pastures in New Zealand and adds to the limited information on the root mass, root turnover and C input under ryegrass-clover pasture systems. Furthermore, this work provided the first measurements of root turnover and C

input to soil during a pasture renewal event involving herbicide. The data from this research will contribute better information for use in modelling and increase the knowledge and understanding of soil C under grazed pasture systems in New Zealand.

Further research on investigating the root dynamics under more diverse pastures with respect to root traits such as diameter, surface area and specific root length within these pastures and how these traits influence the root turnover and C input to soil would be beneficial. Improving the understanding on the quantity of C that is stabilised and the C stabilisation processes in these pasture systems is also important in order to achieve meaningful reductions in atmospheric CO_2 concentrations.

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Chapter 1: Introduction

1.1 Greenhouse gas emission and climate change

Increases in greenhouse gas emissions (CO_2, N_2O and CH_4) to the atmosphere are driving climate change (IPCC 2014). The atmospheric carbon dioxide (CO_2) concentration is approximately 40% greater than the pre-industrial concentration and this increase has been driven by anthropogenic emissions including fossil fuels and land use change. This increase in CO_2 has resulted in the climatic system absorbing energy, leading to warming of the surface of the earth (IPCC 2014). The change of CO_2 in the atmosphere is faster than expected due to natural variability, and has contributed not only to global temperatures increasing, but also changes in patterns of precipitation, more extreme weather events, rising sea levels, and the receding of the arctic sea ice (Hopkins and Del Prado 2007; IPCC 2014; Lal 2009).

Fossil fuels are a large contributor to anthropogenic emissions of CO_2 to the atmosphere, but land use and land use change (particularly agriculture) also contribute to CO_2 emissions (IPCC 2014). Globally, agricultural land (croplands and grazed pastures) covers approximately 40% of the land surface producing food and fibre (Foley et al. 2005). While modern management of this agricultural land has increased production of food there have also been environmental trade-offs such as long term decreases of ecosystem services (Foley et al. 2005). Changes in land use and land use management can result in loss of soil C which reduces the long-term sustainability of agriculture. Agricultural practices such as deforestation, biomass burning, wetland drainage and cultivation can increase the

emissions of CO_2 from soil or decrease C inputs, both of which can result in C loss (Lal 2004). However, other agricultural practices can sequester C and increase the soil C pool, such as re-vegetation of degraded land, and conversion of arable land to forest or grassland (Powlson et al. 2011). Understanding the processes contributing to carbon storage in soils has become of increasing importance during the last few decades due to the need for sequestering C to combat climate change (Rees et al. 2005). There is a specific focus on testing new management approaches for different agricultural land uses that result in C accumulation.

1.2 New Zealand Pasture Systems

Grazed pastures (by dairy cows and dry stock including sheep and beef) cover approximately 51 - 55% of the land surface in New Zealand (MfE 2010; StatisticsNZ 2012). These pastures are predominantly based on the permanent pasture mix of perennial ryegrass (*Lolium perenne L.*) and white clover (*Trifolium repens L.*) (Powell et al. 2007; Dodd et al. 2011; Mackay et al. 2010) and are typically grazed outdoors year-round (MacLeod and Moller 2006).

Management of these pastures have changed substantially in the last two decades with increased fertiliser inputs (particularly N inputs) and stocking rates to increase production of pastures, milk, meat and fibre (MacLeod and Moller 2006). While pasture management practices have increased production and profit, they have also resulted in increased nutrient losses (Clark et al. 2007).

Dairy pastures in New Zealand were suggested to have lost significant amounts of soil C (~700 kg C ha^{-1} y^{-1}) compared to less intensive dry stock grazing systems

(Schipper et al. 2010). However, additional research demonstrated that this loss of C was better explained by losses from specific soil orders (Allophanic and Gley Soils) rather than by grazing intensity (Schipper et al. 2014). A number of hypotheses have been proposed to explain the C loss under grazed pastures such as leaching of dissolved organic matter following solubilisation of C under freshly deposited urine patches (Lambie et al. 2012), increased soil organic matter (SOM) decomposition, and changes in total belowground allocation of C due to grazing management or species cultivar (Bellamy et al. 2005; McSherry and Ritchie 2013; Schipper et al. 2007). However, this past loss of C from soils under agriculture in New Zealand also presents an opportunity to use suitable management practices that may increase the C inputs and hence increase soil C. Furthermore, Beare et al. (2014) demonstrated that many New Zealand soils might be able to store more C, as many of these soils had a saturation deficit, meaning that the measured C content was below their maximum C saturation content.

Increasing the root mass and rooting depth of these grazed pastures has been proposed as an opportunity to increase the C inputs to soil (Dodd et al. 2011a). Perennial ryegrass and white clover pastures in New Zealand are typically shallow rooting species with about 80% of root mass in the top 20 cm of soil (Crush et al. 2005). However, there is still limited data available on the root mass under these pastures in field studies, and even less data on other pasture swards compared to ryegrass-clover. Studies in New Zealand pastures have estimated that between 26 -50% of photosynthetic C of plants is allocated belowground, and increasing the fertility status of the soil can decrease this allocation (Saggar et al. 1997; Stewart and Metherell 1999), possibly due to a smaller root biomass being needed to

gather nutrients (Bloom et al. 1985). If increased nutrient availability decreases root mass, this could have significant implications for C storage in New Zealand agriculture by limiting the C inputs to soil as Rasse et al. (2005) argue that the majority of C stabilised in soil is originally derived from root inputs. However, the limited root mass measurements of New Zealand pastures have shown contrasting effects of nutrient status (Dodd and Mackay 2011b; Saggar and Hedley 2001; Saggar et al. 1997; Saggar et al. 1999; Stewart and Metherell 1999).

Most of the research on New Zealand pastures has focused on a ryegrass and clover mix. Recently, mixed sward pastures, or pastures with greater diversity are becoming of increasing interest to farmers for their tolerance to drought and more consistent annual dry matter production compared to ryegrass-clover pastures, particularly during dry summers (Woodward et al. 2013). These mixed pastures include species such as ryegrass, clover, lucerne (*Medicago sativa* L.), chicory (*Cichorium intybus* L.), plantain (*Plantago lanceolata* L.), and prairie grass (*Bromus wildenowii* L.) and are thought to have greater rooting depth and root mass compared to ryegrass-clover pastures. Whether this increased rooting mass will result in greater soil C is not known. However, many farmers are converting ryegrass-clover pastures to more diverse swards through pasture renewal, by killing the existing sward with herbicide, cultivating and reseeding. Whether this conversion process results in a loss or gain of C is not clear. Gains of C could arise from substantial C inputs from the death of root from the old pasture sward, which are rapidly replaced by growth of the new pasture sward, typically within 2-3 weeks. Losses could occur from decomposition of organic matter following soil disturbance.

There is some evidence that these mixed pastures have increased drought resistance due to greater root depth, and shading of less tolerant species from species such as lucerne (Woodward et al. 2013). With the frequency of extreme weather events (e.g. drought) in New Zealand expected to increase as a result of climate change (Orwin et al. 2015), incorporating these mixed sward or more diverse pastures in existing farming practices may become increasingly favourable. However, there is no information on the rooting dynamics under these types of pastures or how these may regulate the C stocks of soil. Root dynamics such as root mass (dry mass of roots per area, kg ha^{-1}) and root turnover (roots that are produced and die annually) contribute to the quantity of C (kg C ha^{-1}) inputted to soil.

There is limited knowledge on the belowground aspect of pastures, particularly the root dynamics of grazed systems in New Zealand. To develop suitable management practices that can increase soil C in these agricultural soils, it is critical that root dynamics (e.g. root mass) of pasture systems in the field are quantified, and whether these root systems can contribute to soil C. Improving the knowledge of these root dynamics will help to improve our understanding of the C balance of pastures, potentially identifying strategies to increase soil C and offset emissions of other greenhouse gases. It is also important to remember that maintaining or enhancing SOM in soil also contributes to soil quality (Sparling et al. 2003).

1.3 Thesis aims, objectives and hypotheses

The main aim of this thesis was to quantify the root mass and C input to soil under perennial ryegrass and white clover pastures in comparison to mixed swards or more diverse pastures. This research was carried out to better understand the belowground C inputs in New Zealand pasture systems with the aim of increasing C inputs under grazed pastures. To achieve this aim, specific research objectives were:

1) to quantify the changes in seasonal root mass of a perennial ryegrass and white clover pasture in comparison to a more diverse pasture including species such as lucerne, chicory and plantain;
2) to compare rates of root turnover and root C input to soil under a more diverse pasture in comparison with a perennial ryegrass and white clover pasture;
3) to compare the root turnover and C input to soil during pasture renewal (herbicide and direct drill) with that of an existing ryegrass-clover pasture.

General hypotheses to the above research objectives were:

1) Moderately diverse pastures would have greater root mass and rooting depth than perennial ryegrass and white clover pasture due to a greater number of species and more diverse root traits;
2) Moderately diverse pastures would have greater root turnover and C input to soil compared to perennial ryegrass and white clover pasture;
3) The use of herbicide would increase root turnover through plant death, and increase the C input to soil through root decomposition.

1.5 Thesis structure

The structure of this thesis is:

- Chapter 2 is a literature review focussing on root mass, root turnover and C inputs to soil under grazed pasture systems. This review provided a framework from which areas of limited knowledge were identified and further guided the research topics presented in later chapters.
- Chapters 3-5 are experimental work presented in manuscript format addressing the main results of the specific research objectives outlined above. Briefly, Chapter 3 presents seasonal root mass data and Chapter 4 presents root turnover and C input to soil between a ryegrass-clover pasture and a more diverse pasture. Chapter 5 presents root turnover and C input to soil of a ryegrass-clover pasture with and without pasture renewal. Within each chapter the specific methods used are presented and a discussion to relevant literature. At the time of thesis submission, Chapter 3 (McNally et al. 2015) had been accepted and published in Plant and Soil (Vol. 392, 1-2: 289-299) and is presented as the accepted manuscript. As these are presented as papers there is some repetition between chapters so that they can stand as individual studies. Chapters 4 and 5 are yet to be submitted to a peer-reviewed journal.
- Chapter 6 summarises the main results and conclusions of this research and also provides some broader implications of this work and identifies areas where future research could be focussed.

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Chapter 2: Literature Review

2.1 Purpose and structure of literature review

The purpose of this literature review is to overview the contribution roots under pasture systems have on the potential for increasing stable soil carbon (C) (Figure 1). First, a brief overview of the global C cycle, soil C and management strategies for C sequestration will be discussed. The current understanding of the role plant roots play in the C input to soil is then discussed with regard to root mass, root turnover and rhizodeposition. Lastly, an overview of the methods used to study root systems and the inputs of C to soil from plants is presented. There is a specific focus on pasture systems in this review because understanding the root dynamics under these systems is vital in order improve strategies to increase C sequestration and reduce CO_2 emissions.

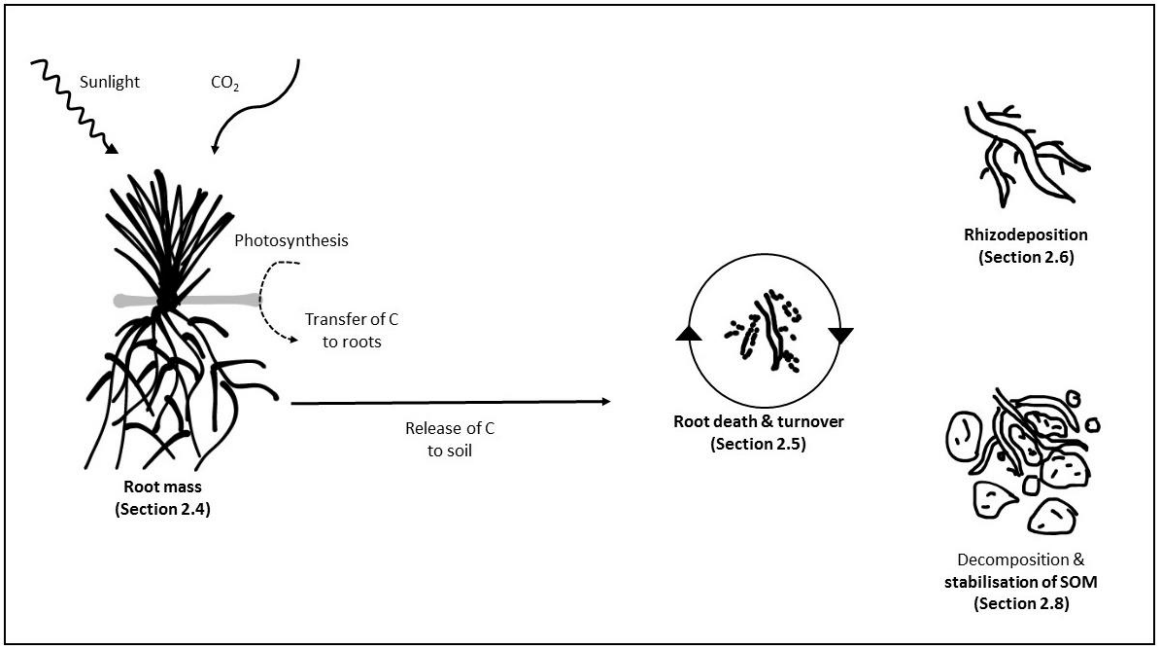

Figure 1 Conceptual diagram of the C flow and C inputs to soil from plant roots. Sections to be discussed are presented under relevant headings.

2.2 The global C cycle and soil C

The global C cycle can be divided into three main reservoirs that control the cycling of C on the earth: oceanic, atmospheric and terrestrial (Batjes 1996). Of these three reservoirs, the oceans contain the largest pool of C with 38000 Pg C, followed by terrestrial ecosystems (C in soil and vegetation) with 2860 Pg C, and then atmosphere with 760 Pg C (Lal 2004). The pool of C within the terrestrial reservoir is much smaller than in the oceans, but is considerably larger than that of the atmosphere. Carbon enters the terrestrial system from the atmosphere through plant photosynthesis and largely is returned to the atmosphere through respiration (microbial, plant and animal) and decomposition of organic matter (Lal 2004).

The balance of C within terrestrial ecosystems can be altered through the direct impact of anthropogenic activities which release greenhouse gases (GHG) to the atmosphere including burning of fossil fuel (Batjes 1996). Anthropogenic activities contributing to the release of GHG's include deforestation, land use change, biomass burning, soil erosion, and environmental pollution (Batjes 1996; Lal 2004). Agricultural activities are a large contributor to GHG emissions globally due to the large land area under which agriculture is carried out on. Consequently, changes in management in anthropogenic activities (mainly agriculture) can change the direction of the C flow to or from the soil.

Agricultural land covers about 40% of the global land surface (Foley et al. 2005; Ramankutty et al. 2008; Suttie et al. 2005), and is dominated by managed grazed pastures with approximately 25% of the land surface used for grazing (Asner et al. 2004). Temperate grasslands contain approximately 18% of the global soil C

stocks and are a substantial store of C (Burke et al. 1997). The C inputs into these agricultural soils can be divided into two major sources: aboveground (stems, leaves, shoots) and belowground (roots and their exudates) (Bolinder et al. 2002). Outputs or losses of C from soils are primarily through microbial respiration of soil organic matter (SOM) but can also be lost from soil through soil erosion and leaching of dissolved organic C (Dawson and Smith 2007) though the ultimate fate of leached and eroded C is not entirely clear.

Efforts have been made to understand the C balance of grazed pasture systems globally, with grazing and management having variable effects on the soil C balance (McSherry and Ritchie 2013). Factors such as soil texture, mean annual precipitation, grass type and grazing intensity explained a large amount of the variation in the grazing effects on soil C stocks with an increase in grazing intensity typically reducing soil C contents (McSherry and Ritchie 2013). The amount of C stored in the aboveground biomass in these grassland systems is considered to be around 10% of the total organic C pool (above and belowground), with the majority of C stored belowground in roots and SOM (Burke et al. 1997). Therefore, understanding the C balance and flows of C within these pasture systems requires accurate assessments of the belowground biomass and turnover. This understanding of belowground input is particularly important in assessing the potential of these pasture systems to increase C sequestration for mitigation of greenhouse gas emissions.

2.2.1 Soil Management Strategies to increase soil C

Soil can act as either a source or sink of atmospheric CO_2 depending on the management, land use, vegetation type or water resources (Lal 2009). When soil acts as a sink, CO_2 is sequestered from the atmosphere mitigating some of the greenhouse gas emissions (Smith 2008).

Soil organic matter (SOM) is primarily a product of plant biomass production (Paterson et al. 2009), whereby photosynthetic CO_2 is converted into above and belowground biomass and then potentially to soil C (Lal 2004). Increasing the SOM pool has been highlighted as an important strategy for enhancing C sequestration (Lal 2004; 2009). Approaches for increasing SOM have been suggested through a number of options and can be simplified through two main conceptual pathways: i) increasing C inputs, and ii) decreasing C losses (Lal 2009).

Methods for reducing losses of C have been focussed on management practices that decrease organic matter decomposition, leaching, and erosion (Lal 2009). This review will focus on approaches for increasing C inputs into soil. Therefore, management options that decrease losses of SOM will not be further discussed but a summary of these management options are discussed in Dawson and Smith (2007), Lal (2009), and Smith (2008).

Increasing the inputs of C to soils under agriculture rely on either growing more biomass in-situ, or recycling biomass produced during the agricultural activity (Lal 2009). Examples of management practices that increase either aboveground

or belowground carbon inputs are: conversion of arable land to grassland or forest, re-vegetation of degraded land, addition of organic materials to soil, conversion of arable cropping to reduced-tillage systems, increasing crop yield through fertiliser applications, stabilization of carbon in the subsoil and use of biochar (Carter and Gregorich 2010; Lal 2009; Powlson et al. 2011). Management strategies that promote root growth are also considered important as root (belowground) carbon has been shown to have a longer residence time in soil than shoot (aboveground) carbon (Rasse et al. 2005). Therefore, many strategies for increasing inputs of biomass to soil explore the use of plants with greater root biomass or greater rooting depth (Kell 2011; Kell 2012; Lal 2009).

It has been suggested that an introduction of deep-rooting vegetation has the potential to increase soil C deeper in the soil horizon (Carter and Gregorich 2010; Kell 2011). The concentration of soil C decreases lower in the soil profile (Batjes 1996) where there is a greater C saturation deficit in New Zealand subsoils (Beare et al. 2014). The saturation deficit refers to the difference between the saturation capacity and the existing soil C concentration in the clay and fine silt fraction of soil (Beare et al. 2014). Therefore, the introduction of deeper rooting systems could result in greater C inputs through greater root mass, turnover and rhizodeposition where there is the greatest potential for additional C storage in subsoils.

Historically, sustaining soil C levels has been thought of in terms of the amount of aboveground residue that needs to be returned to soil annually (Larson et al. 1972; Rasmussen et al. 1980; Rasse et al. 2005). Long term studies on the contribution

of these residues to soil C suggest that they may actually have a limited impact on SOM levels compared to the contribution of root systems (Rasse et al. 2005). As a result, there has been a shift of focus onto the contribution of root systems to soil C. These contributions include root mass, root turnover and rhizodeposition. However, the contributions of roots to soil C under agricultural systems need to be better evaluated and understood to devise suitable management strategies that maximise the carbon storage potential throughout the root-zone.

2.3 The contribution of roots to SOM

Plant roots contribute a considerable proportion of the belowground inputs of C to soil through root turnover and rhizodeposition (Cheng and Gershenson 2007; Jones and Donnelly 2004; Kögel-Knabner 2002). Obtaining a comprehensive understanding of root inputs and the contribution to soil carbon, particularly in grazed pasture systems is difficult because of limited techniques that are suitable to follow C through the plant-root-soil system (Jones and Donnelly 2004). However, this field continues to build interest particularly in its potential applicability in increasing soil carbon and carbon sequestration, and isotope labelling of C has increasingly been used to study the C flow to SOM from plants (Kong and Six 2010; Kuzyakov and Domanski 2000).

Root turnover is broadly defined as the annual fraction of a root system (biomass) that is produced and then lost through root death (Gill et al. 2002). Rhizodeposition is defined as the release of organic compounds from living roots (Nguyen 2003). Both of these processes can promote C storage following stabilisation within soil as discussed in sections 2.6-2.8.

2.4 Root Mass

There are relatively few studies that have quantified root biomass of grazed pasture systems such as those in New Zealand (Crush et al. 2005; Dodd and Mackay 2011b; Matthew 1996; Wedderburn et al. 2010). Studies investigating root mass in New Zealand pastures, have largely been focused on perennial ryegrass cultivars and been carried out in glasshouse studies (Crush et al. 2005; Wedderburn et al. 2010), with fewer reported field studies (Crush et al. 2005; Dodd and Mackay 2011b; McKenzie et al. 1990; Saggar and Hedley 2001; Wedderburn et al. 2010) and both approaches show substantial variation with root biomass ranging between 800–24000 kg DM ha^{-1} in about the top 20 cm of soil (Table 1).

Table 1 Root mass (kg DM ha^{-1}) values for various pasture and grassland systems in New Zealand and globally

Pasture	Location	Depth (mm)	Root Biomass (kg DM ha^{-1})	Reference
Ryegrass-clover	New Zealand	0-120	800 – 2400	Dodd and Mackay (2011b)
Ryegrass-clover	New Zealand	0-200	5900 (Irrigated) 7700 (dryland)	Metherell (2003)
Ryegrass	New Zealand	0-200	463 - 869	Popay and Crush (2010)
Ryegrass-clover	New Zealand	0-200	4700 – 6980	Stewart and Metherell (1999)
Tussock			2680 – 6830	
Pasture species (multiple)	New Zealand	0-100	13670 - 24060	Saggar et al. (1997)
Ryegrass-clover	New Zealand	0-100	11330 - 13310	Saggar et al. (1999)
Pasture	New Zealand	0-100	2730 - 3960	Scott et al. (2012)
Ryegrass-clover	New Zealand	0-250	2000 - 4000	Matthew (1996)
Lucerne-ryegrass	New Zealand	0-300	23000 9000	McKenzie et al. (1990)
Ryegrass-clover				
Pasture	Argentina	0-200	7519 – 14950	Pucheta et al. (2004)
(Grazed)			9368 – 12588	
(ungrazed)				
Lucerne	Uruguay	0-200	900	Gentile et al. (2003)
Chicory		0-300	500	
Pasture	Denmark	0-210	14550 - 20320	Rasmussen et al. (2010)
Ryegrass	Canada	0-300	5530 – 15990	Bolinder et al. (2002)
Lucerne			4280 – 7580	

2.4.1 Depth distribution

Generally, the root distribution of plants will differ depending on the soil environment, water and nutrient resources and competition between plants (de Kroon et al. 2012). A global average of all ecosystems showed that approximately 75% of root mass was found in the top 40 cm of soil (Jackson et al. 1996; Schenk and Jackson 2002). In contrast, the roots of temperate grasslands show shallower rooting depths with 80-90% of root mass found in the top 30 cm of soil (de Kroon et al. 2012; Jackson et al. 1996). Pasture species, in particular, are typically shallow rooting species, for example ryegrass, has about 80% of its root mass in the top 15 cm of soil (Bolinder et al. 2002; Crush et al. 2005). Evans (1978) showed that between 59-81% of roots were found in the top 20 cm of soil for species such as ryegrass, clover, and lucerne grown in New Zealand. Crush et al. (2005) also measured root distributions of a range of forage grasses in New Zealand and the majority of root mass was in the top 30 cm of soil.

Root biomass and rooting depth are also dependent on the species of plant, which in turn effect the carbon storage potential of a soil (Steinbeiss et al. 2008). It is known that there is variation in rooting depth between both plant species and plant cultivars and it has been suggested that plant breeding may allow for greater rooting depth and potentially greater C sequestration in soil (Kell 2011).

2.4.2 Control by nutrient availability and irrigation

It was thought that enhanced nutrient availability would decrease the need for root growth in accordance to the resource balance hypothesis of Bloom et al. (1985). This resource balance hypothesis relates to plants adjusting their growth and life cycle in response to the acquisition of resources such that, if resources are

available (such as through fertiliser use) a plant would need to expend less energy to produce roots for nutrient acquisition (Bloom et al. 1985). Therefore, Dodd and Mackay (2011b) postulated that higher nutrient availability would result in a lower root mass. They made measurements of root mass of perennial ryegrass (*Lolium perenne* L.) and white clover (*Trifolium repens L.*) (referred to as ryegrass-clover) pasture grown under different fertiliser regimes at regular intervals throughout a year. Root mass ranged from approximately 700 – 2400 kg DM ha^{-1} with no difference in root mass between moderate (Olsen P = 24, N = 0 kgN ha^{-1}) and high (Olsen P = 49, N = 400 kgN ha^{-1}) soil fertilities except for two measurement times during spring where the moderate fertility treatment had a greater root mass. Therefore, fertility differences were not enough to drive root exploration in soil and thus root mass was unchanged. However, Saggar et al. (1997) demonstrated greater root mass under high fertility hill country pasture (24000 kg ha^{-1}) compared to low fertility pasture (13670 kg ha^{-1}) during an isotope labelling experiment investigating the partitioning of C in grazed hill country pastures. The high fertility pasture generally received greater amounts of superphosphate fertiliser and had pasture species including perennial ryegrass, poa (*Poa* spp. L.), cocksfoot (*Dactylis glomerata* L.), white clover and other legumes (*T. dubium* Sibth., *T. subterraneum* L.). The low fertility pasture had less superphosphate applied and was composed of a different set of pasture species including brown top (*Agrostis capillaris* L.), sweet vernal (*Anthoxanthum odoratum* L.), crested dogstail (*Cynosurus cristatus* L.) and chewing fescue (*Festuca rubra* L.) (Saggar et al. 1997). Consequently, the species of pasture largely changed between high and low fertility pasture and it is difficult to draw

firm conclusions whether root mass was dependent on nutrient availability or whether change in species was a dominant controller of root mass.

Stewart and Metherell (1999) measured root mass under ryegrass-clover pasture with and without irrigation and superphosphate fertiliser use. Generally there was lower root mass under both irrigated (5090 kg DM ha^{-1}) and superphosphate (4700 kg DM ha^{-1}) treatments compared to when no irrigation (6980 kg DM ha^{-1}) or fertiliser (5890 kg DM ha^{-1}) was used.

2.4.3 Increased species richness

An increase in plant species richness has been shown to increase root mass (Mueller et al. 2013; Tilman et al. 1996) and rooting depth (Mueller et al. 2013). Therefore, increasing root mass through increasing plant diversity has been suggested as one approach to increase carbon storage potential (Steinbeiss et al. 2008). However, the greatest potential appeared to be when the species richness increased from a mono-culture (1 species) to having 2-4 species (Steinbeiss et al. 2008) and many grazed pasture systems often contain several species, e.g. ryegrass-clover.

In New Zealand, pasture species such as ryegrass and clover, are typically shallow rooting species (Crush et al. 2005; Matthew 1996) with a large proportion of root mass in the top 30 cm of soil. While these ryegrass and clover pastures can technically be considered as a mixed pasture (2 species) the number of species within the pastures is low compared to a typical mixed pasture sward which may contain a combination of ryegrass, clover, chicory (*Cichorium intybus* L.), lucerne (*Medicago sativa* L.), plantain (*Plantago lanceolata* L.) and other species.

Recently, in New Zealand, these more diverse pastures have received interest in their potential tolerance to drought (Gerrish 2001; Woodward et al. 2013) due to a perceived greater rooting depth. Furthermore, modelling has indicated that root systems with a high root density and a vigorous taproot growth are likely to maximise nitrate capture (Dunbabin et al. 2003) and may have potential to reduce N leaching (Woodward et al. 2013). However, there are few data on the root mass of these more diverse pastures particularly in permanently grazed systems such as those used in New Zealand.

There is some evidence for differences in root mass in mixtures of lucerne and ryegrass, compared to ryegrass-clover pastures. McKenzie et al. (1990) measured root mass under two lucerne-grass mixtures in Canterbury during an investigation of water use of these pastures. They reported that lucerne and lucerne –grass pastures had greater root mass than ryegrass and ryegrass-clover pastures and that the majority of extra root mass was found in the top 20-30 cm of soil. However, mixtures of lucerne and prairie grass resulted in lower root mass compared to either lucerne or prairie grass monocultures due to competition of water resources and prairie grass shading of lucerne during establishment (McKenzie et al. 1990). Saggar et al. (1997) also measured root mass on what could be defined ‘mixed’ or more diverse pastures with a range of pasture species within the high and low fertility hill country pastures as described above. Both these two studies reported a wide range of root mass measurements under pastures that are mixed, although there was no data on root mass of specifically sown mixed pastures like those reported in Woodward et al. (2013).

More broadly, Adair et al. (2009) reported that increasing species diversity in grasslands increased total belowground carbon allocation and that standing root mass positively influenced the total belowground carbon allocation. Furthermore, mixtures of species may cause the root growth of certain species to be stimulated compared to monocultures which can result in belowground over-yielding (de Kroon et al. 2012). Over-yielding would result in increased root mass, which in turn would have significant implications for C sequestration.

Species may also alter their depth distribution of roots in diverse communities in response to density and presence of nearby roots (de Kroon et al. 2012; Mommer et al. 2010). This adjustment in rooting depth by species resulting in higher deep rooting proportions, was mainly observed in communities with more than 8 species (Mueller et al. 2013). However, communities containing less than 8 species typically displayed rooting patterns similar to the monocultures of those species (Mueller et al. 2013) and thus differences in root mass might not be observed in relatively small increases in diversity.

Diverse communities which can coexist with limited competition between species may also do so due to niche complementarity (Hooper 1998; Loreau and Hector 2001). Differences in rooting depth or root architecture allow species to co-exist with minimal competition for resources as their root systems can access different niches (Schenk 2006). Plasticity in plant phenology results in genetically identical individuals having different phenotypes, and as a result, root systems can differ considerably within species due to environmental conditions (Callaway et al. 2003). This plasticity of roots may contribute to this co-existence between species

with plants adapting to certain environmental conditions by altering their root architectures to suit (Callaway et al. 2003; de Kroon et al. 2012).

It is clear that there is a large variation in the amount of root mass under pasture systems both globally and in New Zealand and our understanding of the root dynamics is still limited. Root systems can differ within species or pasture type depending on environmental conditions such as fertility and irrigation. Therefore, there is an opportunity to extend the understanding of root mass under grazed pastures in New Zealand and particularly that of moderately diverse pastures.

2.5 Root turnover

Root turnover has been defined a number of ways in the literature and summarised by Norby and Jackson (2000). Root turnover was defined as the proportion of root mass that is produced and dies annually using a systems approach. In contrast, Gill and Jackson (2000) defined root turnover as the annual belowground production divided by belowground standing crop and reported in units of y^{-1} (for example, kg ha $^{-1}$ y^{-1}/ kg ha^{-1} = y^{-1}). A value of 1.0 y^{-1} would be equivalent to a root turnover of 1 year (or 365 days) and values < 1.0 y^{-1} would have turnovers slower than 1 year, while values > 1.0 y^{-1} would be faster than 1 year. Tingey et al. (2000) refer to a turnover index as the inverse mean residence time or turnover (y^{-1}) regardless of method used to calculate turnover time. Other methods to determine root turnover have used isotope pulse labelling, and refer to root turnover as the time (days to years) when there is zero labelled isotope (^{13}C) remaining (Scott et al. 2012). Root turnover was measured using a similar isotope method but using the exponential loss of ^{14}C and reported values as days (Saggar and Hedley 2001). Turnover times of pasture species in New Zealand were also

reported in days following minirhizotron measurements (Gibbs and Reid 1992; Reid and Crush 2013).

There is clearly a range of terms and methods used to determine root turnover which is likely to contribute to the wide variety of reported values (Table 2). However, all these methods provide an estimate for the quantity of roots which are produced and die annually, which all reflect the lifespan of roots (Eissenstat and Yanai 2002). Inconsistencies in how root dynamics are reported affect our ability to report root life span and hence turnover times effectively (Eissenstat and Yanai 2002). For the purpose of this review, all units of root turnover will be converted to days regardless of the method used to provide an estimate of root lifespan and hence turnover, though caution is advised against a comprehensive comparison between root turnovers unless a common method was used. The mixed reporting of root turnover is one issue that makes comparison between systems and methods difficult that needs resolving.

Gill and Jackson (2000) summarised root turnover (defined previously) from approximately 190 studies from a wide range of ecosystems and climates. Generally, root turnover increased at higher mean annual temperatures for grasslands and forests and decreased with latitude so that tropical systems had a faster root turnover than higher-latitude sites. Root turnover also varied between plant groups with forest systems typically having slower turnovers than shrublands, grasslands and wetlands. Although a global pattern of root turnover was observed with respect to plant group and climate, they noted that these patterns do not necessarily enable predictions of root turnover with interannual

climate variation at a particular site because of relatively high uncertainties (Gill and Jackson 2000).

There has not been a comprehensive summary of root turnover in grazed pasture or grassland systems. However, a summary of root turnover under some pasture and grassland systems in New Zealand and globally are presented in Table 2. Leifeld et al. (2015) recently measured root turnover in European alpine grassland systems using a ^{14}C radiocarbon method outlined by Gaudinski et al. (2001). This study demonstrated that turnover was influenced by mean annual temperature, but also that root turnover was increased under high and moderate intensity management systems in comparison to low intensity or natural systems. Intensity was defined as the magnitude of biomass removal between high (2-4 cuts per year or >2 livestock units and grazing for most of the season), moderate (1-2 cuts per year, 1-2 livestock units, grazing up to 2 months per year) and low systems (occasional grazing, <1 livestock unit or unmanaged). The results of turnover in these alpine grasslands ranged from 365 days to 6083 days (0.06 – 1.0 y^{-1}), with high or moderate intensity systems having an average root turnover of 760 days (0.48 y^{-1}) and low intensity systems 2607 days (0.14 y^{-1}).

Scott et al. (2012) calculated root turnover using ^{13}C data from Stewart and Metherell (1999) in a ryegrass-clover pasture at the long-term Winchmore trial in Canterbury, New Zealand. Root turnover was generally faster under irrigation and superphosphate use at about 438 days (1.3 y, 0.8 y^{-1}) compared to no irrigation or superphosphate with a turnover of 694 days (1.9 y, 0.5 y^{-1}). However, the greatest difference in root turnover was observed in autumn when the irrigated and

fertilised pastures had much faster root turnover (402 days,1.1 y or 0.9 y^{-1}) compared to pastures receiving no irrigation or superphosphate (767 days, 2.1 y or 0.5 y^{-1}) (Scott et al. 2012). The difference in root turnover with or without irrigation and fertiliser use may in part reflect the root lifespan in response to nutrients and water and the cost of building these roots (Eissenstat and Yanai 2002). There are conflicting studies on whether root lifespan and root turnover, is longer or shorter with nutrient and water availability (Eissenstat and Yanai 2002).

Saggar and Hedley (2001), following isotope labelling of pastures with $^{14}CO_2$, reported root turnover times of approximately 400 days (1.1 y, 0.9 y^{-1}) under a dairy pasture in Manawatu, New Zealand. During this study between 1 % and 8 % of the labelled isotope remained one year after labelling. Saggar and Hedley (2001) also reported a half-life of roots (loss of 50% of label) as the highest in autumn (111 days, 3.3 y^{-1}) and fastest in spring (64 days, 5.7 y^{-1}). Thus, root turnover appeared greatest in spring and slowest in autumn.

Gibbs and Reid (1992) measured root turnover using a field rhizotron method between ryegrass and wheat during a trial at Lincoln, New Zealand. Ryegrass roots displayed a root turnover (life span) of 46 days (7.9 y^{-1}) compared to wheat roots with 59 days (6.2 y^{-1}), with root longevity greater during winter and fastest during spring and summer. However, difficulty in distinguishing between live and dead roots in this method produced considerable uncertainty in the estimates. Reid and Crush (2013) also measured root turnover in ryegrass in a small plot study using a rhizotron method and calculated a scaled root turnover of 44 days (8.3 y^{-1}). These short root life spans would equate to the root system turning over

approximately 8 times per year, which is fast and perhaps unreasonable in comparison to other studies on New Zealand pastures. These rhizotron and minirhizotron methods have limitations in creating an unnatural environment where root production and lifespan may be altered around the transparent window where the camera is located (Eissenstat and Yanai 2002).

Gill et al. (2002) measured root longevity with minirhizotrons under a perennial bunchgrass in the shortgrass steppe, Colorado. In this study, root longevity was correlated to root diameter with roots >4 mm diameter having longer longevity (320 days, 1.1 y^{-1}) than fine roots <2 mm (180 days, 2.0 y^{-1}). Gill and Jackson (2000) also reported the diameter class of forest roots influenced root turnover, with fine roots (<1 mm diameter) having a turnover time of 304 days (1.2 y^{-1}) and roots in the 0-10 mm class having an average turnover of 3650 days (0.1 y^{-1}). Root production and mortality was also highest in the top 20 cm of soil and decreased with soil depth (Gill et al. 2002). However, the method by which root turnover was calculated gave different values. Root turnover was calculated by either dividing root production or root longevity, with maximum root length density giving calculated turnover times of approximately 424 days (0.9 y^{-1}) using root production data and 1043 days (0.4 y^{-1}) using root longevity data. In a similar experiment, root lifespan was measured under different grass species (ryegrass, *Arrhenatherum elatius* L., *Molinia caerulea* L., *Nardus stricta* L.) with differing nutrient availability (Van der Krift and Berendse 2002). Ryegrass displayed the fastest average root lifespan (98 days), followed by *A. elatius* (280 days), *M. caerulea* (371 days) with *N. stricta* having the slowest (406 days). Habitats that were more fertile (high nutrient availability) had significantly shorter root

lifespans than low fertile habitats (Van der Krift and Berendse 2002). Therefore, turnover would be expected to be faster under more fertile or higher nutrient availability compared less fertile systems as was also observed in the Winchmore trial (Scott et al. 2012).

Table 2 Summary of root turnover of pasture and grassland systems. Root turnover is a measure of the time (days) for the root system to be produced and die.

System	Location	Root turnover (days)	Method	Reference
Grassland (Alpine)	Europe	365 - 6083	^{14}C radiocarbon	Leifeld et al. (2015)
Pasture	New Zealand	402 - 803	^{13}C isotope labelling	Scott et al. (2012)
Pasture	New Zealand	93-160	^{14}C isotope labelling	Saggar and Hedley (2001)
Pasture	New Zealand	46	Minirhizotron	Gibbs and Reid (1992)
Crop		59		
Pasture	New Zealand	44	Minirhizotron	Reid and Crush (2013)
Grassland	Co, USA	438 – 1059	Minirhizotron	Gill et al. (2002)
Grass species	Small plot trial, EU	98 - 406	Minirhizotron	Van der Krift and Berendse (2002)

There is clearly a range of terms used in respect to root turnover, these include root turnover, root longevity or root lifespan, which arguably all relate to one another as they are measures of root life/mortality. However, it is also apparent there are differences in root turnover rates depending on the selected method or methods of calculation. Thus, it is difficult and perhaps unreasonable to compare turnover times using different methods. Despite these differences within studies, root turnover was generally influenced by factors such as plant species, mean annual temperature, root diameter, soil fertility and management, and hence it remains difficult to predict root turnover locally from first principles. Therefore,

there is still a need to extend our knowledge of root turnover under pasture systems that are permanently grazed in New Zealand to understand how these root systems may contribute to the C input to soil. While there are some measurements of root turnover in New Zealand based on ryegrass-clover pasture swards, there are no measurements on the root turnover of mixed pastures or moderately diverse pastures. Measurements of these pastures are needed in order to better estimate the potential for C storage and C sequestration under these systems.

2.6 Rhizodeposition and the rhizosphere

The root-soil interface, also defined as the rhizosphere, is considered the site of greatest activity in the soil matrix (Bertin et al. 2003). As stated previously, rhizodeposition is defined as the release of organic compounds from living roots (Kuzyakov and Domanski 2000; Nguyen 2003). Rhizodeposition involves a number of processes such as exudation, secretion, sloughing and lysis of cells and root tissue senescence (Rees et al. 2005). There is limited information on the composition and dynamics of root produced carbon-containing compounds and as a result, the importance of rhizodeposition (root exudates) for the production and stabilisation of soil organic matter is largely unknown (Bertin et al. 2003).

Root exudates primarily consist of organic compounds formed during photosynthesis but also involve the release of ions, oxygen and water into the surrounding soil (Bertin et al. 2003). Root exudates can either lead to net accumulation or consumption of carbon in soil (Rasse et al. 2005) depending on the form and source of the deposits (Johnson et al. 2006). Most exudates produced by roots are rapidly consumed by microbes in the soil (Kögel-Knabner 2002) but

subsequent cycling of dead microbial biomass is considered a significant source of SOM (Miltner et al. 2012). These root exudates can be labile and increase the decomposition rate of existing SOM through priming (Rasse et al. 2005). Priming refers to the change in the decomposition rate of existing SOM with the addition of fresh organic matter which stimulates microbial activity (Fontaine et al. 2003). Plant ecophysiology (plant genetics and physiology, and environment) influences the flux of C from roots which is deposited through rhizodeposition and also the size and morphology of the root system (Nguyen 2003).

Therefore, rhizodeposition is likely to differ between species and also between different environments, though the amount of rhizodeposition that occurs is difficult to measure and is poorly understood (Nguyen 2003). However, estimates of rhizodeposition have been calculated by assuming it to be equivalent to the amount of C in root mass by Rasse et al. (2005). Bolinder et al. (2007) used a similar approach but estimated the C input from rhizodeposition to be 65% of the C in root mass. Studies involving the partitioning and allocation of ryegrass, measured approximately 2800 kg C ha^{-1} input to soil during plant development with root mass (1400 kg C ha^{-1}) and root exudates (1400 kg C ha^{-1}) accounting for roughly half of this input respectively (Kuzyakov et al. 2001).

Rhizodeposition is likely to be a large contributor to the C input to soil, but remains poorly known due to a lack of appropriate methodologies to quantify its magnitude.

2.7 Contribution of roots to SOM

At a broad scale, the first step of C entering the soil from roots involves the transfer of photosynthetically fixed C to roots, and is commonly termed

translocation, but also may be referred to as C allocation (Kuzyakov and Domanski 2000; Saggar et al. 1997). Numerous studies have measured the C allocation between aboveground (shoot) and belowground (root) biomass, though most of these studies have been conducted in wheat and barley (Kuzyakov and Domanski 2000) with fewer studies on the C allocation of pastures in New Zealand (Saggar and Hedley 2001; Saggar et al. 1997; Saggar et al. 1999; Stewart and Metherell 1999). The allocation and translocation of carbon belowground differs depending on the type of system and management with pasture-based systems typically translocating 1.5 – 2 times more carbon than cereal or cropping based systems (Kuzyakov and Domanski 2000). This difference was suggested to be due to pastures and grasslands typically composed of perennial species and usually have a more developed root system, while cereals and crops are usually annual species and allocate a greater proportion of C above-ground (Kuzyakov and Domanski 2000).

Plant roots and rhizodeposits (exudates from roots) are both important sources of carbon for the production of soil organic carbon (Molina et al. 2001) and are thought to contribute more carbon to organic matter than above-ground residues (Lorenz and Lal 2005). Root derived carbon has been suggested to contribute 1.5 to more than 3 times the carbon to soil than shoot derived carbon (Johnson et al. 2006). Root turnover is thought to dominate the carbon inputs of grassland soils compared to other processes such as rhizodeposition, although they both contribute to carbon storage (Soussana et al. 2004). Rasse et al. (2005) summarised a range of studies and reported that roots had residence times in soil

of approximately 2.4 times those of shoots. However, these authors acknowledged that data was sparse.

There have been few specific studies, but Puget and Drinkwater (2001) found greater contribution of root material to soil compared to shoot material in hairy vetch. Denef and Six (2006) also measured a greater potential of root derived C to be stabilised in soil compared to residue (aboveground) derived C during an incubation based experiment. In a long term experiment in Sweden, Kätterer et al. (2011) also noted a greater contribution of root derived C to SOM (2.3 times) compared to aboveground residues.

Plant roots transfer approximately 50% of carbon assimilated during photosynthesis belowground through either root growth or rhizodeposition (Rees et al. 2005). Studies investigating the partitioning of belowground carbon have been conducted in cropping systems, such as cereal and maize crops (Kuzyakov and Domanski 2000) whereas pasture and grazing systems have largely focussed on ryegrass (Butler et al. 2004; Kuzyakov et al. 2001). During the course of one growing season, Rees et al. (2005) estimated the input of root derived carbon ranged between 0.1 and 2.8 t C ha^{-1} for 8 different cropping/pasture species with the highest input under perennial ryegrass. Christensen et al. (2009) also stated the conversion of arable soils to grassland can sequester an extra 0.3-1.9 t C ha^{-1}, and that root residues were responsible for the increase in soil carbon under these grasslands.

Land use and management are also thought to influence the C input to soil. There have been few studies carried out in New Zealand pastures investigating the carbon allocation of pasture systems in relation to fertility status, stocking rate and hill slope (Saggar and Hedley 2001; Saggar et al. 1997; Saggar et al. 1999; Stewart and Metherell 1999). There was greater proportion of C allocation belowground in low fertility pasture (34%) compared to high fertility pasture (26 %), though the high fertility pasture had greater amounts of C allocated to the roots (4432 kg C ha^{-1} high fertility, 2451 kg C ha^{-1} low fertility) (Saggar et al. 1997). Pasture allocated a greater proportion (though lower net input) of C belowground under steep slope classes (4490 kg C ha^{-1} to roots, 555 kg C ha^{-1} to soil) compared to low slope classes (5510 kg C ha^{-1} roots, 930 kg C ha^{-1} soil) in hill country ryegrass-clover pasture (Saggar et al. 1999). On a high fertility dairy pasture, plants generally allocated about 6820 kg C ha^{-1} to roots and 1320 kg C ha^{-1} to soil belowground annually (Saggar and Hedley 2001).

Stewart and Metherell (1999) measured C allocation to roots using a $^{13}CO_2$ pulse labelling experiment in pasture with and without irrigation and superphosphate fertiliser. The allocation of C to roots was greater in the treatment with no superphosphate (50 %) compared to superphosphate use (41 %). Irrigation had no effect on the C allocation belowground. Allocation to roots also varied with season with the greatest C allocation to roots in autumn (51 %) and lowest in spring (39 %). Stewart and Metherell (1999) also measured the allocation of C in a grazed semi-tussock and tussock pasture and found that the allocation of C to roots generally increased with increased stocking rate and grazing frequency.

Christensen et al. (2009) showed that an introduction of pasture to systems previously under cropping resulted in an increase in soil C after conversion. However, increasing the fertility or nutrient availability of a system can also decrease the proportion of C allocation belowground as Saggar et al. (1997) and Stewart and Metherell (1999) measured. This supports the resource balance hypothesis of Bloom et al. (1985) where increased resource allocation to roots would be expected with insufficient nutrient availability. However, total production of the system increased with higher fertility in Saggar et al. (1997), so while the proportion of allocation may be smaller in the higher fertility system, the total allocation of C was still greater so it could be argued that the actual input of C with increased fertility would still be greater.

There is a need to further investigate the contributions of plant roots and shoots to the accumulation of soil organic matter in order to maximise carbon storage in the soil profile (Rasse et al. 2005) and the conversion of residues to SOM has been highlighted as an important research topic (Kätterer et al. 2011).

2.8 Mechanisms and stabilisation of roots on SOM

The ability of a soil to protect organic matter is of great importance when considering soil C sequestration. In order for C to be sequestered it needs to be stabilised and protected from decomposition allowing for longer term storage and gradual soil C accumulation.

A review by Six et al. (2002b) outlines a conceptual model whereby C is stabilised by soil. These SOM protection mechanisms are defined as those whereby C is: i) physically protected from decomposition, ii) associated with fine fractions (silt and clay), or iii) biochemically stabilised through chemically recalcitrant compounds. These mechanisms can be related to different measurable soil fractions which represent pools of C with different residence times or stabilities. Furthermore, physiochemical soil properties define the maximum capacity of these pools to sequester C, suggesting a C saturation limit (Hassink 1997). The C saturation limit results in a finite amount of C able to be stored in soil regardless of increasing C inputs and C saturation of soil appears to follow an asymptotic relationship as outlined by Six et al. (2002b). Based on this C saturation concept, if the soil C content is below this saturation limit, there may be an opportunity to increase soil C by increasing the inputs of C to soil. The difference in the actual soil C content and the saturation point has recently been defined as the C saturation deficit (Stewart et al. 2007).

The conceptual model by Six et al. (2002b) can be related to soil aggregate C dynamics which allow actual measurements of various C pools to be made following a soil fractionation scheme. Various fractionation schemes have been

devised (Del Galdo et al. 2003; Hassink et al. 1997; Six et al. 2002a; Six et al. 2002b; Six et al. 1998), to allow our understanding of management effects on the C stabilisation and saturation of soil. This theory would suggest that once soil C pools are saturated, additional C inputs will not be stabilised in soil. Tisdall and Oades (1982) outlined a soil aggregate hierarchy concept, which was modified by Oades (1984) to suggest microaggregates are formed within macroaggregates which has since formed a large basis of our understanding on C stabilisation and SOM dynamics as reviewed by Six et al. (2004). The dynamics of these soil aggregates are important to the C sequestration and C cycling in soils (Kong et al. 2005; Six et al. 1998).

The turnover of macroaggregates influence the SOM stabilisation by influencing the rate of formation of microaggregates within these macroaggregates (Six et al. 2000). A certain level of macroaggregate turnover is required to have new aggregate formation whereby new unprotected C (usually particulate organic matter or POM) can become protected within aggregates (Six et al. 2004). Furthermore, this protection within aggregates indirectly influences C stabilisation by the access it provides to sites of chemical and physiochemical protection (Balabane and Plante 2004). Most agricultural systems have aggregate turnover rates much faster than the rate of new C input to soil due to management practices that increase aggregate turnover such as disturbance events. This increased aggregate turnover results in a reduced amount of new C being stabilised in soil C pools (Six et al. 2004). However, aggregate turnover is also influenced by factors such as soil fauna and microorganisms, inorganic binding agents and roots. For the purpose of this review, the role of roots will primarily be discussed but the

reader is referred to the review by Six et al. (2004) for further detail on the other factors.

Roots provide an important role on the formation of soil structure as reviewed by Angers and Caron (1998). The penetration of roots influence macropore distribution, alter soil water contents, provide organic compounds which promote soil structure stabilisation through root exudates and stimulate microbial activity which further stabilise soil structure (Angers and Caron 1998). The deposition of C in soil from roots directly and indirectly affect soil structure through either providing compounds which act to cement soil particles into aggregates or stimulate microbial activity which also produce compounds that enhance the formation of microaggregates (Six et al. 2004). Roots also can bind soil particles together by exerting physical forces and localised drying which provide stresses and strains required to form aggregates (Jastrow et al. 1998). The physical enmeshment of roots is such that the root density and the amount of fine roots within a soil may influence the size of aggregate formation, and may even limit the formation of macroaggregates (Jastrow et al. 1998). Root system architecture further influences the uniformity of soil structure stabilisation in the ability to deposit C sources which are utilised in bonding of aggregates (Degens 1997). Systems that have finer roots compared to more coarse roots are suggested to deposit C in a more uniform manner throughout the soil and therefore stabilise more C in aggregates (Degens 1997).

Root activity is also thought to promote SOM stabilisation mechanisms through chemical recalcitrance, physical protection and physio-chemical protection (Rasse

et al. 2005). Chemical recalcitrance is such that specific compounds (such as lignin and suberin) within root tissue cause them to be more resistant to decomposition. Physio-chemical protection and physical protection refer to the protection of C released from roots within aggregates and soil surfaces described above. The fact that roots are thought to enhance these protection mechanisms compared to aboveground plant material is due to the close contact roots have with soil particles. The close contact of roots within the soil also allow protection from metal ions (such as iron and aluminium) by forming organo-mineral complexes though knowledge of these interactions is less well known (Rasse et al. 2005). Increasing the amount of root activity in soil may promote soil aggregate formation, which combined with new inputs of C from roots, may enhance soil C stabilisation mechanisms which would act to increase soil C. Thus, there is a need to further investigate how root dynamics influence the inputs and stabilisation of C into soil.

2.9 Methods used for determining root dynamics and the contribution of root carbon to SOM

While there is clearly a need for investigating the inputs of C to soil through roots, obtaining more information is constrained by appropriate methods. The spatially and temporally complex nature of root systems and difficulties associated with the currently available methods make these systems difficult to study (Bledsoe et al. 1999; Samson and Sinclair 1994). However, information regarding the various parameters of roots such as root biomass, distribution, length and surface area are useful. Root studies can either be non-destructive through the use of minirhizotrons or root windows, or they can be destructive using soil cores or in

growth mesh bags (Rasmussen et al. 2010). Both of these techniques are challenged by the spatial and temporal heterogeneity of root growth in the field. Soil cores are generally considered the most well adapted method for the collective measurement of root biomass, root length, and surface area, although other techniques may be more suitable for a specific parameter (Bledsoe et al. 1999). Root windows, walls and minirhizotrons can be used for the measurement of certain root parameters and cause less disturbance to the rooting environment. However, these techniques also have some disadvantages, in particular that they may cause unusual growing conditions along the edges of these instruments compared to undisturbed soil. For this reason these techniques will not be looked in to in detail and the reader is referred to Bledsoe et al. (1999) for further information.

2.9.1 Soil coring

Soil cores are considered the main method for measuring root biomass through the collection, washing cleaning and weighing of roots. The diameter of the cores is suggested to be at least 5-7 cm with smaller cores susceptible to compaction and can result in extensive severing of roots (Bledsoe et al. 1999). Larger cores are also thought to better sample the spatial heterogeneity of roots in the soil (Bledsoe et al. 1999), though studies on root systems do not use a standard diameter of core. Root biomass (or root mass) is obtained from soil cores by washing soil from roots followed by drying of root material. Root biomass is typically defined as the dry weight of roots per unit area of soil sampled (g cm^{-2}) to a specified depth. Root biomass is often used primarily to understand factors affecting belowground root activities, particularly the distribution of carbon and nutrient acquisition (Bledsoe et al. 1999).

Estimates of root turnover can be made by collecting soil cores through time and measuring the root mass associated with each core (Scurlock et al. 2002). The change in root mass through time is considered as a measure of root production and hence turnover can be estimated. This method of sequential coring through time has advantages of less alteration of root growth than other methods (in-growth cores, rhizotrons) and inexpensive equipment costs (Eissenstat and Yanai 2002). However, this method has problems such as under-estimating the contribution of fine roots as these roots are difficult to fully extract from the soil and likely have a rapid turnover which sequential coring may miss (Eissenstat and Yanai 2002).

2.9.2 Root scanning software

Various software packages coupled to scanning devices (image analysis systems) enable the measurement of root length, root volume, surface area, and diameter of roots extracted from soil. These image analysis systems include WinRHIZO, ROOTEDGE, and Delta-T Scan (Bouma et al. 2000; Himmelbauer 2004) that offer rapid assessment of root characteristics based on complex algorithms. The use of root scanning software allows measurements of various root parameters to occur rapidly and simultaneously which may then be related to processes such as decomposition.

2.9.3 Isotopic methods

Root parameters such as root biomass provide useful information on C mass and distribution but these techniques fall short on determining the rate of C input into soil. Labelling plants with isotopes, such as C isotopes, can provide information on the dynamics and quantity of partitioning (amount of C incorporated) to roots (Saggar et al. 1997).

Three methods of tracing C into the soil from plants are currently used: i) pulse labelling, ii) continuous labelling, and iii) ^{13}C natural abundance (Kuzyakov and Schneckenberger 2004). A summary of the advantages and disadvantages of these three isotopic methods are presented in Table 3. Other methods can also be used for carbon flux measurements in the rhizosphere such as tracing molecular compounds released by roots (Cheng and Gershenson 2007) but are not covered in this review.

Stable isotope notation

Isotopic methods involving the stable C isotopes (^{12}C and ^{13}C) typically use the notation $\delta^{13}C$ which represents the ^{13}C value of a sample relative to the reference standard (PeeDee belemnite, PBD) and is expressed by equation 1:

$$\delta^{13}C = 1000\frac{R_{sample}-R_{standard}}{R_{standard}}\ ‰ \text{ ……..(Eqn 1)}$$

where R_{sample} and $R_{standard}$ equal the ^{13}C:^{12}C abundance ratios of the sample and standard (PDB). Negative values of $\delta^{13}C$ indicate that the ^{13}C is less abundant than the reference standard. These values of $\delta^{13}C$ can be used to calculate a C input from a known source, for example roots into different pools such as soil (Equation 2).

$$C_{input} = (\delta^{13}C_{sample} - \delta^{13}C_{initial}) / (\delta^{13}C_{source} - \delta^{13}C_{initial}) \ldots\ldots\text{(Eqn 2)}$$

where C_{input} is the fraction of new C in the pool of interest, $\delta^{13}C_{source}$ is the $\delta^{13}C$ for the source (e.g. root), $\delta^{13}C_{sample}$ and $\delta^{13}C_{initial}$ are the $\delta^{13}C$ values of the sample at the time of interest and the background value respectively (Staddon 2004).

Pulse labelling

Pulse labelling refers to a method whereby plants are labelled with either ^{11}C, ^{13}C or ^{14}C within transparent chambers for a short time before the isotope is traced through the plant-soil system. This method of labelling can either be carried out as a one-off labelling or over various labelling events. Typically, isotope enrichment is calculated as the difference to the background label within the plant or soil. The enrichment of isotope that ends up in soil C pools or the C respired is considered to be plant derived, and hence this method can be used to determine the C input from plants to soil or organic matter pools (Kuzyakov and Schneckenberger 2004).

However, this method cannot be used to determine C balances of an entire ecosystem due to the short time frame of labelling, nor can it be used to distinguish sources of respired C which contains the label (Cheng and Gershenson 2007). The C assimilated within the plant can only roughly be determined and the allocation of C only refers to the C within the short timeframe that the isotope was labelled (Kuzyakov and Schneckenberger 2004). However, a series of pulses applied to plants over regular intervals have been used to provide reasonable estimates of belowground C input (Kuzyakov and Schneckenberger 2004; Saggar et al. 1997; Saggar et al. 1999). Root turnover has also been estimated using this pulse labelling method in pasture systems (Saggar and Hedley 2001; Scott et al. 2012).

The isotope pulse labelling method (using ^{13}C and ^{14}C) has been used in various studies on C dynamics on soil (Denef and Six 2006; Kong et al. 2011; Kong and Six 2012; Kong and Six 2010; Kuzyakov and Domanski 2000; Staddon et al. 2003) including New Zealand pasture systems measuring the C allocation in the pasture-soil system (Saggar and Hedley 2001; Saggar et al. 1997; Saggar et al. 1999; Stewart and Metherell 1999).

Continuous labelling

Continuous labelling is similar to that of pulse labelling though it differs in the fact that plants are grown within chambers and constantly labelled with isotope usually from the first emergence of shoot growth until the sampling date. While this method is much more expensive and requires special equipment the total amount of C assimilated within the plant is known, allowing a better estimate C of allocation within the plant and C balance in the system (Kuzyakov and Schneckenberger 2004). Studies involving this form of labelling are limited due to the expensive setup of equipment required (Kuzyakov and Domanski 2000).

^{13}C natural abundance

Natural abundance methods are based on the discrimination between ^{13}C and ^{12}C isotopes during assimilation of CO_2 of different photosynthesis types. This discrimination occurs based on the specific enzymes involved in C3 (Rubisco) and C4 (phosphoenol pyruvate carboxylase) plants (Kuzyakov and Domanski 2000). This isotopic discrimination results in SOM which develops under C3 and C4 vegetation having different isotopic signatures with SOM under C3 vegetation typically around -27‰ and C4 around -14‰. This method is useful for measurements of C3 vegetation grown in a soil that had been covered with C4 vegetation or vice versa. The change in the isotopic discrimination causes newly

incorporated C to have a different isotopic signature to the older C. This method is also easy to carry out under field conditions as it requires no equipment. However, the main disadvantage of this method is that it relies on a change in C3-C4 plant-pairs which are uncommon under natural field conditions (Kuzyakov and Domanski 2000). The use of this method in New Zealand is less practical than isotope labelling methods as the majority of pastures are based on perennial ryegrass and white clover which are both C3 plants (MacLeod and Moller 2006). Plants that are C4 are used but intermittently (such as maize) (MacLeod and Moller 2006) and would likely be too short to alter the isotopic signature of soil C.

Table 3 Advantages and disadvantages of various isotopic techniques in studying carbon dynamics of root systems. Adapted from (Cheng and Gershenson 2007).

Method	Advantages	Disadvantages
Pulse labelling	Gives information on C pathways of plant. Label preferentially found in labile C pools.	Unable to do ecosystem C balances. Unable to distinguish between C respired from roots and C resulting from microbial mineralization of root C.
Continuous labelling	Allows for C budgets. Label distributed homogenously through plant. Can estimate C flux through soil microbial biomass.	Expensive and difficult. Does not distinguish between root respiration and rhizosphere decomposition of root material.
Natural abundance	Relatively simple. Does not require radioactive isotope. Can distinguish between soil and plant C decomposition.	Can only distinguish large differences in system due to high level of noise.

2.10 Summary

This review has highlighted that there is a need to further investigate root mass and root turnover under pasture systems in New Zealand. While there are some measurements of root mass and root turnover on ryegrass-clover pastures, there are relatively few field studies. Furthermore, there are no studies on the root dynamics of more diverse pasture swards containing species such as ryegrass, clover, lucerne, chicory and plantain. There is also limited and highly variable data on the root turnover and the C input to soil in these pastures, particularly those of highly productive dairy systems and no data for diverse pastures. Quantification of root mass under pastures commonly grazed by dairy cows and the subsequent root turnover of this root mass would enable a better understanding of the C inputs from roots to soil in these pasture systems. There is limited data available on field studies investigating root mass under ryegrass-clover pastures grazed by dairy cows, and very limited data on root mass in diverse pasture swards. Therefore, investigating whether there are differences between these pasture systems and whether there are seasonal changes in root mass are needed.

Root turnover has been measured in many different systems and has been demonstrated to be influenced by many factors such as plant type, temperature, management, and root diameter. However, while broad generalisations of root turnover can be made at a global scale, there is difficulty in predicting root turnover at smaller scales. Therefore, to better understand root turnover under certain climates or managements, more measurements need to be made. In New Zealand, relatively few studies have been carried out on root turnover under

pasture systems, and even fewer in highly productive dairy systems. Furthermore, no measurements have been made on more diverse pasture systems.

There is also limited information on the inputs of C to soil under these pasture swards that are permanently grazed. Inputs of C and how they might be stabilised in long term soil C pools are important to better understand the C balance of these pastures and to potentially provide strategies to increase C sequestration.

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Chapter 3: Root carbon inputs under moderately diverse sward and conventional ryegrass-clover pasture: implications for soil carbon sequestration

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3.1 Abstract

Background and aims

A strategy to increase soil C under pasture-based systems is to increase the root mass inputs or increase rooting depth of plants. Our objective in this study was to measure the seasonal dynamics of root mass and C inputs under two different pasture types (ryegrass-clover vs moderately diverse) that differ in plant diversity and which are commonly used in New Zealand agriculture.

Methods

This study was carried out on an existing plant diversity field trial containing 6 replicate paddocks of both moderately-diverse and ryegrass-clover pastures. Soil cores (0-100-200-300 mm sections) were collected seasonally across one year and individual root traits assessed from all species.

Results

The moderately diverse pasture had greater root mass (5320-9350 kg ha^{-1}) than the ryegrass-clover pasture (3810-5700 kg ha^{-1}) for all seasons and had greater root mass lower in the soil profile. A secondary objective demonstrated no significant difference in root mass between high and low sugar ryegrass cultivar. Increased root mass results in an estimated increase of C input to the soil of about 1203 kg C ha^{-1} (0-300 mm depth) under the moderately diverse pasture, excluding root exudates. Root trait measurements demonstrated a greater diversity of root traits in the moderately diverse sward compared to the ryegrass-clover pasture.

Conclusions

Moderately diverse pasture systems offer scope to increase soil C under grazed pastures through increased root mass inputs and rooting depth.

Keywords: Grazed pastures, root mass, soil C, moderately diverse pasture, ryegrass-clover

3.2 Introduction

Increasing soil carbon may play a critical role in mitigating greenhouse gas emissions. Soils contain the largest amount of carbon (C) in the terrestrial ecosystem with roughly twice the amount of C stored in the soil as found in the atmosphere (Batjes 1996; Powlson et al. 2011) and three times as much as in vegetation (Smith 2008). Consequently, relatively small changes to the soil C pool can influence the global C balance. Changes in agricultural management practices that may increase the soil C pool are being considered as an approach for sequestering C from the atmosphere and mitigating agricultural C emissions (Smith 2004).

Agricultural land covers approximately 40% of the global land surface (Smith et al. 2008) of which 70% is grasslands or grazed land (Soussana and Lüscher 2007). The soil C pool of these agricultural ecosystems is dependent on the balance between relatively high rates of C inputs and outputs (Paustian et al. 2000). Inputs to the soil C pool in grazed pasture systems are primarily through above and belowground plant biomass turnover and through returns of animal excreta (derived from plant production), while outputs are largely through heterotrophic respiration. Imported feed and manure can also act as a source of C into these grazed systems. Increasing the inputs of C to a soil is hypothesised to increase the soil C pool up until a C saturation point (Six et al. 2002b). Soil C saturation is a soil's maximum ability to store C irrespective of changes in inputs (or when inputs are non-limiting) (Six et al. 2002b). Surface soil layers are more likely to be near the C saturation point, given higher inputs. Deeper soil layers have less C input and thus the C content of soils usually declines with soil depth (Jobbágy and Jackson 2000). Hence, lower layers in the soil profile are likely to be further from C saturation. Even if surface soils are close to saturation, there is an opportunity to increase the C content of soils with depth by increasing the inputs of C deeper in the soil profile.

Plant roots make significant contributions to soil C under grassland and grazed pastures. Roots provide a C input to the soil C pool through root turnover and rhizodeposition (Farrar et al. 2003). Root turnover is considered a key component of C sequestration in soils (Matamala et al. 2003; Norby et al. 2004) with root longevity or turnover influenced by root traits such as specific root length (SRL) (Eissenstat et al. 2000; Roumet et al. 2006). Studies have demonstrated that roots

contribute more to soil C and in particular stabilised C, than aboveground biomass (Kätterer et al. 2011; Kong and Six 2010), with the mean residence time of root-derived C in soil approximately 2.4 times that of aboveground C inputs (Rasse et al. 2005). Assuming that both turnover and rhizodeposition are proportional to root mass, then increasing the C inputs to soil through either increasing total root mass, or increasing the depth distribution of roots in the soil profile are potential strategies for increasing soil C inputs and hence soil C stocks. The use of deep-rooting species has been suggested as a method to increase soil C at depth (Crush and Nichols 2010; Dodd et al. 2011a; Powlson et al. 2011). Increasing root inputs could be achieved by selecting plants with greater root biomass or rooting depth (Kell 2011) or potentially by increasing species diversity or richness of a pasture. A number of studies have demonstrated that increasing species richness can increase aboveground ecosystem productivity (Tilman et al. 1996) with the most diverse and productive plant communities displaying the deepest rooting distributions and greater root biomass (Mueller et al. 2013). Therefore, there is the potential to increase C sequestration by increasing the plant diversity of a grassland system (Steinbeiss et al. 2008).

High productivity New Zealand pastures are dominated by perennial ryegrass (*Lolium perenne* L.) and white clover (*Trifolium repens* L.), which typically exhibit shallow rooting depths (Bolinder et al. 2002; Crush et al. 2005). Adding other species into the pasture sward such as chicory (*Chicorium intybus* L.), lucerne (*Medicago sativa* L.), and plantain (*Plantago lanceolata* L.) results in swards of greater species diversity compared to conventional ryegrass-clover swards. The addition of a mixed species pasture to the farm system provides

advantages such as increased drought resilience, greater productivity, more even distribution of annual dry matter (DM) production and feed quality, and greater stability compared to ryegrass-clover based pastures (Gerrish 2001; Woodward et al. 2013). While there is some information on the root mass of ryegrass-clover pastures in New Zealand (Dodd et al. 2011a; Dodd and Mackay 2011b; Matthew 1996; Saggar et al. 1997), there is little data on root biomass under moderately diverse pastures. Furthermore, if moderately diverse pastures are considered to be a potential management option to increase C input to soil, then understanding the rooting characteristics such as rooting depth and the proportion of fine/coarse roots are also important.

A secondary objective afforded by the chosen site was to compare the effects of high and low sugar perennial ryegrass cultivars on root structure in both low and moderate diversity swards. High sugar ryegrass cultivars are defined by an increase in water soluble carbohydrate in the aboveground herbage and have received interest in pastoral systems due to their ability to potentially increase milk production and reduce the excretion of urinary nitrogen (Cosgrove et al., 2007; Cosgrove et al., 2009; Edwards et al., 2007). However, the effect of these high sugar ryegrass cultivars on belowground root mass is poorly understood. This study took advantage of an existing field-scale trial that compared dry matter yield, pasture persistence and milk solids production between a ryegrass-clover and a moderately diverse pasture (Woodward et al. 2013). The main objective of this study was to quantify the root structure and dynamics of a moderately diverse pasture in comparison with a low species diverse (conventional ryegrass-clover)

pasture. If the more diverse pastures consistently contribute more C to soil, this has significant implications for pasture systems globally.

3.3 Materials and Methods

3.3.1 Site Description

The study was conducted at Scott Farm, a research dairy farm owned and operated by the New Zealand dairy research organisation DairyNZ. The farm is located 7 km northeast of Hamilton in the Waikato region, North Island, New Zealand (37°46'13.62''S, 175°22'40.64''E). Thirty year mean annual rainfall and temperature obtained from a weather station within 6 km of the site was 1117 mm and 13.8 °C respectively (NIWA, 2015). The soil type of the study location was classified as a Matangi silt loam (Typic Orthic Gley Soil) (Hewitt 1993; Mudge et al. 2011). The study site was located within an existing plant diversity trial containing a number of 0.5 ha paddocks. These paddocks were rotationally grazed year round with an average stocking rate of 3 cows ha^{-1} and generally received 150 kg N ha^{-1} y^{-1} (Rutledge et al. 2014) and maintenance fertiliser (P= 35 kg ha^{-1} y^{-1}, K= 117 kg ha^{-1} y^{-1}, S= 50 kg ha^{-1} y^{-1}) (Woodward et al. 2013).

The study focussed on four treatments each containing 3 replicates in a randomised block design. The treatments consisted of a ryegrass-clover sward, high sugar ryegrass-clover sward, moderately diverse pasture and a moderately diverse pasture containing the high sugar ryegrass cultivar. Ryegrass-clover refers to a perennial ryegrass and white clover pasture commonly used in New Zealand grazed systems. The moderately diverse pasture refers to a perennial ryegrass and white clover pasture with the addition of chicory, plantain, and lucerne. The high sugar ryegrass refers to the ryegrass cultivar (*'Abermagic-AR1')* of the pasture

which contains a higher water soluble carbohydrate content than other ryegrass cultivars (Cosgrove et al. 2009), while all other species present in the pasture sward remained unchanged.

Establishment of this plant diversity trial was carried out between February and April, 2010. The cultivation method involved 2 applications of a glyphosate-based herbicide, followed by mouldboard ploughing, power harrowing and seed sowing using a roller till. Dairy shed effluent (40 mm), lime (2000 kg ha^{-1}), and maintenance fertiliser application (550 kg ha^{-1} 'Superten', 50 kg ha^{-1} NaCl, 35 kg ha^{-1} "CalMag") occurred between the first herbicide and power harrowing stage (Woodward et al. 2013). Seeding rates were 38.5 kg ha^{-1} for the mixed pasture (10 kg ha^{-1} perennial ryegrass [*Lolium perenne* L.*, 'One50-AR1'*], 2 kg ha^{-1} white clover [*Trifolium repens* L.*, 'Kopu II'*], 15 kg ha^{-1} prairie grass [*Bromus wildenowii* L.*, 'Atom'*], 2 kg ha^{-1} chicory [*Cichorium intybus* L.*, 'Choice'*], 1.5 kg ha^{-1} plantain [*Plantago lanceolata* L.*, 'Tonic'*], 8 kg ha^{-1} lucerne [*Medicago sativa* L.*, 'Torlesse'*]) and 23 kg ha^{-1} for the ryegrass-clover (18 kg ha^{-1} perennial ryegrass ['One50-AR1'], 5 kg ha^{-1} white clover ['Kopu II']) (Woodward et al. 2013). Although prairie grass was included in the pasture establishment, by the time this study was carried out the prairie grass species composition was very low to non-existent (Woodward *et al.*, 2013) and was not observed in the field; hence it was omitted from the analysis of this study.

3.3.2 Sample collection

Root samples were collected four times through the year in April, July, and October 2012, and February 2013 (Southern Hemisphere autumn, winter, spring and summer respectively) two years after pasture establishment. For each

sampling, ten random sample sites in each paddock were located while ensuring no sample was taken within 5 m of any fence line or water trough. At each sample site, the aboveground species composition within a 0.25 m^2 quadrant was recorded by estimating the relative abundance of each species (% foliar cover). For the % foliar cover, 'other' was defined as the observation of species that were not included in each respective treatment, or bare ground. Species evenness was calculated based on a Shannon-Weaver index using aboveground species composition whereby:

$Evenness = \Sigma\, pi * \ln(pi) Hmax,$

where p_i is the proportion of each species abundance and $H_{max} = \ln(S)$. S is equal to the species count of each treatment (including 'other").

Soil cores (50 mm diameter, 300 mm depth) were taken from the centre of the quadrant and the core partitioned into 3 depth increments (0-100, 100-200, and 200-300 mm) and kept separate for individual processing. Therefore, each seasonal sampling consisted of 360 samples (4 treatments, 3 replicates, 10 cores, 3 depths). Samples were refrigerated at 4°C until root extraction.

3.3.3 Root Biomass

Root biomass was washed from soil with water, collecting all root material retained on a 250 μm sieve. Samples were first passed through a 2 mm sieve with water to loosen the soil particles ensuring all water was collected. Root material was floated off and collected using a 250 μm sieve. It is acknowledged that some very fine root material may have been lost through the 250 μm sieve but the contribution of this missing root material after additional searching was estimated to be approximately <5 % of total root mass. Root material was dried in a fan forced oven at 65°C for at least 48 hours until constant weight. Root dry weights

were converted to an equivalent mass per hectare of soil surface (kg ha^{-1}) using the cross sectional surface area of the soil core. Root tissue was analysed for total C and N with a Vario ER cube elemental analyser (Elementar, Hanau, Germany).

3.3.4 Root turnover and C input

Root turnover was calculated as the difference in the maximum and minimum root masses within each pasture treatment during one year using a method modified from Scurlock *et al.*, (2002). Assuming that root mass production and subsequent decline occurs seasonally, then the difference between the maximum and minimum root mass values over an annual basis can be used to estimate the root turnover and C input. The difference of root turnover between each treatment was then used to obtain the difference in root C input between the two pastures. The C input from this root turnover was calculated using a community-weighted root C content for each pasture. The community-weighted mean (CWM) root C content for each pasture was calculated as the % C of root material weighted by the relative aboveground abundance of each species (Laughlin 2011). However, this method of calculating C input is conservative because it does not account for any contribution of C from rhizodeposition which may account for up to 2.5 × the contribution from root turnover (Johnen and Sauerbeck 1977; Kuzyakov and Domanski 2000; Kuzyakov and Schneckenberger 2004). CWM traits in each plot were calculated as $\sum_{i=1}^{S} t_i \, p_i$, where t_i is the mean root trait of species i, and p_i is the relative aboveground cover of species i.

3.3.5 Root trait measurements

Root traits (root length, diameter, surface area, volume) were obtained following extraction of individual plants of each species (ryegrass, clover, chicory, plantain, lucerne) in July 2012 and October 2013. Individual plants (n=6) were isolated and extracted down to 300 mm keeping the bulk of soil intact around the root system where possible. Soil was gently shaken and washed from the root system using water, ensuring that the root profile remained as intact as possible. Roots were evenly spread in a water layer on a transparent tray (400 x 300 mm) and digital images taken at a resolution of 600 dpi (dots per inch) using a flatbed scanner (Epson Expression). Root images were analysed for total root length, average root diameter, projected area, surface area and root volume using WinRHIZO image analysis software (Regent instruments Inc., Montreal, Canada). Root parameters were standardised by root mass to give specific root length (SRL) and specific surface area (SSA), while the root mass and volume was used to calculate root density. An estimate of the total root length (km m^{-2}) for both pastures in the top 300 mm was calculated using a CWM approach modified from Laughlin (2011). Root length was calculated by multiplying the mean SRL for each pasture by the corresponding root mass using the equation: Total root length (km m^{-2}) = mean SRL (km kg^{-1}) × Root mass (kg m^{-2}), where mean SRL was a CWM calculated as noted previously.

3.3.6 Statistical Analysis

Repeated measures ANOVA was used to analyse the temporal dynamics of total root mass (0-300 mm) using season, pasture type (ryegrass-clover and moderately diverse) and ryegrass cultivar as factors. The results of the repeated measures ANOVA showed that the high sugar ryegrass cultivar had no significant effect on total root mass ($F_{1,8} = 1.750$, $P = 0.222$). Therefore, root mass data was subsequently pooled based on pasture type (moderately diverse vs. ryegrass-clover) to give 6 replicates of each and a second repeated measures ANOVA carried out. If an overall effect of pasture type was detected, then one-way ANOVA was used within each season to determine how root mass differed between pasture types. Root mass data was log transformed to meet the variance assumptions of the ANOVA. Root length data was analysed using repeated measures ANOVA using season and pasture type as factors. The results of the root turnover/C input were analysed using a one-tailed *t*-test for statistical significance between pasture type. For all tests, $\alpha = 0.05$. ANOVA and the *t*-test was performed using R (version 2.15.0) and repeated measures ANOVA was performed using STATISTICA (version 10).

3.4 Results

3.4.1 Root mass

Root mass varied seasonally ($F_{3,30}$ = 6.0, P = 0.0025) for both pastures where root mass peaked in the summer with the lowest root mass during winter (Figure 2a). There was consistently greater root mass to 300 mm depth under the moderately diverse pasture compared to the ryegrass-clover pasture across all seasons ($F_{1,10}$ = 17.208, P = 0.002), with average differences for autumn, winter, and spring and summer of 980, 1700, 700 kg ha^{-1}, 3700 kg ha^{-1}, respectively (Figure 2a). The 0-100 mm depth had the greatest difference between the pastures during summer with 2080 kg ha^{-1} more root mass under the moderately diverse pasture ($p<0.05$) (Figure 2b).

In the 100-200 mm depth, there was significantly more root mass (1.5 to 2 times, depending on season; $p<0.05$) for the moderately diverse pasture compared to the ryegrass-clover pasture across all seasons. Autumn and summer showed the greatest treatment differences in root mass with 857 and 952 kg ha^{-1} more root mass under the moderately diverse pasture (Figure 2c). In the 200-300 mm depth, there was on average double the root mass in the moderately diverse pasture compared to the ryegrass-clover in winter and summer ($p<0.05$), with the greatest treatment difference also occurring in summer with 650 kg ha^{-1} more root mass in the moderately diverse pasture (Figure 2d). The sugar cultivar had no significant effect on total root mass under both ryegrass-clover and moderately-diverse pastures (data not shown) and all further analysis and results were combined to give 6 replicates of both moderately-diverse and ryegrass-clover pasture.

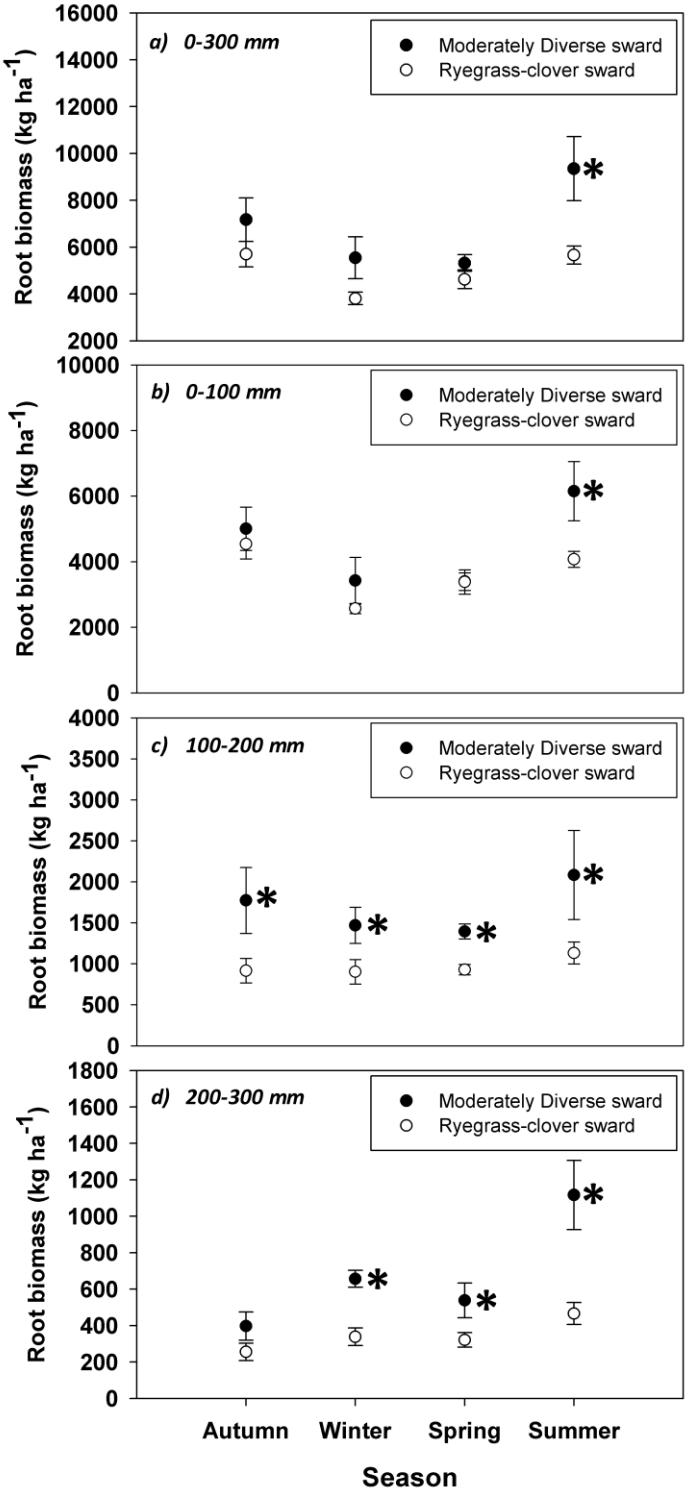

Figure 2 Root biomass for the moderately diverse sward (black filled circle) and ryegrass-clover sward (black outline, open filled circle) in each season and for the various soil depths, a) 0-300 mm, b) 0-100 mm, c) 100-200 mm, d) 200-300 mm. *denotes statistical significance of $p<0.05$ between the pasture types, error bars represent 1 SE. Note differences in scale of biomass axes.

3.4.2 Root traits and species abundance

The species composition, as measured by aboveground cover, varied throughout the year (Table 4). The moderately diverse pasture had a greater species evenness than the ryegrass-clover pasture across all seasons with the exception of summer (Table 4). The moderately-diverse pasture also had greater species richness based on the abundance of the sown species. Both pastures had a high contribution of the class "other" (i.e. weeds, dead or bare ground) in the summer sampling period which coincided with one of the worst summer droughts in recorded history (Porteous and Mullan 2013). Seasonal weather data collected from a weather station within 6 km of the site was compared to 30 year averages (NIWA, 2015). Rainfall (3 month cumulative rainfall) for autumn (254 mm cf. 296 mm), winter (393 mm cf. 320 mm) and spring (247 mm cf. 274 mm) was similar to the 30 year mean. However, the summer period of this study was well below the 30 year mean (50.8 mm cf. 228.7 mm) (NIWA, 2015). Air temperatures during this study were generally cooler in autumn (10.4°C cf. 12°C), winter (9.1°C cf. 10.1°C) and spring (14.4°C cf. 15°C) while summer was warmer than average (19°C cf. 18.1°C) (NIWA, 2015).

Specific root length (SRL) ranged from 1.9 cm g^{-1} for lucerne to 67.8 cm g^{-1} for ryegrass (Table 5). The species which displayed lower SRL (lucerne and chicory) were typically associated with the presence of taproots and had a smaller proportion of fine roots (<2 mm diameter) in comparison to ryegrass and clover which had a higher SRL and a greater proportion of fine roots. Ryegrass and clover generally had greater specific surface area (SSA) and a smaller average root diameter compared to the lucerne and chicory. Root density, or the mass per

volume of root material, was smallest for chicory (0.10 g cm^{-3}) and greatest for lucerne (0.22 g cm^{-3}), while ryegrass, clover and plantain all had similar densities (0.13 - 0.16 g cm^{-3}).

Estimated total root length varied seasonally (P = 0.001) though there was no difference between pasture type to 30 cm depth (P = 0.410) with length ranging from 20-25 km m^{-2} during winter to a maximum of between 30-50 km m^{-2} root length during the summer period (Figure 3).

Table 4 Seasonal aboveground species abundance (% surface cover) and species evenness of the two pastures (moderately diverse and ryegrass-clover, n = 6). Values in parentheses represent 1 SE.

	Autumn	Winter	Spring	Summer
Moderately diverse				
Ryegrass	31 (7)	33 (2)	41 (1)	35 (3)
Clover	12 (2)	18 (1)	11 (1)	8 (1)
Plantain	12 (3)	15 (2)	13 (2)	7 (2)
Chicory	19 (3)	12 (2)	20 (2)	11 (2)
Lucerne	20 (5)	13 (2)	8 (1)	14 (4)
Other	5 (2)	10 (2)	7 (1)	24 (3)
Evenness*	0.93	0.95	0.89	0.91
Ryegrass-Clover				
Ryegrass	52 (4)	45 (8)	66 (3)	42 (4)
Clover	42 (6)	44 (6)	27 (4)	29 (6)
Other	6 (1)	11 (2)	7 (1)	29 (3)
Evenness*	0.79	0.88	0.73	0.99

*Species evenness calculated using a Shannon-Weaver index.

Table 5 Average root parameters for the individual plant species (0-300 mm depth) found within the moderately diverse pasture (n=6). Values in parentheses represent 1 SE.

	Total C (%)	Total N (%)	C:N	Specific root length (m g^{-1})	Specific Surface Area (cm^2 g^{-1})	Root Density (g cm^{-3})	Average root diameter (mm)	% of root length < 2 mm diameter
Ryegrass	40.1	1.5	26.7	67.8 (4.0)	670 (25)	0.13 (0.01)	0.32 (0.01)	99.9
Clover	36.3	2.9	12.5	47.5 (2.0)	510 (23)	0.15 (0.01)	0.34 (0.01)	99.7
Plantain	40.9	1.8	22.7	17.0 (2.0)	220 (17)	0.16 (0.01)	0.54 (0.06)	99.0
Chicory	41.7	1.3	32.1	2.9 (0.6)	63 (6)	0.10 (0.01)	0.91 (0.2)	94.1
Lucerne	44.2	2.8	15.8	1.9 (0.4)	45 (6)	0.22 (0.02)	1.10 (0.2)	91.0

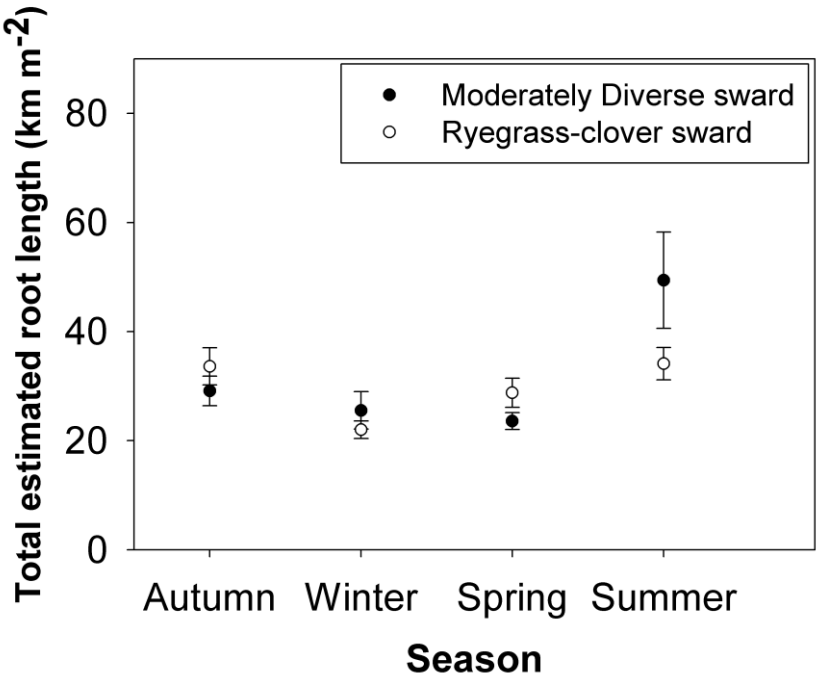

Figure 3 Seasonal estimated total root length (km m-2) between the moderately diverse and ryegrass-clover pasture, calculated as community weighted means using individual species specific root length (SRL), aboveground species composition and total root biomass values. Error bars represent 1 SE.

3.4.3 Root turnover and C input

Estimated annual root turnover was higher in the moderately diverse pasture (5411 kg ha^{-1}) compared to ryegrass clover (2672 kg ha^{-1}) with a difference of 2739 kg ha^{-1} root mass to 300 mm depth (Table 6). This difference equated to a greater annual C input to soil of 1203 kg C ha^{-1} under the moderately diverse pasture (0-300 mm) in comparison to ryegrass-clover, though this difference was not significant (P = 0.05) following propagation of errors. The estimated C input from root turnover was significantly greater in the moderately diverse pasture at all individual depths (0-100, 100-200, 200-300 mm) following a one-tailed t test, with an additional 829 (P = 0.04), 360 (P = 0.04), and 221 (P = 0.02) kg ha^{-1} of C respectively.

Table 6 Comparative root turnover (kg DM ha-1) for the moderately diverse and ryegrass-clover pastures for the various soil depths, the corresponding difference in root DM and subsequent C input for one year. Values presented in parentheses represent 1 SE.

	0-300 mm	0-100 mm	100-200 mm	200-300 mm
Moderately diverse	5411 (1347)	4120 (847)	1483 (367)	771 (195)
Ryegrass-Clover	2672 (460)	2263 (376)	652 (102)	250 (48)
Difference	2379 (1091)	1857 (747)	831 (415)	521 (163)
C input*	1203 (431)	829 (295)	360 (164)	221 (64)
p-value	0.05	0.04	0.04	0.02

* Calculated using average root DM of 41% C for moderately diverse and 38% C for ryegrass-clover sward. Calculated using a CWM approach using aboveground species composition and %C of root DM for individual species.

3.5 Discussion

3.5.1 Root mass

The moderately diverse pasture had a greater root mass and greater rooting depth throughout the measured profile compared to the ryegrass-clover based pasture across all seasons supporting the initial hypothesis. The greatest difference of root mass between the two pasture types were observed during summer with the lowest difference occurring during winter. The difference in root mass and root turnover between the two pasture types to 300 mm depth equated to an estimated greater C input of around 1203 kg ha^{-1} under the moderately diverse pasture. In contrast, there was no difference between high and low sugar ryegrass cultivar on root mass. This was not surprising as the principles behind the use of high sugar ryegrass cultivars are to increase milk production or live weight gain while reducing the excretion of urinary nitrogen (Cosgrove et al. 2009).

While there are no other measurements of root mass of moderately diverse pastures in New Zealand, the root masses obtained for ryegrass-clover pastures in this study were similar to other studies carried out on ryegrass-clover systems in New Zealand (Dodd and Mackay 2011b; Matthew 1996; Saggar et al. 1999) where root mass estimates under ryegrass-clover pasture show a range of 700 to 13000 kg ha^{-1}. A New Zealand-based study on lucerne and lucerne-grass mixtures found that lucerne was deeper rooting and had higher root yields deeper in the soil compared to ryegrass (McKenzie et al. 1990).

The observed seasonal pattern of root growth has been observed in other studies based on ryegrass-clover pastures (Dodd and Mackay 2011b; Matthew 1996;

Saggar and Hedley 2001). It should be noted that the summer sampling in this study coincided during a significant seasonal drought. During this summer period, the combination of below average rainfall and above average temperatures (NIWA, 2015) resulted in the potential evapotranspiration deficit being the highest on record (MPI, 2013). A drier than average November, and lower than average rainfall over the 2012/13 summer resulted in below average pasture growth rates for the period of November 2012 to April 2013 (MPI, 2013).

Chapman et al. (2013) found that inter-annual variation in pasture growth aboveground is driven by climatic variability in dairy producing regions such as the Waikato. Therefore, it would be expected that belowground variation in root mass would also be seen, and that the sampling year in this study likely occurred in an extreme climatic year as observed with the summer drought. The effect of the drought over the summer period may have contributed to the observed differences in root mass between the two pastures due to increased root mortality during drought stress particularly in species such as perennial ryegrass that are considered to have low drought tolerance (Wang and Bughrara 2008). However, the influence of weather variation on root mass and turnover is poorly understood under pasture systems in New Zealand.

The moderately diverse pasture also displayed greater variance in the root mass values than the ryegrass-clover pasture likely due to the spatial variability of tap-rooted species (chicory and lucerne) within the paddock (Gentile et al. 2003). In this study, lucerne was also found to contain the greatest average root density compared to the other species measured. Therefore, samples containing lucerne

would give greater root mass for a given volume of root material, while the chicory root mass would be smaller for a given root volume compared to the other species due to lower root density. These differences in root density may also contribute to the greater variability of root mass under the moderately diverse compared to the ryegrass-clover pasture, which displayed a more uniform pasture composition.

In addition to greater total root mass, the moderately diverse pasture had nearly double the root mass lower in the soil profile (100-200 and 200-300 mm) compared to the ryegrass-clover pasture. However, approximately 90-95% of the observed root mass was in the upper 200 mm, based on the depth distribution of the roots under both swards to 300 mm depth. The observed depth distribution in this current study follows trends of other studies based on ryegrass-clover pasture systems with greater than 80% of root mass found in the top 200 mm of soil (Crush et al. 2005; McKenzie et al. 1990).

This increased root mass and rooting depth distribution under the moderately diverse pasture compared to the ryegrass-clover sward could be partly explained by an increase in species richness. Previous studies have demonstrated that diverse plant communities are associated with the deepest distributions of plant biomass (Mueller et al. 2013; Tilman et al. 1996) and that higher root biomass production resulted from increased plant species richness (Steinbeiss et al. 2008; Tilman et al. 2001). This increased productivity with plant diversity has been suggested to be due in part to niche complementarity. Niche complementarity occurs when multiple species coexist because they partition resource and habitat

requirements, which results in more efficient resource acquisition and therefore higher net ecosystem productivity (Ashton et al. 2010; Loreau and Hector 2001; Tilman et al. 2001). The increased root mass under the moderately diverse pasture could be partly explained by niche complementarity of the species found within that pasture due to the individual species ability to access different resources or habitats, for example rooting depth.

However, as the ryegrass-clover and moderately diverse pastures in this study are very similar in terms of species richness (2 cf. 5), the increase in root mass may also be driven by key species found within the moderately diverse pasture (Grime 1998). Species such as lucerne and chicory that have approximately 50% of roots in the top 300 mm of the soil profile (Bolinder et al. 2002; Gentile et al. 2003) are likely to have a deeper rooting distribution compared to ryegrass with approximately 80% of root mass found in the top 150 mm of soil (Bolinder et al. 2002; Crush et al. 2005). In the study by Bolinder *et al.* (2002) it was found that lucerne had significantly more roots in the 300-450 mm layer compared to the other forage species studied, including ryegrass. Gentile *et al.* (2003) found that lucerne and chicory roots were still present below 0.9 m. Thus, lucerne and chicory may be the species driving the greater root mass and rooting depth in the moderately diverse pasture.

3.5.2 Root turnover and C input

The estimate of C input values under the ryegrass-clover sward (2672 kg DM ha^{-1}, 1015 kg C ha^{-1}) were consistent with the findings of Saggar *et al.* (1999) who found annual inputs of C to soil from belowground inputs to be in the range of 555-930 kg C ha^{-1} following isotopic labelling of a ryegrass-clover-based pasture. The root turnover was greater under the moderately diverse pasture compared to

the ryegrass-clover pasture in all the soil depths measured as a result of greater seasonal root mass production. This greater root production/turnover led to an estimated increased C input to soil by about 1203 kg C ha^{-1} yr^{-1} to a depth of 300 mm by the moderately diverse pasture. However, high variability following error propagation meant that we could not show a significant effect of the total input in the 0-300 mm depth, though individual depths displayed greater estimated C input. We also acknowledge that not all of this additional C would be stabilised as soil C but this estimate can provide a relative comparison on differences of the root C input between the two pasture swards.

Dodd *et al.* (2011a) suggested that in New Zealand soils there was scope for increasing root mass below the 100 mm depth where the C content is likely to be further from C saturation. C saturation level is the maximum amount of C that can be stored in a soil and is dictated by soil mineralogical and textural properties (Six et al. 2002b; Stewart et al. 2007). A recent paper by Beare *et al.* (2014) estimated that New Zealand soils have a larger C saturation deficit lower in the soil profile. Therefore, the greater C input in the 100-300 mm soil depth under the moderately diverse pasture in the current study is likely to also occur in soil further from a C saturation limit compared to the 0-100 mm depth. Thus, the moderately diverse pasture could be considered a management option to increase the C input to soil where it is also further from C saturation compared to the ryegrass-clover sward.

One limitation in the method used to estimate root turnover was that it does not account for C inputs from rhizodeposition. Rhizodeposition is the broad term encompassing a range of processes such as root exudates and cell sloughing where C enters the soil (Jones et al. 2009). The contribution of C released from roots

through rhizodeposition has been estimated to be up to 2.5 times the C that is found in root mass (Johnen and Sauerbeck 1977). Therefore, the actual carbon input in this study is likely to be larger under both pastures if we also take into account of rhizodeposition than that estimated based on root mass alone.

3.5.3 Root traits

The root traits for the individual species demonstrated that the ryegrass-clover pasture typically had smaller root diameters resulting in greater SRL and a greater proportion of fine roots per unit length. The additional species found in the moderately diverse pasture displayed greater diversity in root traits compared to the ryegrass-clover and typically had a smaller proportion of fine roots due to the presence of species with larger root diameters. However, the estimated total root length was generally similar between the two pastures showing that the ryegrass-clover had just as much root length as the moderately diverse pasture despite having lower root mass. Root morphology may be important in the stabilisation of C through their influence on C inputs and soil aggregation. Jastrow *et al.* (1998) demonstrated that roots were important in the restoration of macroaggregate structure in a restored prairie with the root density and root distribution influencing the aggregate size and formation. Fine roots were thought to act as a physical network that entangles soil particles and helps to stabilise macroaggregates (Jastrow et al. 2007). Within these macroaggregates, microaggregates are formed whereby C can be physically protected from decomposition within these microaggregates and C stabilisation can occur (Six et al. 2004). Furthermore, root diameter influences the decomposition and turnover of roots, where smaller fine roots (<2 mm diameter) have a faster decomposition and turnover (Pacaldo et al. 2014). The implications of greater SRL, finer roots,

and similar total root length under the ryegrass-clover pasture is that this pasture may contain species which have faster root turnover and subsequent C input to the soil than the species found in the moderately diverse pasture. The objective was to investigate whether there were differences in the root mass under these two pasture swards, however, there is also a need to further investigate the total C inputs under these two swards in regard to the root dynamics and root turnover.

3.6 Conclusion

This study measured greater root mass and greater rooting depth under a moderately diverse pasture compared to a ryegrass-clover pasture under a permanently grazed dairy system. The increased root mass suggested an additional annual C input of 1203 kg C ha^{-1} under the moderately diverse pasture. Whether or not a proportion of this extra C is stored in the soil needs to be determined to make statements about increased soil C stocks. However, these results indicate scope for enhancing soil C sequestration by moderate increases in functional plant types. If the additional C input proves to be consistent across a range of New Zealand soil types then the moderately diverse pastures would provide a management option for increasing soil C input under permanently grazed systems.

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Chapter 4: Root turnover and Root C input to soil under a mixed sward and ryegrass-clover pasture

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Sam McNally, Louis Schipper, Daniel Laughlin, Susanna Rutledge, Mike Dodd and Johan Six decided on the experimental design. Sam McNally collected and analysed samples, carried out statistical analysis and was responsible for the writing of the manuscript. Louis Schipper was the primary reviewer of this work with Daniel Laughlin, Susanna Rutledge, Mike Dodd and Johan Six providing additional comments to improve the manuscript. Daniel Laughlin further provided assistance with statistical analysis.

4.1 Abstract

Increasing organic matter inputs from roots and the turnover of roots have been highlighted to be important sources to the soil C pool. Ryegrass-clover pastures under permanent grazing largely dominate New Zealand agriculture. Previous studies have indicated greater root inputs from more diverse pasture swards in comparison to ryegrass-clover pastures. The objective of this study was to measure the root turnover and C input to soil under a moderately diverse pasture compared to a ryegrass-clover pasture commonly used in New Zealand agriculture. Pastures were labelled using an isotope pulse labelling method in the field within 1 m^2 clear chambers, whereby $^{13}CO_2$ was taken up following photosynthesis during 5 daily labelling events throughout a 5-week period. The labelled ^{13}C was subsequently measured in the roots (0-100, 100-200 mm depth) and soil (0-100 mm) at regular intervals to calculate root turnover (138 day

period) and C input (88 day period). This study coincided with a period of severe soil moisture stress (summer drought). There was no difference between root turnover rates of the two pastures and these pastures had a mean turnover time of approximately 276 days. The C input from roots ranged from 3 kg C ha^{-1} d^{-1} to 230 kg C ha^{-1} d^{-1} for the two pastures averaging inputs of 58 kg C ha^{-1} d^{-1} over the 88-day period. The two pastures displayed similar root turnover and C inputs to soil during a period of drought. The inputs of C to soil in this study are higher than those reported in other studies. This discrepancy was likely caused by increased root death during the summer drought in the current study. Nonetheless, this study provides the first values of C input and root turnover under moderately diverse pastures under grazed agriculture in New Zealand and will be useful for understanding their potential in C sequestration of soils.

4.2 Introduction

Soil contains the largest pool of carbon (C) in the terrestrial ecosystem, estimated at approximately twice that of the atmosphere (Batjes 1996; Powlson et al. 2011) and approximately three times that found in vegetation (Smith 2008). Changes to this pool of soil C can influence the CO_2 content of the atmosphere in a positive or negative way depending on whether the soil acts as a sink or source of C. Land under agricultural management has the most potential for human manipulation of its C sink status and has been shown to be both a sink and a source depending on various management factors (e.g. irrigation, cultivation) (Lal 2009).

Increasing the soil C pool under agriculture is being investigated as a potential strategy to mitigate some of the greenhouse gas emissions. Increases in the soil C pool require an increase in either the rate of C input to soil and/or a reduction in

the rate of loss of C from soil. These C losses are dominated by soil microbial respiration (Paustian et al. 2000), although they can also occur via erosion, leaching (Dawson and Smith 2007) and photodegradation (Rutledge et al. 2010). Ideally, additional C inputs need to be stabilised in slow turnover soil organic matter fractions to reduce the risk of future losses due to disturbance.

Inputs to the soil C pool are mainly through plant biomass (above- and below-ground tissue turnover), transfer of C in organic exudates from roots and the recycling of biomass through herbivores, such as excreta from grazing animals (Chirinda et al. 2012). Inputs of C to soil can be processed by soil fauna (Philippot et al. 2013) so that the newly added C becomes incorporated into the soil as soil organic matter (Kuzyakov and Domanski 2000). Changes in the amount of these inputs of C to soil depend largely on land use and land management.

Plant roots are a key component in the soil ecosystem (Bardgett et al. 2014; Solly et al. 2013), as they are a main source of C to the soil pool and influence soil microbial activity and decomposition processes (Philippot et al. 2013). Plant roots have been shown to have a greater contribution to soil C than that of aboveground biomass (Kätterer et al. 2011; Kong and Six 2010; Rasse et al. 2005) as roots are in direct contact with the soil and potential sites for C stabilisation. Furthermore, root activity in soil enhances soil organic matter protection mechanisms through a range of processes such as physio-chemical protection of root derived compounds on soil minerals and physical protection of root hairs within soil aggregates (Rasse et al. 2005). Consequently, increased root inputs through greater root

biomass and turnover has been proposed as a potential pathway to increase soil C (Lal 2009; Kell 2011; Kell 2012).

Root turnover is important for C and nutrient cycling in terrestrial ecosystems (Aerts et al. 1992) and combined with root exudation acts as one of the main sources of C to the plant-soil cycle (Leifeld et al. 2015). Root turnover is defined as the annual replenishment of new roots from those that die or are sloughed off by physical forces (Cheng et al. 1991; Leifeld et al. 2015). Root turnover is sensitive to mean annual temperature and management practices, with turnover accelerated under higher temperatures (Leifeld et al. 2015) and thus, can vary for a given plant species or land management depending on local climate conditions. Under the same climatic conditions, turnover has been shown to be accelerated under more intensive grazing conditions (higher stocking rates) (Klumpp et al. 2009; Pucheta et al. 2004) and with increased fertiliser use or irrigation (Stewart and Metherell 1999). Root turnover is also influenced by root traits such as diameter, with fine roots (<2 mm) having faster turnover than more coarse roots (Gill et al. 2002; Joslin et al. 2006; Matamala et al. 2003). Root turnover is also affected by species richness with more diverse grassland communities displaying less root loss and hence turnover than monocultures, although selection effects can partly explain this reduced root mortality in diverse communities where species with the longest root turnovers become the dominant species (Mommer et al. 2015).

While there are some measurements of root turnover under perennial ryegrass dominant pastures in New Zealand (Dodd and Mackay 2011b; Reid and Crush

2013; Saggar and Hedley 2001; Scott et al. 2012), there are no measurements of root turnover for more diverse pasture communities increasingly being used under New Zealand conditions. Moderately diverse pastures have traditionally been of interest to farmers for their potential tolerance to drought and for providing better consistency of annual dry matter production (Gerrish 2001; Woodward et al. 2013). Furthermore, there is some evidence that ruminants grazing moderately diverse pastures have reduced urinary nitrogen, which has implications for reducing N leaching and presumably N_2O emissions (Woodward et al. 2013).

McNally *et al.* (2015) measured greater root biomass under a moderately diverse pasture compared to a ryegrass-clover pasture under permanently grazed agriculture in New Zealand. Net root turnover and C input to these soils over one year was estimated to be greater under the moderately diverse pasture. However, these estimates were calculated from differences in seasonal root mass measurements and are an indirect measure of root turnover. The measurements of root turnover under these moderately diverse pastures, in comparison with conventional ryegrass-clover pastures are important to better understand the opportunity for diverse pastures to alter C sequestration. An alternative, more direct and likely more quantitative approach for measuring root turnover is through the use of isotope pulse labelling using $^{13}CO_2$ (Scott et al. 2012). In this technique, plants enclosed in transparent chambers are labelled with $^{13}CO_2$ through photosynthetic uptake, with a proportion of the ^{13}C being translocated belowground. The decline of ^{13}C within the root tissues sampled over time is analysed to give estimates of root turnover.

The objective of this study was to compare the root turnover and C input to soil under two pasture types commonly used in New Zealand agriculture, using the ^{13}C stable isotope labelling method. The pasture types were the same perennial ryegrass-white clover dominant and moderately diverse swards investigated by McNally *et al.* (2015). Based on the seasonal root mass dynamics observed in that study, it was hypothesised that there would be faster root turnover and greater C input to soil under the moderately diverse pasture compared to the ryegrass-white clover pasture.

4.3 Methods

4.3.1 Site Description

The study was conducted at Scott Farm, a dairy research farm owned and operated by the New Zealand dairy research organisation DairyNZ. The farm is located 7 km northeast of Hamilton in the Waikato region, North Island, New Zealand (37°46'13. 62''S, 175°22'40. 64''E). Thirty year mean annual rainfall and temperature obtained from a weather station within 6 km of the site were 1117 mm and 13.8 °C, respectively (NIWA 2015). The soil type at the study location was a Matangi silt loam (Typic Orthic Gley Soil) (Hewitt 1993; Mudge et al. 2011). The concentration of total C and N in the surface soil (0–100 mm) was 7.7% and 0.72% respectively (Mudge et al. 2011). Total porosity (v/v) of the Ap horizon (0–250 mm) was 0.66 m^3 m^{-3}, field capacity (-10 kPa) 0.54 m^3 m^{-3}, the lower limit of readily available water (-100 kPa) 0.43 m^3 m^{-3}, and permanent wilting point (-1500 kPa) was 0.25 m^3 m^{-3} (Mudge et al. 2011). The study site was located within an existing plant diversity trial containing a number of 0.5 ha paddocks (Woodward et al. 2013). These paddocks were rotationally grazed year round with an average stocking rate of 3 cows ha^{-1} and generally received 150 kg N ha^{-1} y^{-1} (Rutledge et al. 2014) and maintenance fertiliser (P= 35 kg ha^{-1} y^{-1}, K= 117 kg ha^{-1} y^{-1}, S= 50 kg ha^{-1} y^{-1}) (Woodward et al. 2013).

The current study focussed on two treatments within the plant diversity trial, each containing 3 replicate paddocks in a randomised block design. The treatments consisted of a perennial ryegrass (*Lolium perenne* L.) and white clover (*Trifolium repens* L.) pasture referred to hereinafter as "ryegrass-clover"; and a more diverse pasture containing perennial ryegrass, white clover, prairie grass (*Bromus*

willdenowii Kunth.), chicory (*Cichorium intybus* L.), lucerne (*Medicago sativa* L.) and plantain (*Plantago lanceolata* L.), referred to hereinafter as "moderately diverse".

The plant diversity trial was established between February and April 2010. Prior to this trial, all the paddocks within the trial were grazed by dairy cows on a ryegrass-clover based pasture. During establishment, the paddocks were all cultivated with two applications of a glyphosate-based herbicide, followed by mouldboard ploughing, power harrowing and seed sowing using a roller till. Dairy shed effluent (40 mm), lime (2000 kg ha^{-1}), and maintenance fertiliser application (550 kg ha^{-1} 'Superten', 50 kg ha^{-1} NaCl, 35 kg ha^{-1} "CalMag") occurred between the first herbicide and power harrowing stage (Woodward et al. 2013). Seeding rates were 23 kg ha^{-1} for the ryegrass-clover (including 18 kg ha^{-1} perennial ryegrass cv. '*One50-AR1*', 5 kg ha^{-1} white clover cv. '*Kopu II*') and 38.5 kg ha^{-1} for the moderately diverse pasture (including 10 kg ha^{-1} perennial ryegrass cv. *'One50-AR1'*, 2 kg ha^{-1} white clover cv. *'Kopu II'*, 15 kg ha^{-1} prairie grass cv. *'Atom'*, 2 kg ha^{-1} chicory *cv. 'Choice'*, 1.5 kg ha^{-1} plantain cv. *'Tonic'* and 8 kg ha^{-1} lucerne *cv. 'Torlesse'*) (Woodward et al. 2013). Although prairie grass was included in the pasture establishment, the prairie grass species cover was very low to non-existent by the time this study was carried out (Woodward et al. 2013) and was not observed in the field during this study.

4.3.2 Isotope pulse labelling

To determine turnover of roots, isotope pulse labelling of aboveground plant biomass was carried out using methods adapted from Stewart and Metherell (1999), Denef and Six (2006) and Kong and Six (2010).

A single plot (1 m^2) was established in each of the three replicate paddocks for both treatments described above so that there were 6 plots in total (2 treatments x 3 replicates). Plots were fenced off (~2.5 m^2) to exclude cattle prior to and for the duration of the study to ensure that grazing had not taken place on the plots at least 2 weeks prior to labelling. Aboveground herbage was cut to a residual stubble of 40 mm the week prior to labelling.

Isotope pulse labelling was carried out using 0.28 m^3 clear Perspex chambers (28 cm high) sealed to the ground using polyethylene film sheeting and sand bags (Appendix 1a). The clear chamber allowed photosynthesis to continue during labelling. Air was circulated using an electric fan mounted within the chamber and the CO_2 concentration was monitored during the labelling within the chamber using an infrared gas analyser (LI-8100A; LI-COR, Lincoln, USA). After the chamber was sealed and the CO_2 concentration had decreased below ambient (400 ppm) due to plant photosynthesis, a pulse of $^{13}CO_2$ was generated inside the chamber. $^{13}CO_2$ was evolved within the chambers using an aqueous solution containing approximately 1 g of $Na_2{}^{13}CO_2$ (99 atom %) and the addition of H_2SO_4 acid. The acid was injected into a vial containing the aqueous $Na_2{}^{13}CO_2$ solution through a rubber septum installed into the top of the chamber (Appendix 1b). The chambers were removed once the CO_2 concentration dropped well below ambient

concentration (to ~100 ppm). Each labelling occurred in the morning for approximately 1 hour. Chambers were replaced in the evening of the labelling to capture $^{13}CO_2$ loss from plant respiration during the night when photosynthetic uptake ceased. Chambers were removed the following morning once the CO_2 concentration had dropped below ambient concentration and $^{13}CO_2$ had been taken up again. Plots were labelled once weekly over a 5 week period between 10 December 2012 and 10 January 2013 (early summer, Southern Hemisphere) giving a total of 5 labelling events per plot.

4.3.3 Sampling and Measurements

Soil cores and herbage samples were collected before (background) and after isotope labelling from within the 1 m^2 plots. Samples were collected after labelling on 0, 7, 14, 21, 28, 35, 58, 88, 108 and 138 days (January – April 2013) after the last labelling event. Soil samples were collected from random locations within each plot using a 50 mm diameter soil core (200 mm depth) driven in using a maul. Two soil cores per sampling event were taken per plot and the core incremented into 100 mm depths and each depth bulked. Roots were separated visually from the soil with the aid of an 8 mm sieve and then roots washed with deionised water and dried in a 60°C oven to a constant weight (~48 hrs). Aboveground herbage was harvested during each sampling from a 100 cm^2 area directly above each collected core. The herbage of the entire plot (1 m^2) was harvested (40 mm residual) to coincide with the grazing regime of the corresponding paddock within one week after labelling. All herbage collected was removed from the plots and dried to constant weight in a 60°C oven. Samples of plant material (root and aboveground herbage) were ground finely using a ball mill grinder.

Soil was air-dried following sieving and a sub-sample ground for isotopic and elemental C analysis. Soil moisture contents were determined following oven drying a sub-sample of field soil at 105°C for 24 hours.

Soil, root, and herbage samples were analysed for elemental C and isotopic C concentrations using a Europa Scientific ANCA-SL elemental analyser (Europa Scientific Ltd, Crewe, UK) coupled to a 20-20 Stable Isotope Analyser mass spectrometer (Europa Scientific Ltd, Crewe, UK). The results of the isotopic analysis were expressed as $\delta^{13}C = \left[\left(\frac{^{13}R_{Sample}}{^{13}R_{Standard}}\right) - 1\right]$, where $^{13}R = {}^{13}C/{}^{12}C$ and the standard was relative to Pee Dee Belemnite (PBD).

Monthly climate data (mean air temperature, total monthly rainfall and volumetric soil moisture content) for the corresponding year of the study and 30-year means (for air temperature and rainfall) were collected from the Ruakura climatological weather station approximately 6 km from the site (NIWA 2015).

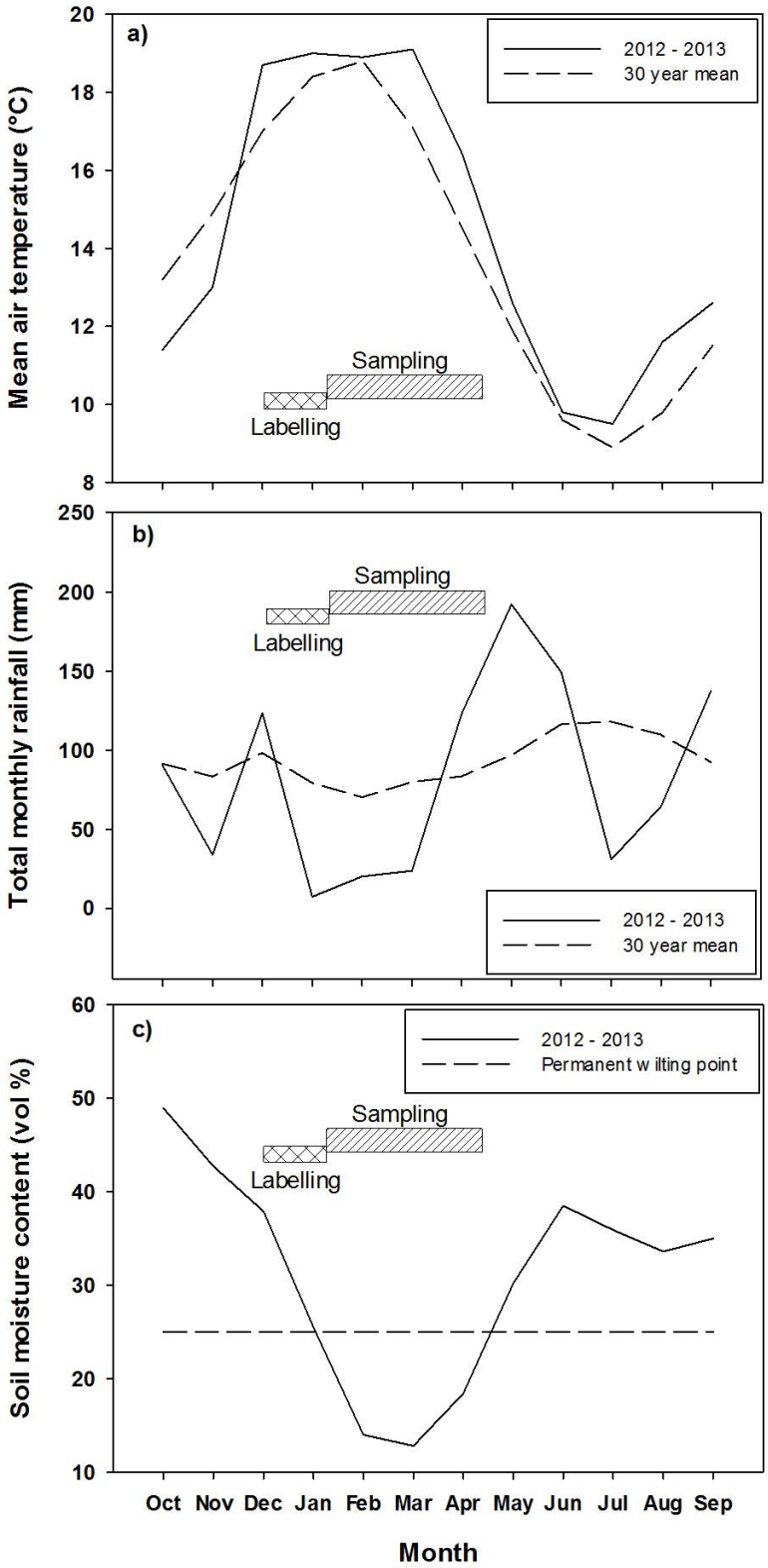

Figure 4 a) Mean air temperature (°C), b) Total monthly rainfall (mm), c) Volumetric soil moisture content, 0-100 mm depth, between October 2012 to September 2013 and compared to the 30-year mean (for temperature and rainfall). Data were obtained from a climatological weather station within 6 km from the site (NIWA 2015). Labelling and sampling periods are shown. Horizontal line on c) is the permanent wilting point.

4.3.4 Calculations and statistical analysis

Root turnover time in days for 0-100 mm and 100-200 mm depth was calculated using a method similar to that of Scott *et al.* (2012) whereby the ratio of isotope enrichment relative to peak enrichment was linearly regressed against the time since peak enrichment. The linear decline in ^{13}C isotope was used to calculate the *x* intercept when *y*=0 for the linear regression equation to estimate root turnover time (Scott et al. 2012).

The input of C from roots to the bulk soil (0-100 mm depth) was determined by first calculating the proportion (*f*) of soil C derived from the ^{13}C label (roots) using a method similar to Kong and Six (2010) whereby:

$$f = \frac{^{13}C_{soil} - ^{13}C_{natural\ abundance}}{^{13}C_{root} - ^{13}C_{natural\ abundance}}$$

where $^{13}C_{soil}$ = $\delta^{13}C$ of the soil sampled after labelling, $^{13}C_{root}$ = $\delta^{13}C$ of the root material from the same sample as $^{13}C_{soil}$, and $^{13}C_{natural\ abundance}$ = $\delta^{13}C$ of the background soil sample taken before any labelling occurred.

To determine the input of new C from the roots into soil, the derived *f* value was multiplied by the total C of the soil (Kong and Six 2010).

Calculation of the C input to soil was confounded by some negative values of the *f* value described above. Negative values were obtained when the soil or root sample ($^{13}C_{soil}$ or $^{13}C_{root}$) in question had a $\delta^{13}C$ value lower than that of the original background $\delta^{13}C$ value ($^{13}C_{natural\ abundance}$). This may occur when soil cores on a particular sampling date did not intersect with roots that had been adequately labelled. This effectively would calculate through to a negative C input, which

was assumed not to be possible. To account for these negative values, the average daily C inputs of ryegrass-clover and moderately diverse pastures were calculated by conservatively assuming negative values to be a zero input. ^{13}C data for soil and roots are presented in Appendix 1c and 1d.

An analysis of variance was performed on the root turnover data with pasture type (ryegrass-clover and moderately diverse) as a factor. Repeated measures ANOVA was performed on the soil C input data using pasture type and time after labelling (in days) as factors. All statistical analysis was carried out with R (version 3.2.0) using the Rcmdr package (Fox 2005). For all tests $\alpha = 0.05$.

4.4 Results

4.4.1 Soil moisture content

During the experiment air temperature was above normal, and rainfall far below-normal (Figure 4a,b). The low rainfall combined with high summer temperatures resulted in a decrease of soil moisture contents of both pastures following isotope labelling (Figure 4c and 5). The ryegrass-clover pasture dropped from approximately 29% moisture content to below wilting point (25%) by 14 days after labelling. The moderately diverse sward neared wilting point between days 14 and 28 but never dropped below wilting point. Generally, the moderately diverse pasture had greater soil moisture contents than the ryegrass-clover, although this was not a statistically significant difference.

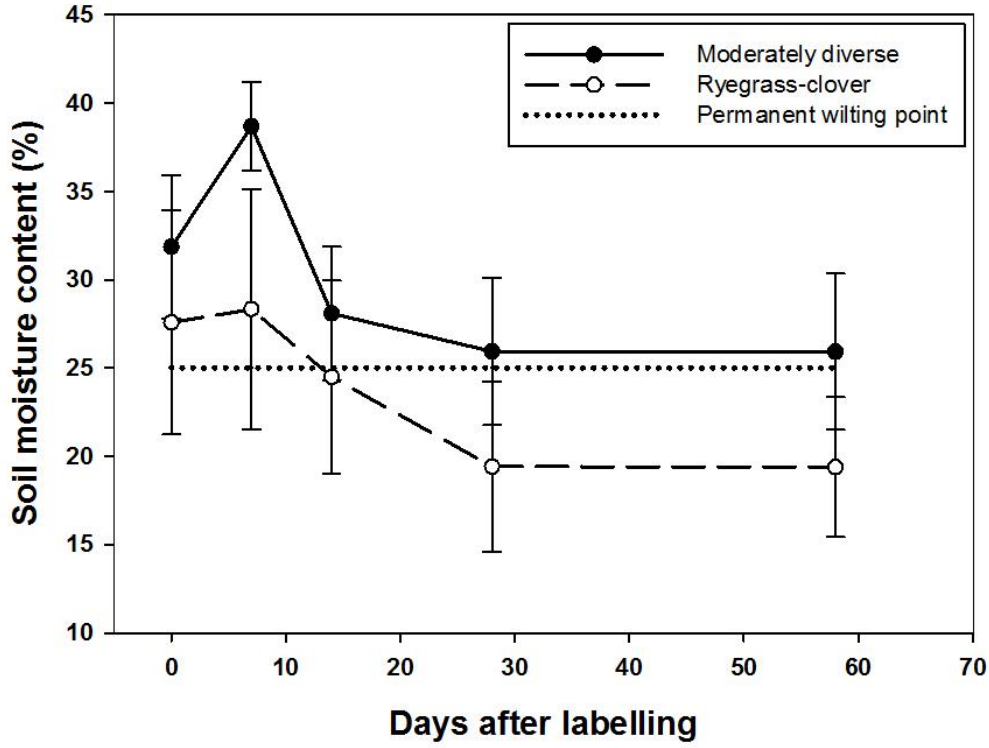

Figure 5 Soil moisture content for moderately diverse (black filled circles) and ryegrass-clover (open circles) pastures up to 58 days after labelling. Horizontal line represents permanent wilting point (25%). Error bars are 1 standard error (n=3). Data after day 58 was not available but is presented from climatological station in Figure 4c.

4.4.2 Root turnover

Background $\delta^{13}C$ concentration of the roots ranged from -28.70 to -30.63 ‰. Maximum peak enrichment measured in roots was -3.61 ‰ and average peak enrichment for roots of both pastures was -18 ‰. Peak enrichment under both pasture types occurred between 0 and 7 days after the final isotope labelling and then declined back to the background $\delta^{13}C$ with time (Appendix 1c). There was considerable variability in $\delta^{13}C$ values through time in the collected root material of both pastures. This variability was likely due to the spatial distribution of roots in the soil and the difficulty associated in sampling the spatial variability of roots with only two cores per sampling interval. The decline in $\delta^{13}C$ of the moderately

diverse pasture at 0-100 mm depth was not significant (P = 0.06; 0-100 mm, Figure 6a). Despite this variability, the $\delta^{13}C$ decline through time for the ryegrass-clover pasture was significant (P = 0.02; 0 -100 mm, Figure 6b). Although the moderately diverse pasture did not have a significant slope, the line of best-fit equation was used to calculate a best estimate of root turnover (Table 7). Root turnover at 0-100 mm depth was generally slower under the moderately diverse sward (298 days, Figure 6a) compared to the ryegrass-clover sward (260 days, Figure 6b). Due to the substantial variability in $\delta^{13}C$ in the collected root material through time, the statistical ability to detect possible differences in root turnover between pasture types was weak. Therefore, results of both pastures were combined prior to linear regression analysis, which allowed an estimation of an average root turnover of 276 days for roots at 0 – 100 mm depth (Figure 6c, Table 7). When data from the two pastures were combined the $\delta^{13}C$ decline through time was highly significant (P = 0.003).

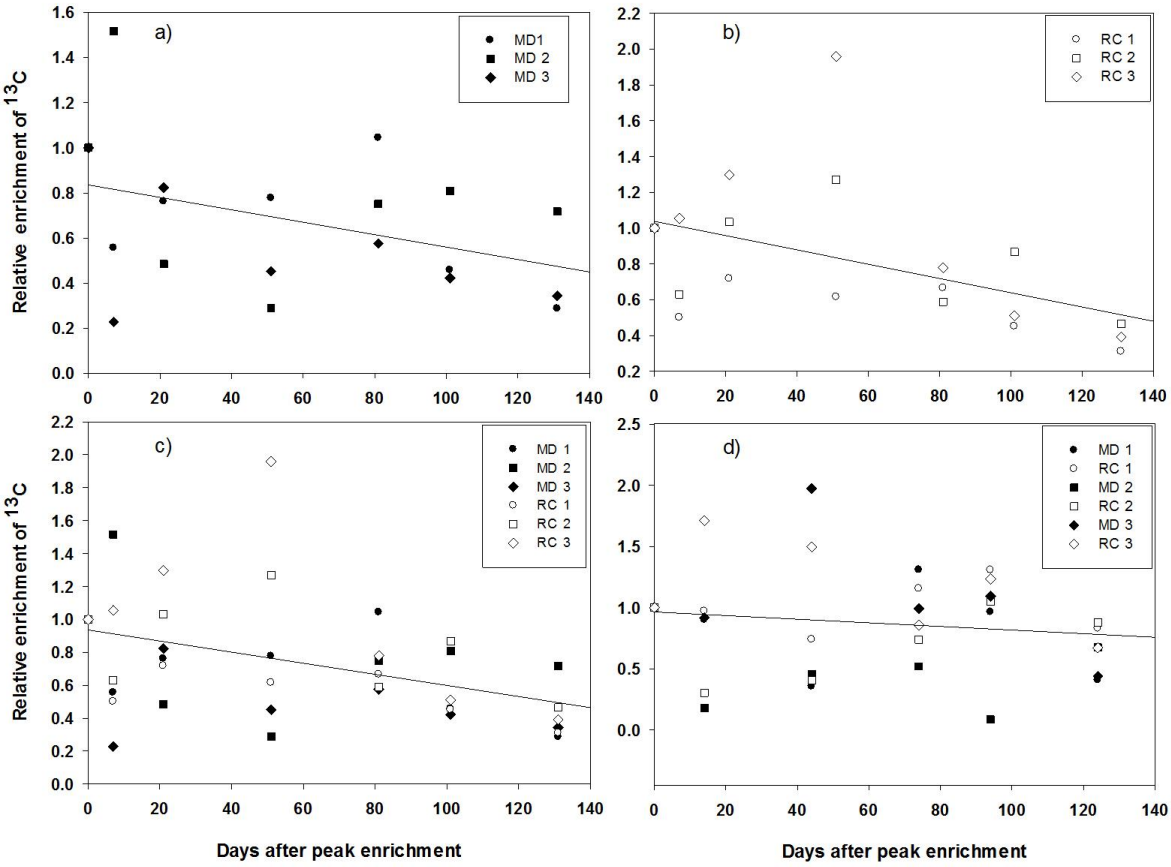

Figure 6 Relative isotope (^{13}C) enrichment of roots through time after peak enrichment for a) the moderately diverse (MD) pasture (0-100 mm depth, n=3), b) ryegrass-clover (RC) pasture (0-100 mm depth, n=3), c) combined MD and RC plots (0-100 mm depth, n=6) and d) combined MD and RC plots (100-200 mm depth, n=6). The linear equation of line of best fit is used to solve for x when y = 0 to give an estimate for root turnover. The line of best fits for the respective figures were a) $y = -0.0028x + 0.8351$, P=0.06, b) $y = -0.004x + 1.0383$, P=0.02, c) $y = -0.0034x + 0.9367$, P=0.003, d) $y = -0.0015x + 0.9644$, P=0.35.

There was greater variability in the relative isotope enrichment ratio of roots for the 100-200 mm depth through time compared to the 0-100 mm depth. This variability resulted in a decline of $\delta^{13}C$ through time that was not significant even when data from both pastures were combined (P=0.35; Figure 6d). Hence the data was insufficient to make any confident statement on root turnover time at this depth. However, using data as they were, root turnover in the 100-200 mm depth was approximately 643 days when combining the results of the two pasture swards.

Table 7 Root turnover time (days) for the moderately diverse pasture, ryegrass clover and the combined pastures. Values in parentheses represent the respective p-value for each category.

	0 – 100 mm	100 – 200 mm
Moderately-diverse	298 (0.06)	n/a
Ryegrass-clover	260 (0.02)	n/a
Combined	276 (0.003)	654 (0.35)

4.4.3 C input to soil

The C input from the roots (0-100 mm depth) ranged from approximately 3 kg C ha^{-1} d^{-1} up to a maximum of 230 kg C ha^{-1} d^{-1} with an average C input of 90 kg C ha^{-1} d^{-1} for the moderately diverse pasture and 25 kg C ha^{-1} d^{-1} for the ryegrass-clover pasture. The moderately diverse sward generally had a greater C input across all sampling periods with the exception of the sample 7 days after labelling (Figure 7). The C input between 14 and 28 days post labelling was on average 200-230 kg C ha^{-1} d^{-1} input for the moderately diverse pasture compared to 25-70 kg C ha^{-1} d^{-1} for the ryegrass-clover pasture. However, there was a very large variability in the C input between treatments and repeated measures ANOVA of the C input showed no significant difference (P = 0.10) between the two pasture types (Figure 7) or with sampling time (P = 0.55). Therefore, results of the C input for both pastures were combined and the C input calculated giving a combined average C input for the two pastures of 58 kg C ha^{-1} d^{-1} over an 88 day period.

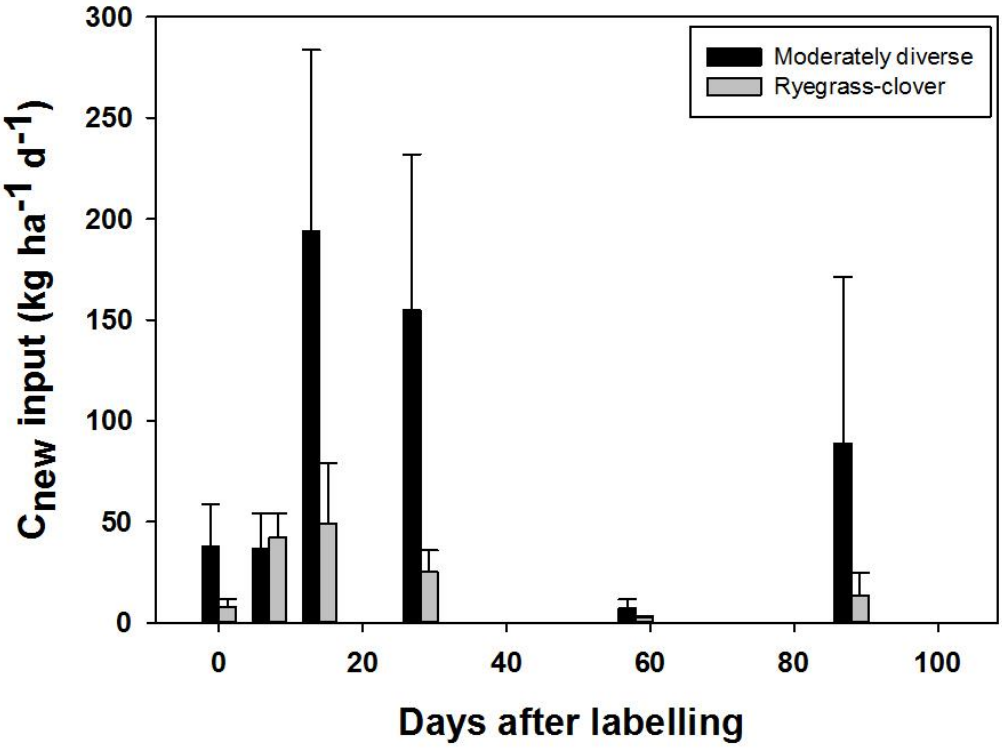

Figure 7 C input to soil (kg ha^{-1} d^{-1}; 0-100 mm depth) through time after isotope labelling under both ryegrass-clover and moderately diverse pastures. Error bars are 1 standard error (n=3). Overall there was no significant difference between pasture types (*P = 0.12*).

4.5 Discussion

4.5.1 Root turnover

The hypothesis for this study was that the moderately diverse sward would have increased root turnover in comparison to the ryegrass-clover pasture. This hypothesis was based on the study of McNally et al. (2015) who observed greater root mass and what appeared to be greater seasonal root mass change over a year. However, the results of the current study did not support this concept, as a significant difference in root turnover between the two pasture swards was not detected.

There has been a wide range of estimates for root turnover in pastures reported in the literature, likely due to large differences in management regimes. The average root turnover time of 276 days measured in the current study was shorter than that measured by Scott et al. (2012). The root turnover measured in the current study would be expected to be faster due to more intensive management with higher nutrient inputs, stocking density, and more frequent grazing than the Scott et al. (2012) study. The study by Scott et al. (2012) calculated root turnover rates using $\delta^{13}C$ data originally collected by Stewart and Metherell (1999). These turnover rates under ryegrass-clover were between 400-800 days (1.1 to 2.2 years) depending on fertiliser or irrigation use. They measured increased root turnover with the addition of phosphate fertiliser or irrigation under the ryegrass-clover pastures in comparison to dryland or unfertilised pastures. The farm in the current study generally had greater fertiliser inputs compared to that used in Scott et al. (2012), which may have contributed to greater root turnover observed in this study. Furthermore, pastures at Winchmore did not receive N fertiliser inputs in contrast to Scott Farm where N fertiliser inputs were generally 150 kg ha^{-1} y^{-1} (Rutledge et al. 2014; Woodward et al. 2013). Leifeld et al. (2015) also noted that soils with a lower N status generally had slower root turnover. Higher intensity systems were also associated with higher nutrient inputs, greater stocking density and more frequent grazing intervals and as a result had faster root turnover times (Leifeld et al. 2015).

Root turnover is also influenced by mean annual temperature (Gill and Jackson 2000; Leifeld et al. 2015). The turnover rates in this study would be expected to be faster than those of Scott et al. (2012) as their study site was in the Canterbury

region of the South Island of New Zealand with a slightly lower mean annual temperature of 11.1°C (Fraser et al. 2012) compared to 13.8°C at Scott Farm (Mudge et al. 2011; NIWA 2015). Gill and Jackson (2000) showed that root turnover rates increased with increasing mean annual temperature. With more temperate conditions, it could be expected that better plant growth with faster root turnover would be expected year round compared to that of a cooler climate.

Another factor that may have increased the root turnover rate in this study compared to those measured by Scott et al. (2012) was soil moisture content. This study coincided with a dry period where soil moisture contents decreased below the permanent wilting point for a sustained period of time (Figure 4c and 5). Wedderburn et al. (2010) reported that ryegrass responded to the onset of drought by initially increasing root growth presumably to search for more water, but this was followed by a rapid increase in root death as the drought continued. The onset of drought in the current study may have caused an increase in root turnover compared to that expected over the same period of time under average climate conditions.

In contrast, others have measured much faster root turnover rates in New Zealand pastures. Saggar and Hedley (2001) measured root turnover times between 128-160 days using a ^{14}C labelling method on a grazed dairy farm, while Gibbs and Reid (1992) and Reid and Crush (2013) measured turnover times of around 44-46 days for ryegrass plants using a minirhizotron method. However, minirhizotrons, have a tendency to overestimate the fine root turnover times compared to isotopic methods (Pritchard and Strand 2008; Tierney and Fahey 2002).

Root turnover was estimated to be greater under the moderately diverse pasture (438 days) compared to ryegrass-clover pasture (694 days) using data from McNally et al. (2015) and using a method (turnover = annual belowground production / average standing root mass) similar to Gill and Jackson (2000). However, this estimate was based on root mass measurements, which may not have accounted for the dynamic nature of root turnover (Yuan and Chen 2012). Although the isotope method used in this study provides another method of root turnover compared to using root mass measurements (Yuan and Chen 2012), the high variability in the results made it difficult to draw conclusions on differences in turnover rates between pasture swards.

The lack of clear statistical difference between the two swards may also reflect that both pastures are based on a similar base of plant species. The moderately diverse pasture, in this study, contained ryegrass and clover as well as lucerne, chicory and plantain. Because ryegrass and clover are present in both pasture swards, it might be expected that the results of root turnover would be similar. Given the difficulty in representing all the species in each sampling, due to the spatial variability of the species, it is perhaps not surprising that the root turnover rates were difficult to distinguish. The greater variability of root distribution of the moderately diverse pasture compared to the ryegrass-clover (McNally et al. 2015) was also likely contributing to the greater spatial variability of roots from the individual species contributing to each core. In particular, several of the species in the moderately diverse sward have large taproots (lucerne, chicory) that are difficult to adequately sample given the small area of the sample plots. Consequently, there was difficulty in fully representing all the individual species

contributing to the moderately diverse pasture for every sampling based on the size of the labelled plot and the number of cores taken. However, if the root turnover rates between the two pasture swards were similar, and given the moderately diverse pastures contained greater root biomass (McNally et al. 2015), a greater input of root material (C input) could still be expected under the moderately diverse pastures compared to the ryegrass-clover pasture. However, this tentative conclusion requires further investigation using a similar method with improved sensitivity and perhaps greater replication.

Root turnover times in this study may also be influenced by root diameter which would be expected to be different between the two pasture swards due to the presence of tap rooted species (lucerne and chicory). There have been numerous studies that suggest that root diameter is important for root turnover rates, although these have mostly been conducted in forest systems (Janssens et al. 2002; Joslin et al. 2006; Matamala et al. 2003). Fine roots are thought to have faster turnover rates (Gill et al. 2002; Gill and Jackson 2000; Janssens et al. 2002), and are more likely important for C inputs to soil compared to roots of thicker diameter. It was initially hypothesised that more diverse pasture swards, with species such as lucerne and chicory that have larger root diameters, lower specific root lengths and surface areas, would have very different turnover times than the ryegrass-clover sward.

McNally et al. (2015) analysed some key root traits for the species within the moderately diverse and ryegrass-clover pastures. Ryegrass and clover species were found to have higher specific root length, larger specific surface area,

smaller average root diameters, and a greater proportion of roots less than 2 mm in diameter. Lucerne and chicory typically had greater root diameters, smaller specific surface area and lower specific root length due to their tap-rooted nature and generally coarser roots. However, the root diameters of all the species within the two pastures in the previous study all displayed a high proportion of roots less than 2 mm diameter (91% for lucerne to 99.9% for ryegrass; (McNally et al. 2015). Therefore, as all species had a large proportion of fine roots, it is perhaps not surprising that the root turnover times were similar for the two pastures. It is also acknowledged that this method would not have clearly distuinguised between root diameter classes and is more an integral of multiple root diameters. Further investigations on the influence that root diameter has on the root turnover and root decomposition would be beneficial to understanding how roots contribute to C sequestration.

4.5.2 C input to soil

The C input to soil (0-100 mm depth) ranged from 3 kg C ha^{-1} d^{-1} up to a maximum of 230 kg C ha^{-1} d^{-1} with an average input of 90 kg C ha^{-1} d^{-1} and 25 kg C ha^{-1} d^{-1} over 88 days for the moderately diverse and ryegrass-clover pastures respectively. In general, it appeared that the moderately diverse pasture had a greater daily C input between 7 and 28 days after isotope labelling compared to the ryegrass-clover pasture, however, this difference was not significant. The increase in C input between 14-28 days after labelling (Figure 7) coincided with the soil being under severe soil moisture stress and having soil moisture contents lower than permanent wilting point (Figure 4c and 5). The second increase in C input between 58-88 days (Figure 7) after labelling coincided with rainfall re-

wetting the soil. No statistical difference in C input to soil could be detected between the two pastures, and a combined average C input of 58 kg C ha^{-1} d^{-1} was measured over 88 days.

The values for C input in this study appear to be generally greater than measured in other studies of pasture swards (Saggar and Hedley 2001; Saggar et al. 1997; Saggar et al. 1999), however, as this study did not make measurements for the different seasons, it is not reasonable to extrapolate data to C input on an annual basis. Consequently, C inputs from other studies have been converted to a daily input for comparison. Saggar and colleagues (1997; 1999; 2001) conducted a series of studies using ^{14}C pulse labelling measuring C inputs to roots and in high and low fertility systems and for different slope classes. Following ^{14}C isotope labelling, Saggar and Hedley (2001) measured an input of between 10 - 40 kg C ha^{-1} d^{-1} to the roots and a C input to soil of approximately 5 – 10 kg C ha^{-1} d^{-1} under a dairy pasture depending on season. On hill country soils, Saggar et al. (1997) measured lower inputs of between and 1.5 – 2.5 kg C ha^{-1} d^{-1} for low and high fertility systems. Pastures with higher nutrient inputs allocated more C to roots and soil than pastures with lower nutrient inputs. Saggar et al. (1999) measured daily C input to the soil of between 2 (steep slope) and 7 (low slope) kg C ha^{-1} d^{-1} with a greater C inputs on low slope pastures compared to steeper slope pastures. The pastures on steeper slopes allocated less C to the roots and soil than pastures on the lower slope.

The current study at Scott Farm would be considered most comparable to the dairy system in Saggar and Hedley (2001) and the low slope and high fertility

system of Saggar et al. (1997) and Saggar et al. (1999). Carbon inputs to soil were measured under a high fertility dairy farm near Palmerston North, New Zealand receiving annual inputs of 90 kg N ha^{-1} and 40 kg P ha^{-1}, and average rainfall of 962 mm (Saggar and Hedley, 2001). The current study at Scott Farm, received similar amounts of P fertiliser, higher rates of N fertiliser and greater average rainfall (1117 mm compared to 962 mm). Pasture production (11600 kg DM ha^{-1} moderately diverse & 10900 kg DM ha^{-1} ryegrass-clover) during the labelling year for the current site was lower than that reported for Saggar and Hedley (2001) of 16020 kg DM ha^{-1}.

The hill country studies of Saggar et al. (1997; 1999) were carried out on the Ballantrae Hill country research station grazed by sheep. Although P fertiliser rates were higher at Ballantrae compared to Scott farm, the applied N was much higher in the current study at Scott Farm. Therefore, the findings of this study with an average daily C input of 58 kg C ha^{-1} d^{-1} (for both pastures) are high but not unreasonable given higher N inputs and flat land and the severe soil moisture stress in comparison to the findings of Saggar and Hedley (2001) and Saggar et al. (1997; 1999). The higher average inputs reported in this study was partly due to some very large calculated inputs on certain sampling periods. The maximum input of 230 kg C ha^{-1} d^{-1} and the elevated inputs of C to soil was likely due to the severe soil moisture stress during drought and substantial root death. Reid and Crush (2013) and Wedderburn et al. (2010) demonstrated that ryegrass responded to drought with increased root growth in the short term to seek out water. As soil moisture continued to be low there was then a large increase in root death. The increased C inputs between days 14 and 28 after labelling could have been due to

death of roots following the moisture status of soil dropping below permanent wilting point.

Root mass in this study increased between background sampling (late spring) and the end of labelling (Day 0), before a large decline in root mass when soil moisture contents decreased (Appendix 1e). Root production and the subsequent seasonal decline agreed with previous measurements of McNally et al. (2015) for the same study site, where maximum values of root mass were observed in summer, following spring growth, before a decline to a minimum in winter. With the moderately diverse pasture having greater root mass and deeper roots compared to ryegrass-clover (McNally et al. 2015), it might be simply expected that the C input to soil from roots would also be greater under the moderately diverse pasture. However, it was noted that ryegrass contributed more to the belowground C pools than clover in a study on ryegrass-clover in Denmark (de Neergaard and Gorissen 2004). In the current study all plots had at least 50% cover of ryegrass. Therefore, as both pastures are based on a ryegrass-clover pasture, the lack of statistical difference could be due to the importance that ryegrass has in the C input to soil compared to the other species.

Ryegrass had the greatest specific root length and surface area of all the species in the two pastures and also the greatest proportion of fine roots (<2mm) (McNally et al. 2015). The greater surface area and root length may mean that relative importance of ryegrass to the C input to soil may be greater than the other species present in the moderately diverse pasture. Ryegrass typically has a low resilience to severe moisture stress (Wang and Bughrara 2008) and as a result the C inputs

observed between days 14 and 28 may be caused by the death of the ryegrass roots and the subsequent input of C from this root turnover.

The measurements of C assimilated to roots during labelling by Saggar and Hedley (2001) under a dairy farm (10 – 40 kg C ha^{-1} d^{-1}) could support the elevated C input to soil in this study (average C input of 58 kg C ha^{-1} d^{-1}) being due to root death, assuming similar C allocation to roots in the current study. Root mass measurements in this study could also explain, although not account for all, of the large C inputs to soil from root death measured in this study (Appendix 1e). Estimations of the C input from the below ground production/turnover of roots from various studies on New Zealand pastures (Dodd and Mackay 2011b; McNally et al. 2015; Scott et al. 2012) is approximately 5 kg C ha^{-1} d^{-1}. However, these values did not include rhizodeposition from roots which would be a substantial contributor to the C input to soil (Nguyen 2003). Therefore, it would be expected that the actual C input of soil to be greater than the 5 kg C ha^{-1} d^{-1} but would also be dependent on other factors such as season and pasture production. Further research to better quantify the C input to soil under moderately diverse pastures would be beneficial.

4.6 Conclusion

The objective of this study was to better understand the root turnover and C input to soil under moderately diverse pastures compared to ryegrass-clover pastures. No difference in root turnover and C inputs between the two pastures was detected likely because both pastures contained a substantial cover of ryegrass, which is likely contributing most of the C input into the soil. However, based on the large variability of results and the severe drought that occurred during this

study, it was difficult to determine whether there was no difference or whether the variability in the results was too large to observe a difference. Further research is needed to investigate the root turnover and C input to soil in regard to root traits of individual species and across a range of seasons and climatic conditions in order to make better statements on the C sequestration potential of the moderately diverse pastures.

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Chapter 5: Root turnover and C input following spray-off and pasture renewal compared to an existing ryegrass-clover sward.

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Sam McNally, Louis Schipper, Daniel Laughlin, Susanna Rutledge, Mike Dodd and Johan Six decided on the experimental design. Sam McNally collected and analysed samples, carried out statistical analysis and was responsible for the writing of the manuscript. Louis Schipper was the primary reviewer of this work with Daniel Laughlin, Susanna Rutledge, Mike Dodd and Johan Six providing additional comments to improve the manuscript. Daniel Laughlin further provided assistance with statistical analysis.

5.1 Abstract

Increasing the turnover of roots is considered to be one method that may increase C inputs to soil and potentially soil C storage in pastures. A common practice in grazed pastures is to periodically renew the pasture swards and this includes the use of herbicide to kill the existing sward. The use of herbicide is expected to increase root inputs and turnover as plant death occurs. However, the effect of herbicide on root turnover and the subsequent C input to soil has not been quantified. Therefore, the objective of this study was to quantify the impact of pasture renewal on the root turnover and C input to soil of ryegrass-clover pastures. Pastures were labelled in the field using a ^{13}C isotope pulse labelling method within 1 m^2 clear chambers, where plants take up ^{13}C via photosynthesis. Five labelling events were consecutively carried out over 5 days in 3 replicate

paddocks of two treatments (2 plots per paddock). The pasture sward in one plot of each paddock was sprayed with herbicide (glyphosate) and then direct drilled with seed into the soil to re-establish the sward. The ^{13}C was measured in roots and soil (0-100 mm) at regular intervals over an 89-day period in both treatments. There was a rapid root turnover in the treatments that had been sprayed with herbicide resulting in an initial root turnover time of 17 days. Subsequently, root turnover slowed to about 524 days, similar to the unsprayed pastures, which had a root turnover of 585 days. The faster initial root turnover resulted in a greater cumulative C input to soil over 89 days with roughly two times the C input in the sprayed treatment (3238 ± 378 kg C ha^{-1}) compared to the unsprayed treatment (1726 ± 540 kg C ha^{-1}). The use of glyphosate during pasture renewal increased root turnover and resulted in a greater cumulative C input to soil over the short term but the amount of this input that would be stabilised in the soil is still to be known. This study provides the first values of root turnover and C input to soil during a pasture renewal event in New Zealand.

5.2 Introduction

Agriculture covers a large area of land globally producing food and fibre (FAO 2013; Lal 2004), and is also a source of greenhouse gases: CO_2, CH_4 and N_2O (Johnson et al. 2007). Increasing organic matter inputs to soil is considered to be one strategy for increasing soil C under agricultural management so that the land acts as a sink for CO_2 (Johnson et al. 2007; Lal 2009). Within agricultural land, temperate grasslands account for approximately 10-12% of the global soil C pool (Jones and Donnelly 2004; Lal 2004). Accumulation of C in these grassland ecosystems typically occurs below ground, with changes in C under these systems resulting from either land use change or grassland management (Soussana et al.

2004). One method to achieve increasing C inputs into grassland soils is by increasing root mass, rooting depth and root turnover (Kell 2012; Lal 2009).

In New Zealand, the use of year-round, permanently grazed pastures dominates the agricultural industry covering approximately 51-55% of the land surface. Dairy farms account for approximately 30% of pastoral land (StatisticsNZ 2012), and are largely located on flat, most productive land (MacLeod and Moller 2006; Rutledge et al. 2015). The other pastures are generally on hill country with much lower grazing intensity, typically grazed by sheep and beef. Dairy farms rely on productive pastures to remain profitable, and one way of ensuring pastures remain productive is to periodically renew pastures (Brazendale et al. 2011) with new mixes of more competitive, productive and persistent species (Brazendale et al. 2011; Clark et al. 2007). During renewal, the existing pasture sward typically is killed with a herbicide, and may be cultivated before reseeding. Killing the existing sward will likely result in a large input of C to soil as roots die and then decompose. In contrast, lack of C fixation by photosynthesis while pasture cover is absent (Rutledge et al. 2014; Willems et al. 2011) and enhanced microbial respiration due to soil disturbance during cultivation will likely result in C loss (Lal 2004; Rutledge et al. 2014; Smith et al. 2008). Consequently, the inputs of C to soil from pasture roots that have been sprayed with herbicide during pasture renewal events may be balanced by losses during the period of no or little photosynthesis and cultivation. However, the net effects of these inputs and decomposition on soil C have not been measured.

The initial step in pasture renewal involves spraying with herbicide, such as glyphosate, to reduce competition of established pasture on seedlings (Thom et al. 2011). Glyphosate inhibits an enzyme in the shikimic acid pathway, which disrupts the production of key aromatic amino acids and results in plant death (Baylis 2000). Consequently, after spraying there are likely to be large inputs of C to soil as roots, in particular, start to degrade. It is estimated that approximately 30% of photosynthetically fixed C passes through this shikimate pathway (Baylis 2000; Maeda and Dudareva 2012). It might be expected that a short-term disruption in the C translocation in plants would cause a change in the amount of the C transferred to the soil through root processes such as rhizodeposition, but the main input of C following herbicide use is likely to be from root death and turnover.

There is a general consensus that soil disturbance events such as frequent cultivation result in a loss of C from soil (Baker et al. 2007) but the effects of periodic cultivation are less well understood (Conant et al. 2007). In one of few studies, Rutledge et al. (2014) measured a short term (~40 day) C loss during a pasture renewal event (80 – 400 g C m^{-2}) involving herbicide and cultivation (ploughing), though the site recovered this loss after re-establishment of the new pastures within the year (Rutledge et al. 2015). The type of cultivation is also important and direct drilling is generally thought to result in less soil C loss compared to more intensive tillage because of lower soil disturbance (Paustian et al. 2000).

The net impact of pasture renewal on the input of C from plants to soil is unclear. It is unknown whether or not there is a net input of C during pasture renewal due to potentially offsetting effects of C loss during pasture renewal and large inputs of C following root death. Therefore, the objective of this study was to compare the root turnover and C input to soil during pasture renewal (herbicide application and direct drill) with that of an unsprayed ryegrass-clover pasture commonly used in New Zealand agriculture. This study was carried out using an isotope pulse labelling method followed by soil and root sampling.

5.3 Methods

5.3.1 Site Description

The study was conducted at Scott Farm, a research dairy farm owned and operated by the New Zealand dairy research organisation DairyNZ. The farm is located 7 km northeast of Hamilton in the Waikato region, North Island, New Zealand (37°46’13. 62’’S, 175°22’40. 64’’E). Mean air temperature, mean annual rainfall and soil moisture data (Appendix 2a) were recorded at the Ruakura climatological station approximately 6 km from the site (NIWA 2015). The soil type of the study location was a Matangi silt loam (Typic Orthic Gley Soil) (Hewitt 1993; Mudge et al. 2011). The concentration of total C and N in the surface soil (0–100 mm) was 7.7% and 0.72% respectively (Mudge et al. 2011). Total porosity (v/v) of the Ap horizon (0–250 mm) was 0.66 m^3 m^{-3}, field capacity (10 kPa) 0.54 m^3 m^{-3}, the lower limit of readily available water (100 kPa) 0.43 m^3 m^{-3} and permanent wilting point (1500 kPa) was 0.25 m^3 m^{-3} (Mudge et al. 2011).

The current study was sited on 3 replicate paddocks (0.5 ha) of perennial ryegrass (*Lolium perenne* L.) and white clover (*Trifolium repens* L.) pasture grazed by dairy cows, which will now be referred to as "ryegrass-clover". These paddocks were rotationally grazed year-round with an average stocking rate of 3 cows ha^{-1} and generally received 150 kg N ha^{-1} y^{-1} (Rutledge et al. 2014) and maintenance fertiliser (P= 35 kg ha^{-1} y^{-1}, K= 117 kg ha^{-1} y^{-1}, S= 50 kg ha^{-1} y^{-1}) (Woodward et al. 2013). This study was conducted between October 2014 and January 2015. The pasture sward last underwent pasture renewal in early 2010.

5.3.2 Isotope pulse labelling

To measure turnover of roots, $^{13}CO_2$ isotope pulse labelling of aboveground plant biomass was carried out using methods adapted from Stewart and Metherell (1999), Denef and Six (2006) and Kong and Six (2010). Two plots (1 m^2) were established side-by-side (1 m apart) in each of the three replicate paddocks so that there 6 plots in total (2 plots x 3 replicates). Plots were fenced (~2.5 m^2) to exclude cattle prior to and for the duration of the study to ensure that grazing had not taken place on the plots at least 2 weeks prior to labelling. Aboveground herbage was cut to a residual of 40 mm the week prior to labelling.

Isotope pulse labelling was carried out using 0.28 m^3 clear chambers (28 cm high) sealed to the ground using polyethylene film sheeting and sand bags (Appendix 2b, 2c, 2d). The clear chamber allowed photosynthesis to continue during labelling. Air was circulated using an electric fan mounted within the chamber and the CO_2 concentration was monitored during labelling within the chamber using an infrared gas analyser (LI-8100A, LI-COR, Lincoln, USA). After the

chamber was sealed and the CO_2 concentration had decreased below ambient (400 ppm) due to plant photosynthesis, a pulse of $^{13}CO_2$ was generated inside the chamber. $^{13}CO_2$ was evolved within the chambers using an aqueous solution containing approximately 1 g of $Na_2{}^{13}CO_2$ (99 atom %, Sigma Aldrich) and the addition of H_2SO_4 acid. The acid was injected into a vial containing the aqueous $Na_2{}^{13}CO_2$ solution through a rubber septum installed into the top of the chamber. The chambers were removed after approximately 40 – 60 minutes, once the CO_2 concentration dropped below ambient concentration (~100 ppm) as the label was taken up through plant photosynthesis. Chambers were replaced over the labelled plants that evening (before sunset) to capture $^{13}CO_2$ loss from plant respiration during the night when photosynthetic uptake ceased. Chambers were removed the following morning once the CO_2 concentration had dropped well below ambient concentration (~100 ppm) measured by an IRGA and plants had taken up the respired CO_2 again through morning photosynthesis. The re-covering of labelled plots overnight was carried out to maximise the total ^{13}C uptake by plants by recapturing overnight respiratory losses. Chambers were briefly removed (5 mins), resealed including a fresh aqueous solution of $Na_2{}^{13}CO_3$ and then spiked with a new pulse of $^{13}CO_2$ by addition of acid. Plots were labelled following this method for 5 consecutive days over one week between the 20 October 2014 and the 24 October 2014 (Spring, Southern Hemisphere) giving a total of 5 labelling events per plot.

5.3.3 Pasture renewal

After the last labelling (24 October) one plot in each replicate paddock was randomly chosen to go through a pasture renewal event involving herbicide and direct drilling of seed. Selected plots were sprayed (day 0) using 1.5 kg ha^{-1}

glyphosate (active ingredient) as normally used by farmers. Reseeding of the sprayed plots was carried out 7 days after spraying (31 October 2014). Aboveground biomass was cut and removed from all plots prior to direct drilling of seeds to simulate grazing by cows, a common practice after spraying. This aboveground biomass removal would also have minimised any contribution from aboveground shoots to soil C. Direct drilling was simulated on a small scale by using a disk hand edger to form the seed channels (120 mm apart and approximately 20 mm deep). Ryegrass seed was sown directly into the channels at a rate of 18 kg ha^{-1} and clover seed were broadcast sown across the entire plot at a rate of 3 kg ha^{-1}. After seeding, the plot was raked to simulate power harrowing. Emergence of seedlings occurred between the 7th and 19th November 2014. The second plot in each paddock was not altered in any way except for removal of aboveground biomass at the same time as the sprayed plots. Plots that were sprayed and pasture renewed are referred to as "sprayed" and plots that were not altered are referred to as "unsprayed".

5.3.4 Measurements and Sampling

Soil cores and herbage samples were collected before (background) and after isotope labelling from within all the 1 m^2 plots. These samples were collected 0, 4, 7, 14, 26, 28, 35, 42, 48, 55 and 89 days after the last labelling event. Soil samples were collected randomly within each plot using a 50 mm diameter soil core (200 mm depth) driven in using a maul. Previous work at this site demonstrated that 90% of measured root mass down to 300 mm depth was in the top 200 mm of soil for ryegrass-clover (McNally et al. 2015). Two soil cores were collected in each sampling event for each plot and the core divided into 100 mm depths and each depth bulked for the two cores. Roots were separated visually

from the soil with the aid of an 8 mm sieve and then roots washed with deionised water and dried in a 60°C oven to a constant weight (~48 hrs). The herbage of all plots (1 m^2) that was harvested (40 mm residual) within one week after labelling (but before direct drilling of the new sward, see previous section) and dried to constant weight in a 60°C oven. Subsequent harvests of aboveground material occurred during collection of cores by either removing vegetation from a 100 cm^2 area directly above the core or by harvesting all plots to coincide with grazing of the surrounding paddock. Sub-samples of plant material (root and aboveground herbage) were ground finely using a ball mill grinder. Soil was air-dried following sieving and a sub-sample also ground for isotopic and elemental C analysis.

Soil, root, and herbage samples were analysed for elemental C and isotopic C concentrations using a Europa Scientific ANCA-SL elemental analyser (Europa Scientific Ltd, Crewe, UK) coupled to a 20-20 Stable Isotope Analyser mass spectrometer (Europa Scientific Ltd, Crewe, UK). The results of the isotopic analysis were expressed as $\delta^{13}C = \left[\left(\frac{^{13}R_{Sample}}{^{13}R_{Standard}}\right) - 1\right]$

where $^{13}R = {}^{13}C/{}^{12}C$ and the standard is relative to Pee Dee Belemnite (PBD). Soil moisture contents were determined following oven drying a sub-sample of field soil at 105°C for 24 hours.

5.3.5 Calculations and statistical analysis

Root turnover was calculated using a method similar to that of Scott et al. (2012) whereby the ratio of isotope enrichment relative to peak enrichment was linearly regressed against the time since peak enrichment. The linear decline in ^{13}C isotope was used to calculate the *x* intercept when $y = 0$ for the linear regression equation to estimate root turnover time when all the label has been lost (Scott et al. 2012).

A piecewise regression model (Toms and Lesperance 2003) was applied to the sprayed treatment because root turnover rate appeared to change abruptly over the time of sampling. This resulted in two equations before and after the break point and we solved for *x* (days) when *y* = 0 (complete root turnover) to give estimates of root turnover. The breakpoint in the piecewise regression was determined by the data, not by an *a priori* choice.

The input of C from roots to the bulk soil was determined by first calculating the proportion (*f*) of soil C derived from the ^{13}C label (roots) using a method similar to Kong and Six (2010) whereby:

$$f = \frac{^{13}C_{soil} - ^{13}C_{natural\ abundance}}{^{13}C_{root} - ^{13}C_{natural\ abundance}}$$

where $^{13}C_{soil} = \delta^{13}C$ of the soil sample of interest, $^{13}C_{root} = \delta^{13}C$ of the root material, and $^{13}C_{natural\ abundance} = \delta^{13}C$ of the background soil sample taken before any labelling occurred.

To determine the input of new C from the roots into soil, the derived *f* value was multiplied by the total C of the soil (Kong and Six 2010).

Calculation of the C input to soil was confounded by some negative values of the *f* value describe above. Negative values occurred when the soil or root sample ($^{13}C_{soil}$ or $^{13}C_{root}$) in question had a $\delta^{13}C$ value lower than that of the original background $\delta^{13}C$ value ($^{13}C_{natural\ abundance}$) before labelling. This may occur when soil cores on a particular sampling date did not intersect with roots that had been adequately labelled. Even small negative values (which could happen randomly) calculate through to relatively large negative C inputs, which were assumed not to be possible. To account for these negative values, the average daily C inputs of

ryegrass-clover and moderately diverse pastures were calculated by conservatively assuming negative values to be a zero input. The C input to soil in the 100-200 mm depth was affected by a large number of negative values so that it was not possible to sensibly calculate the C inputs during each sampling. ^{13}C root and soil data is presented in Appendix 2e, 2f and 2g.

Repeated measures ANOVA was performed on the soil C input data using pasture type (sprayed vs. unsprayed) and labelling time (days after labelling) as factors. Paired *t*-tests were also performed on the C input data for all sampling periods. All statistical analysis was carried out using R (version 3.2.0) with the "segmented" (Muggeo 2008) and "Rcmdr" (Fox 2005) packages. For all tests $\alpha = 0.05$.

5.4 Results

5.4.1 Root turnover

Background $\delta^{13}C$ root values ranged from -30.49‰ to -29.28‰ before labelling and subsequently peak enrichment of roots following labelling had an average peak enrichment of -7 ‰ (Appendix 2e). Peak enrichment occurred on the last day of labelling (day 0) for both treatments before declining back towards background $\delta^{13}C$ values. The $\delta^{13}C$ of aboveground herbage displayed large isotope enrichment at the end of the last labelling (+150 – 200 ‰) compared to background enrichment levels (-30 ‰) (Appendix 2h). Despite the high enrichment of the plants, there was considerable variability in the $\delta^{13}C$ of the roots of individual samples throughout the sampling. This variability was likely due to the spatial variability of roots in the soil in relation to the aboveground plant

material and the difficulty in capturing this variability with only two cores per sampling. Despite this variability, there was a significant decline in the relative isotope enrichment ratio through time for the sprayed treatment (P= 0.039; 0 – 100 mm, Figure 8a) but not significant (slope and regression) for the unsprayed treatment (P=0.463; 0 – 100 mm depth, Figure 8a). While the unsprayed treatment did not have a significant slope, the line of best-fit equation was used to calculate a best estimate of root turnover (Table 8).

The decline of relative enrichment ratio for the sprayed pasture was better represented by a piecewise regression model (P = 0.0016; Figure 8a) compared to the linear regression model (P = 0.039; Figure 8a). There was rapid root turnover (17 days) between days 0 (spray-off) and 11 days (break point in piecewise regression) followed by a slower turnover rate (524 days) from day 11 to 89 (Table 8).

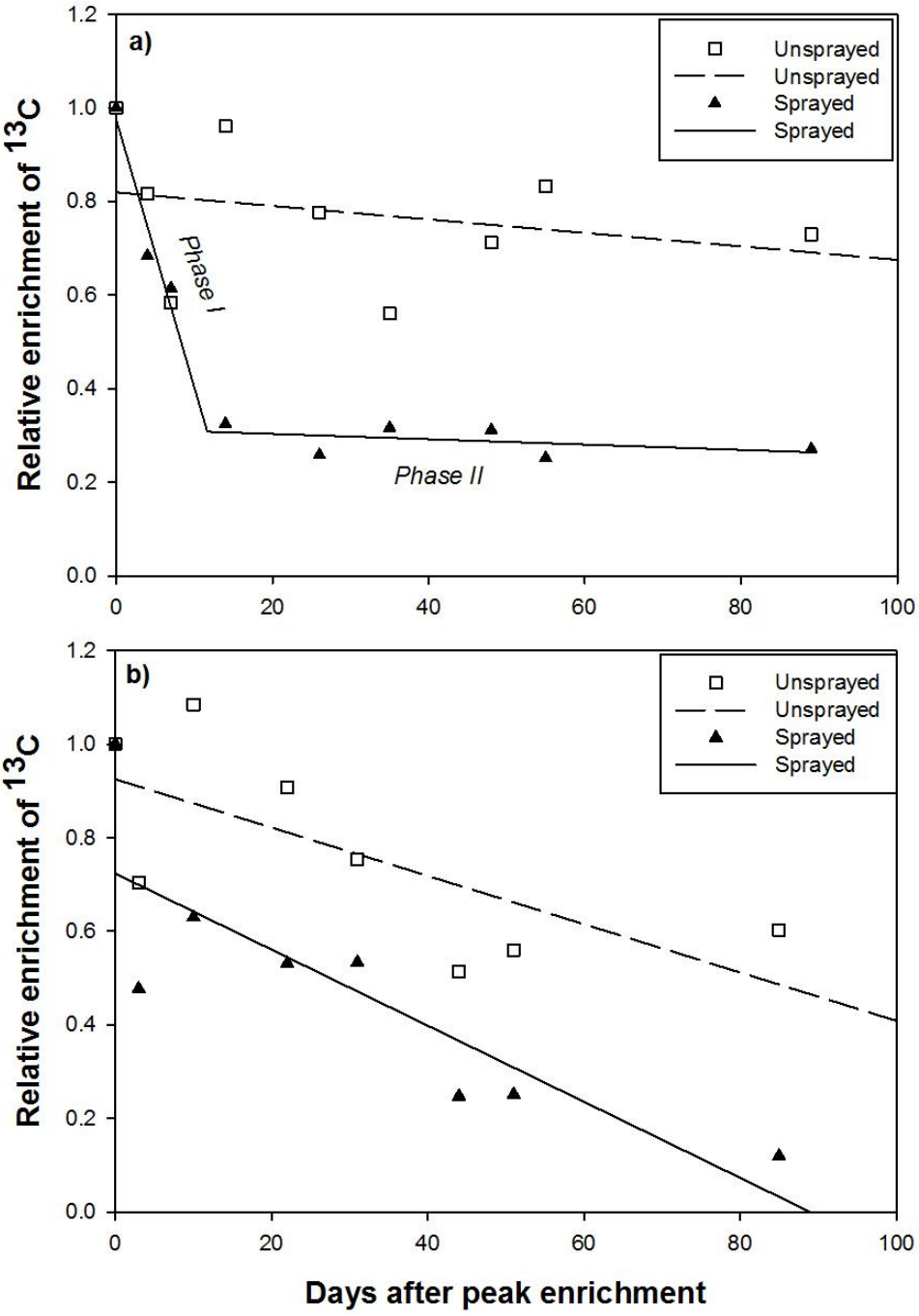

Figure 8 Relative isotope enrichment ratio of roots after peak enrichment for ryegrass-clover pasture with (sprayed, n=3) and without (unsprayed, n=3) the use of herbicide for a) 0 – 100 mm depth and b) 100-200 mm depth Each point is the average of three replicates. Line of best fit for a) equals y = - 0.0014 x + 0.8194, P=0.463 for the unsprayed, and for the sprayed treatment a piecewise regression (P=0.0016) with fitted breakpoint at 11 days was best defined by 2 phases, phase I (0-11 days) y = - 0.0568 x + 0.974, phase II (11 – 89 days) y = - 0.0006 x + 0.3141. For b) the line of best fit equals y = - 0.0052 x + 0.925, P=0.053 for the unsprayed, and y = - 0.0081 x + 0.724, P=0.009 for the sprayed treatment. Linear equations were used to solve for x when y = 0 to give an estimate or root turnover.

Root turnover for 100-200 mm depth was approximately 2 times faster in the sprayed treatment (89 days) in comparison to the unsprayed treatment (178 days; Table 8). The linear regression of the sprayed treatment was significant (*P*=0.009; Figure 8b) but the unsprayed treatment was marginally not significant (*P*=0.053; Figure 8b).

Table 8 Summary of the root turnover (days) under ryegrass-clover pastures (0-100 mm, 100-200 mm) with (sprayed) and without (unsprayed) the use of glyphosate.

	0-89 days	0-11 days*	11-89 days*
Unsprayed			
(0-100 mm)	585	n/a	n/a
(100-200 mm)	178	n/a	n/a
Sprayed			
(0-100 mm)	103	17	524
(100-200 mm)	89	n/a	n/a

* Determined by fitting piecewise regression for sprayed plots with break point of 11 days. n/a – not applicable as no piecewise regression was fitted.

5.4.2 C input to soil

The C input from the roots to soil for individual plots ranged from approximately 0.5 kg C ha^{-1} d^{-1} up to a maximum of 264 kg C ha^{-1} d^{-1} for both treatments. Average C input was 36 kg C ha^{-1} d^{-1} for the sprayed pasture and 19 kg C ha^{-1} d^{-1} for the unsprayed ryegrass-clover pasture (Table 9). There was no significant difference between treatment (P = 0.12) or time after labelling (P = 0.19) following repeated measures ANOVA, reflecting variability in data. However, results of paired *t*-tests, showed that the sprayed off pasture had a greater C input ($p<0.05$) across all sampling periods with the exception of the samples taken at 0 and 4 days after labelling was completed (Table 9). Samples taken at day 0 were before spraying so it would not be anticipated that there would be any differences. However, there was a greater C input under the unsprayed pasture compared to

the sprayed pasture 4 days after the last labelling (P= 0.04). Between days 7 and 89 after labelling the C input was always greater under the sprayed off pasture compared to the unsprayed treatment (Table 9). The net C input under both pastures was approximately 960 kg C ha^{-1} between 0 and 26 days after labelling. However, from the sampling at day 14 onwards, there was between 2 to 3 times the C input in the sprayed treatment compared to the unsprayed treatment, such that after 89 days there was approximately double the total C input to soil (P=0.002) under the sprayed pasture (3238 kg C ha^{-1}) compared to the unsprayed pasture (1726 kg C ha^{-1}). C inputs for the 100 – 200 mm depth are not shown due to the inadequate data (see methods section on data analysis).

Table 9 C input (kg ha^{-1} & kg ha^{-1} d^{-1}) to soil from roots (0-100 mm) for the sprayed and unsprayed treatment plots (n=3) after days since final labelling. Data within parentheses are one standard error and p-values are from a paired t-test between treatments (sprayed, unsprayed) at each sampling within the same replicate paddock (n=3). Daily inputs calculated at time 0 are based on the input over 5 days of labelling.

Treatment		0	4	7	14	26	35	48	55	89	Total C input
Sprayed	kg ha^{-1}	108(104)	132(111)	176(97)	305(182)	238(155)	381(95)	357(180)	629(403)	912(400)	3238(378)
	kg ha^{-1} d^{-1}	22(21)	33(28)	59(32)	44(26)	20(13)	42(11)	27(14)	90(58)	27(12)	
Unsprayed	kg ha^{-1}	226(214)	303(159)	105(55)	189(75)	140(119)	143(83)	216(83)	127(53)	277(78)	1726(540)
	kg ha^{-1} d^{-1}	45(43)	76(40)	35(18)	27(11)	12(10)	16(9)	17(6)	18(8)	8(2)	
p-value		0.08	0.04	0.02	0.04	0.03	0.02	0.02	0.05	0.02	0.002

5.5 Discussion

5.5.1 Root turnover

At a coarse level, spraying pasture with glyphosate (herbicide) during pasture renewal resulted in increased root turnover (average of 103 days) compared to the unsprayed ryegrass-clover pasture sward (585 days) in top 100 mm. However, piecewise regression demonstrated two different rates of root turnover in sprayed pastures: i) turnover immediately following the spray off with glyphosate (phase I) and ii) turnover during the re-emergence of the new pasture (phase II). The rapid root turnover of about 17 days (phase I) was much faster than the unsprayed root turnover and the secondary root turnover in phase II. This secondary root turnover (phase II) following pasture re-emergence (14-26 days after labelling) was 524 days (Figure 8a).

Glyphosate would be expected to increase the root turnover in comparison to existing pasture. Glyphosate typically takes effect over a period of 10 days (NPIC 2010), during which time the herbicide inhibits the shikimate pathway resulting in plant death (Baylis 2000), and therefore, root death which would likely increase root turnover. The root turnover in the 100 - 200 mm depth was also faster in the sprayed treatment compared to the unsprayed treatment.

As expected, the rapid turnover time of 17 days for the sprayed roots (phase I) was much faster than any previous measurements of root turnover of ryegrass systems (Gibbs and Reid 1992; Reid and Crush 2013; Saggar and Hedley 2001; Stewart and Metherell 1999). However, it is difficult to compare root turnover of the sprayed ryegrass to root turnover in other studies where root turnover was

measured when plants remained alive. This rapid root turnover under the sprayed treatment was much faster than that of the unsprayed pasture and that of unsprayed pasture during measurements on the same site the previous summer (Chapter 4).

Root turnover under the unsprayed ryegrass-clover pasture was approximately 585 days, and similar to the turnover time during phase II of the sprayed treatment. It is important to note that the root turnover for the unsprayed ryegrass-clover in this study was calculated using a non-significant linear regression model as a best estimate. The inability of this current methodology to detect slow turnover is an acknowledged weakness that might be overcome with greater sampling density. However, an estimate of root turnover was calculated to provide a comparison to the sprayed treatment. The unsprayed pasture and second phase in the sprayed treatment had slower root turnover times (0-100 mm) than those determined during isotope labelling at the same site the previous summer (Chapter 4). In this earlier study, ryegrass-clover had root turnover times of approximately 276 days during a summer period with severe moisture stress which likely increased root turnover due to plant death during drought. Current measurements of root turnover were not made during a period of moisture stress and so likely explain the difference in measurements between these two studies at the same site. A period of moisture stress did occur between January and February 2015 but this was after sample collection in this study.

Root turnover times of this study fall between measurements of other studies on ryegrass-clover in New Zealand (Table 2; Chapter 2.5). Saggar and Hedley (2001)

using ^{14}C isotope labelling measured root turnover of between 128 and 160 days under a high fertility dairy pasture. In contrast, Scott et al. (2012), measured turnover times of between 400-800 days under ryegrass with different fertiliser and irrigation management following ^{13}C pulse labelling.

The turnover time during phase II (sprayed) and the unsprayed pasture are also similar to turnovers measured in other studies (Scott et al. 2011; McNally, 2015). The similarity between the phase II (sprayed) and unsprayed turnover times may be due to the new un-labelled roots in the sprayed treatment interfering with the ^{13}C signal that was measured. With the observed appearance of the new pasture at 26 days after peak enrichment, the isotope signal would likely be diluted by the new unlabelled roots. These new roots were not able to be distinguished from the older roots that would have been sprayed and as a result the contribution from the new roots (unlabelled) would have likely decreased the ^{13}C enrichment of the roots. However, the slower turnover calculated after the break point in piecewise regression might also reflect a slower more passive root decomposition pool in comparison to the initial rapid turnover of roots. Fine roots would be expected to turnover and decompose more rapidly than more coarse roots (Gill and Jackson 2000) and hence the rapid turnover seen in phase I might be a measure of fine root turnover with phase II a measure of turnover of coarse roots.

Wardle et al. (1994) measured root decomposition in ryegrass following application with glyphosate using litterbags. Ryegrass had relatively slow root decomposition with approximately 50% of root material remaining after 338 days that was attributed to the fibrous and resilient nature of ryegrass roots. Therefore,

the initial rapid turnover seen in the sprayed treatment may reflect the turnover of the fine roots following glyphosate application, followed by the coarser roots which had a slower turnover. However, as root diameter was not measured in this study and because of the difficulties in sampling very fine roots it is not possible to draw a firm conclusion on the effect of root diameter on turnover.

5.5.2 C Input to soil

There was generally greater daily and net C input to soil in the sprayed treatment compared to the unsprayed pasture with approximately double the C input to soil after 89 days (3238 ± 378 kg C ha^{-1} compared to 1726 ± 540 kg C ha^{-1}). This was in agreement with the much faster root turnover in the sprayed treatment.

The C input under the unsprayed pasture had an average C input of 19 kg C ha^{-1} d^{-1} over 89 days. This input was larger than previous measurements under ryegrass-clover pastures in New Zealand. Saggar and Hedley (2001) measured C input in a high fertility dairy pasture of between 5 kg C ha^{-1} d^{-1} to 10 kg C ha^{-1} d^{-1} following ^{14}C pulse labelling. The high fertility dairy farm studied by Saggar and Hedley (2001) received annual inputs of 90 kg N ha^{-1} and 40 kg P ha^{-1} and had a 30-year average rainfall of 962 mm. Carbon inputs from roots to soil for hill country pasture have generally reported to be even lower, with between 1.5 kg C ha^{-1} d^{-1} to 7 kg C ha^{-1} d^{-1} (Saggar et al. 1997; Saggar et al. 1999). However, these later two studies were both measured in hill country systems, which have much lower fertiliser inputs (125 – 325 kg single superphosphate ha^{-1}) in comparison to a flat land dairy system in the current study. The current study at Scott Farm, received similar amounts of P fertiliser, but higher rates of N fertiliser (150 kg ha^{-1} y^{-1}) and greater average rainfall (1117 mm). It is perhaps not unreasonable that the

unsprayed pastures in the current study would have larger C inputs than these studies of dairy and hill country pastures (Saggar and Hedley 2001; Saggar et al. 1997; Saggar et al. 1999; Stewart and Metherell 1999) that had lower N inputs and annual rainfall.

The greater C input under the sprayed pasture was likely due to increased inputs to soil from higher root turnover. The inputs of C into soil also likely followed the 2 phases observed in the root turnover. Glyphosate causes plant death, and hence, root death (Baylis 2000; NPIC 2010), which would act to increase the inputs of C to soil in the short term. There was no difference in C input between the two treatments at day 0 and importantly a greater input under the unsprayed pasture at day 4 compared to the sprayed pasture. Glyphosate was applied at day 0 after sampling of the two plots, so the C input to soil for that sampling would not be expected to have yet been influenced by the glyphosate. The greater input under the unsprayed pasture at day 4 was likely due to an initial reduction in photosynthesis and C allocation to roots following application of glyphosate to the sprayed treatment. Glyphosate inhibits the shikimate pathway of which about 30% of photosynthetically fixed C passes through (Baylis 2000; Maeda and Dudareva 2012). An inhibition of this pathway would immediately start to decrease the flow of C through this pathway resulting in a reduction in plant growth and transport of C to roots, but root death and accelerated degradation is likely delayed for between 4 and 20 days while glyphosate takes effect (NPIC 2010). Consequently, C inputs into the soil would initially be less in the sprayed treatment than the unsprayed treatment. The measured C input to soil after the sampling at day 4 was always greater under the sprayed pasture compared to the unsprayed pasture. This

increased C input in samples between days 7 and 89, was always about 2-3 times the input in the unsprayed pasture and likely is the result of increased root death and decomposition of roots.

The total C input after 89 days under the sprayed treatment (3238 kg C ha^{-1}) was not unreasonable considering there was approximately 9000 kg DM ha^{-1} root mass at the time of spray off, which equates to approximately 3600 kg C ha^{-1} (Appendix 2i). Therefore, by causing this standing root mass to die and turn over through the use of herbicide would theoretically cause a large input of C to soil. However, it is not reasonable to suggest all of this root mass remains in the soil, as a large portion of this C input would be expected to be lost through microbial respiration which was not measured in this study. Wardle et al. (1994) measured slower decomposition of ryegrass root tissue following herbicide use, so it is also unreasonable to suggest that all the root material would decompose so rapidly to produce this C input.

During pasture renewal (including spraying and cultivation), C inputs through photosynthesis stop while plant growth is killed or suppressed. However, microbial activity would continue during this period, which typically results in a loss of C until plant growth resumes to offset this microbial respiration by fixing new C through photosynthesis (Rutledge et al. 2014). Given the pasture renewal in this study was carried out over a short timeframe and pasture emergence was observed within 26 days after spray-off, the losses of C while no photosynthesis is occurring would likely be minimal compared to if a longer cultivation period had occurred. Rutledge et al. (2014) measured a short-term C loss following

cultivation and pasture renewal at the same study site at paddock scales using eddy covariance and full C budgets, and demonstrated a recovery of lost C on an annual scale (Rutledge et al. 2015). Therefore, given minimal C loss during the renewal stage and good pasture regrowth, a pasture renewal event may increase the C input to soil by approximately twice that of an existing pasture in the short term. However, the amount of that C which would be stabilised into long-term soil C pools requires further investigation. Future research should also focus on the stabilisation of this fresh C input within soil aggregates by using techniques such as soil physical fractionation.

5.6 Conclusion

The objective of this study was to determine the root turnover and C input to soil in a ryegrass-clover pasture during a pasture renewal event (herbicide and direct drill) using ^{13}C isotope pulse labelling. The results demonstrated that there is an initial rapid root turnover of 17 days (phase I, 0-11 days after spraying) under the sprayed treatment, followed by a slower root turnover of 524 days during phase II (12 – 89 days after spraying). The phase II turnover time compared well with 585 days measured for the unsprayed pasture although this latter turnover rate was poorly defined. The initial rapid turnover was likely due to rapid root death and decomposition of fine roots following application of herbicide. The increased root turnover following herbicide use resulted in a greater cumulative C input to soil in the sprayed treatment (3238 ± 378 kg C ha^{-1}) compared to the undisturbed pasture (1726 ± 540 kg C ha^{-1}) over 89 days. However, it is unclear how much of this C would be stabilised long term in the soil.

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Chapter 6: Summary and Conclusions

Atmospheric concentrations of greenhouse gases (CO_2, CH_4, and N_2O) have risen to the highest levels in recent years due to anthropogenic emissions. Consequently, changes in the climate are being observed due to surface warming caused by these emissions (IPCC 2014). While burning of fossil fuels is a major contributor to total greenhouse gas emissions, agriculture, land use and land management have contributed roughly 30% to these greenhouse gas emissions (Burney et al. 2010; Cole et al. 1997).

Soils contain the largest pool of C in the terrestrial ecosystem, and can either act as either a sink or source of CO_2 to the atmosphere. Agricultural land covers a large percentage of the global land surface (40%). Within agriculture, land use and land use change contribute to these anthropogenic emissions of CO_2 with practices such as deforestation, burning of biomass, drainage of wetlands and cultivation all increasing emissions of CO_2 (Lal 2004). However, changes in management practices can also increase the soil C pool and act as a sink for atmospheric CO_2. These practices include re-vegetating degraded land, and reducing cultivation events by converting arable land to forest or grassland (Powlson et al. 2011).

In New Zealand, agriculture covers between 51 – 55% of the land surface and the majority of this land is primarily used for grazing of dairy cows, beef and sheep (MfE 2010; StatisticsNZ 2012). This agricultural land is an important source of greenhouse gas emissions with approximately 50% of New Zealands total emissions being attributed to agricultural emissions of N_2O, CH_4 and CO_2. While

N_2O and CO_2 emissions can be attributed to soil processes, it is important to note that in agriculture, CH_4 is largely attributed to ruminant digestion rather than soil processes. Management of these pastures has changed substantially in recent decades with increased fertiliser input and higher stocking rates to maximise production and profitability (MacLeod and Moller 2006). Furthermore, certain land uses and soil types in New Zealand have been shown to have lost C during this period (Schipper et al. 2010), however, the causes for these losses were largely unknown. Losses of soil C under certain land management present an opportunity to again increase the C in these soils by changes in management. Indeed, Beare et al. (2014) argue that many of New Zealand soils have a saturation deficit and are below their potential upper C concentration (C saturation). Increasing the root mass and rooting depth under these pastures has been proposed as a strategy to increase the C inputs in New Zealand soils (Dodd et al. 2011a). Soil deeper in the soil profile has a greater C saturation deficit (Beare et al. 2014) and increasing inputs of C to depth through roots may offer considerable scope for increasing soil C.

The majority of grazed pastures in New Zealand are based on perennial ryegrass and white clover mix, but these pastures are typically shallow rooting with about 80% of the root mass in the top 20 cm of soil (Crush et al. 2005). Recently, the use of mixed pasture swards, containing a greater number of species, has become of interest to farmers for their perceived increased drought tolerance and more consistent annual dry matter production (Woodward et al. 2013). With the number of extreme weather events, such as drought, forecasted to increase as a result of climate change (Orwin et al. 2015), the use of these pastures may become more

common. The use of these mixed sward pastures have also been hypothesised to increase the root mass and rooting depth of pastures due to the greater number of species and species with larger root systems compared to the ryegrass-clover pastures. However, there is limited information available on the root dynamics of pastures in New Zealand and no information on root mass and root dynamics for the mixed sward pastures. Improving the understanding of root dynamics under these pasture systems is important in order to better assess the potential for C sequestration in the grazed pasture systems.

The main objectives for this research were:

1) to quantify the changes in seasonal root mass of a perennial ryegrass and white clover pasture in comparison to a more diverse pasture including species such as lucerne, chicory and plantain;

2) to compare rates of root turnover and root C input to soil under a more diverse pasture in comparison with a perennial ryegrass and white clover pasture;

3) to compare the root turnover and C input to soil during pasture renewal (herbicide and direct drill) with that of an existing ryegrass-clover pasture.

6.1 Evaluation of Thesis Objectives

Chapter 3: Root carbon inputs under moderately diverse sward and conventional ryegrass-clover pasture: implications for soil carbon sequestration

This chapter specifically focussed on the root mass and seasonal change in root mass under a ryegrass-clover and a moderately diverse pasture sward. The central hypothesis was that the moderately diverse pasture would have greater root mass and rooting depth than the ryegrass-clover pasture due to a greater number of species and more diverse root traits. To test this hypothesis, soil cores were taken seasonally over one year and root mass measured following washing of roots from soil. Root traits of individual species were also analysed using WinRHIZO after extraction of plants. In addition, the change in seasonal root mass was used to calculate the root production and turnover to allow an estimate of the C input to soil to be made. Root mass varied seasonally from about 3500 kg ha^{-1} to 14000 kg ha^{-1} in the moderately diverse sward and 3000 kg ha^{-1} to 7600 kg ha^{-1} in the ryegrass-clover sward in 0 – 300 mm depth. The greatest root mass for both pastures was generally observed in the summer sampling, and this sampling was also when the greatest difference between root mass between pastures was observed. The range in root mass was similar to previous measurements made for ryegrass-clover pastures, although these studies had a wide range of root mass (700 – 24060 kg ha^{-1}; (Dodd and Mackay 2011b; Matthew 1996; Saggar and Hedley 2001; Saggar et al. 1997; Saggar et al. 1999).

This study provides the first measurements of root mass in moderately diverse pastures in New Zealand. The moderately diverse pasture had greater root mass across all seasons and greater rooting depth compared to the ryegrass-clover pasture. Due to the greater root mass under moderately diverse pasture there was

an estimated greater C input to the soil of approximately 1000 kg C ha^{-1} y^{-1}. This supported the hypothesis that there would be greater root mass and a greater C input to soil under the moderately diverse pasture compared to the ryegrass-clover pasture. However, this method of calculating the root turnover and hence C input using sequential soil cores can result in large errors in estimates as this approach does not fully capture fine root turnover or rhizodeposition (Milchunas 2009; Ziter and MacDougall 2013).

The greater root mass of moderately diverse pastures might be explained by the increase in diversity (Steinbeiss et al. 2008; Tilman et al. 2001; Tilman et al. 1996) but also could be due to the presence of specific species (Grime 1998). Studies in grasslands have demonstrated that more diverse communities had greater root mass compared to monocultures or low diverse communities (Tilman et al. 2001; Tilman et al. 1996). This increase in root diversity was suggested to occur due to niche complementarity, where species are able to coexist by partitioning resources and accessing different habitats, such as shallow compared to deeper rooting plants (Hooper 1998; Loreau and Hector 2001). However, there has also been suggestion that the increase in root mass could be driven by a dominant species (Grime 1998).

The root trait measurements demonstrated that lucerne and chicory in the moderately diverse pasture had lower specific root length and greater root diameter than the ryegrass and clover species. This indicated that the ryegrass and clover species had a greater proportion of fine roots. The presence of taproots in

lucerne and chicory increase the root diameter, decrease the proportion of fine roots, but increase the rooting depth.

The large variation of root mass within each seasonal collection for both pastures, particularly the moderately diverse pasture was likely due to the spatial heterogeneity of root distribution of individual species collected in each core. The roots of lucerne had higher root density (mass per volume) than all other species, meaning that for any given volume of root material, lucerne would have a greater mass. Differences in root densities likely also explain some of the variation in root mass through seasons. This variation in root mass may also indicate that the differences between the two pastures may be driven by a particular species, such as lucerne.

A major drought occurred during the summer sampling and this was when the greatest difference in root mass was observed between the two pasture swards. It is unclear whether the difference in root biomass between the two swards was directly attributable to this drought. Climatic factors such as annual rainfall and temperature have been identified as important controllers of root growth and production. Ryegrass and clover are shallow rooting species compared to lucerne and chicory. Therefore, the latter species are likely to be less affected by severe moisture stress. Possibly, for a different year with average rainfall and temperatures, the seasonal difference in root mass may not have been as large, and estimated C input to soil would be smaller.

The focus of this work was on the root mass under these two pastures which was used to estimate the C input to soil based on the seasonal production and decline of root mass. However, since this work was completed, I recognise that potential for re-allocation of C in roots to shoots may have occurred and decreased the root mass and C in the soil. Roots act as storage organs of resources such as C, and these reserves have been recognised as being involved during recovery after defoliation and persistence of pasture (Avice et al. 1997). These reserves of C have been suggested to be re-mobilised following defoliation by grazing to support re-growth of aboveground materials (Briske et al. 1996; Donaghy and Fulkerson 1998; Johansson 1993; Paterson and Sim 1999). However, other studies have demonstrated that defoliation of grasslands results in an increase of root litter inputs (Ziter and MacDougall 2013). Therefore, the role of this root to shoot re-allocation of C in these moderately diverse and ryegrass-clover pastures needs to be better assessed before any firm statement on the seasonal root production and turnover can be used to give estimates of C input to soil.

Chapter 4: Root turnover and root C input to soil under moderately diverse and ryegrass-clover pastures

The root sampling approach applied in Chapter 3 did not allow direct measurement of root turnover or C input to soil. The objective of Chapter 4 was to quantify and compare the root turnover and C input under moderately diverse and ryegrass-clover pastures. The hypothesis for this research was that the moderately diverse pasture would have greater root turnover and C input to soil, based on the indirect measurements of root turnover of Chapter 3. An isotopic method was used to address this objective by labelling plants with ^{13}C during photosynthesis and tracing the ^{13}C through the plant-root-soil system.

Results of the labelling demonstrated there was no difference in root turnover between the moderately diverse (298 days) and ryegrass-clover pasture (260 days) with a combined average root turnover time of 276 days. These root turnover times were faster than other measurements in New Zealand pastures using a similar ^{13}C labelling method (Scott et al. 2012) where root turnover was between 400 – 800 days (1.1 – 2.2 years). In contrast, other measurements reported faster root turnover times for ryegrass-clover pastures of between 128-160 days using ^{14}C isotope labelling (Saggar and Hedley 2001) and about 45 days using minirhizotrons (Gibbs and Reid 1992; Reid and Crush 2013). This study provides the first measurements of root turnover for moderately diverse or diverse pastures. The C input to soil also showed no difference between the two pastures with inputs of approximately 58 kg C ha^{-1} d^{-1} for an 88 day period. This C input was larger than other studies of New Zealand pastures where between 1.5 – 10 kg C ha^{-1} d^{-1} was measured across several studies of ryegrass-clover pasture (Saggar and Hedley 2001; Saggar et al. 1997; Saggar et al. 1999). Pastures in those studies

all received less N inputs than the pastures in the current study and some were on less intensively used hill country which could potentially explain the greater C input found in the current study. The total C input in this study averaged for both pastures was approximately 5100 kg C ha^{-1} over the entire 88 day period, which is very high compared to the total annual input of 1320 kg C ha^{-1} measured by Saggar and Hedley (2001) on a different dairy pasture. However, this study also occurred during a period of summer drought when there was severe moisture stress. This moisture stress likely increased root death and root turnover, resulting in larger C inputs to soil than might be expected in more benign climate conditions.

There was a large variation in the $\delta^{13}C$ values that were measured, which resulted in some negative C inputs calculated (see Chapter 4 for further details). Furthermore, the soil in this study had high total C concentrations (7-8%) so it was also likely that the labelling did not result in sufficient isotope label entering the soil to raise the background C levels enough to detect a difference. The method of labelling where plots were labelled once weekly for 5 weeks may not have caused a large enough pulse of ^{13}C (enrichment) in the soil to raise above the background C content of the soil. Previous studies using this isotopic approach at the Winchmore Research Station (Metherell 2003; Scott et al. 2012; Stewart and Metherell 1999) did not focus on the C transfer to soil, presumably because of similar limitations. However, those soils had much lower C contents (around 3-5%) and therefore a greater proportion of soil C may have been labelled. Therefore, future labelling studies may be best carried out on soils with lower C contents in order to get a greater proportion of labelled C into the soil C.

The number of cores taken during each sampling also may also have been insufficient to fully capture the spatial variability of the isotope enrichment within the soil. Two cores (50 mm diameter) per plot per sampling were taken and ideally the number of cores would be increased to capture greater spatial variability of plants and soil C. However, the size of the plots limited the number of cores that could be extracted with the larger diameter corer. Smaller diameter cores would have allowed more cores to be taken within the plots, but the spatial variability of the roots and species (particularly tap rooted species) would have been less well represented.

Another limitation during this study was effectively representing all the species within the moderately diverse pasture with each sampling. With only two cores collected each sampling, it may not have been possible to capture all the species with every sampling. This effectively meant that a large number of samples collected would have been dominated by ryegrass-clover. Ideally, every core or sample would have a contribution from all the species that matches the species composition of the entire pasture. This would help ensure that the C input and root turnover would reflect the entire pasture rather than a specific species immediately above each core at any given time. However, as the species composition changed during season (Chapter 3), the C input would likely change if the dominant species was responsible for the majority of C input. Therefore, while assessing the input of C under these more diverse pastures, it would be beneficial to determine the C input from individual species to determine whether a certain species is providing the dominant input of C or whether all species contribute equally.

To assess the C input under these diverse pastures a mesocosm type approach may be better suited. If a series of mesocosms were established with the same species composition and all labelled simultaneously, this would enable a separate mesocosm to be extracted at each sampling such that every sample would have roughly the same contribution from all species each sampling. However, there would be limitations using this type of approach also such as increased cost of equipment setup and greater labour involved.

The high variability of root turnover precluded identifying smaller differences between these two pasture swards. However, this lack of detectable difference may also be because there was no actual difference. If this was the case, there would be limited scope for increasing C in soil using the current mix of additional pasture species in the moderately diverse pasture swards. This lack of a difference is perhaps not surprising since both pastures are based on a ryegrass-clover base and both had a substantial cover of ryegrass-clover. Although there may be greater root mass under the moderately diverse pastures, the lack of a difference in C input to soil suggests that there may only be limited benefit to soil C under these pastures, or more time is needed to measure changes in C contents of soil from root decomposition. However, other added benefits of these moderately diverse pastures, such as increased drought tolerance through a more diverse root system mean these pastures are still of importance to farmers and land management. An improved pasture sward during drought conditions may help to minimise soil C loss (through microbial respiration) during periods where plant growth is stopped or suppressed.

Chapter 5: Root turnover and C input under a perennial ryegrass and white clover pasture with and without pasture renewal involving herbicide

The previous chapter measured the root turnover and C input from roots to soil of diverse swards and ryegrass-clover pasture. To convert from traditional ryegrass-clover pasture to more diverse swards, or renew the ryegrass-clover sward, requires removal of existing pasture either by cultivation and/or with herbicide and then reseeding. The objective of this chapter was to determine whether the use of herbicide and subsequent direct drill seeding during pasture renewal increased root turnover and resulted in an increased C input due to root death compared to an existing ryegrass-clover pasture. The main hypothesis of this study was that the use of herbicide would increase root turnover through plant death, and increase the C input to soil through root decomposition.

Isotope labelling methodology used in this experiment was the same as that used in Chapter 4, except for a change in the timing of the labelling. Rather than labelling weekly for 5 weeks, this study labelled plants daily over a 5 day period, which increased the relative plant enrichment. In Chapter 4, the relative enrichment ($\delta^{13}C$) of aboveground plant material increased from background levels (-30 ‰) to about +30 ‰. However, by increasing the frequency of labelling in the last experiment, plants were enriched to approximately +200 ‰. This increased enrichment resulted in increased average enrichment of the root tissue in this study (-7 ‰) compared to Chapter 4 (-18 ‰), although the enrichment in the soil was still similar for both studies. This improvement in the isotope labelling method during this study, where pastures were labelled daily for 5 days, would be recommended for future studies. Increasing the enrichment of the plant-root-soil

system may be further improved in soils with high background C contents by an additional increase in the number of labelling events.

The results of this study demonstrated that root turnover increased rapidly following application of herbicide with a turnover time of 17 days. This root turnover rate is much faster than that found in any of the previous studies on pasture systems in New Zealand (Gibbs and Reid 1992; Reid and Crush 2013; Saggar and Hedley 2001; Scott et al. 2012), because these other studies are on living pasture and not did not include substantial plant death following herbicide application. After this initial turnover, a secondary turnover time of about 524 days was observed. This estimated secondary turnover time was likely influenced by the re-emergence of new roots following pasture renewal, with pasture re-emergence occurring within 26 days after spraying with herbicide. The unsprayed pasture had a root turnover of 585 days, which was similar to the secondary turnover in the sprayed treatment. It is important to note that the root turnover of the unsprayed pasture was calculated using a non-significant regression, likely due to large variability in the $\delta^{13}C$ data. Therefore, while this root turnover time is a best estimate, caution is needed when using this value to compare to other studies.

However, the turnover times calculated in this study are in rough agreement to the measured turnover times in Chapter 4 and also to the turnover times measured by Scott et al. (2012) of 400-800 days in a ryegrass-clover pasture, suggesting that this estimate is not unreasonable. The two phases of root turnover could also suggest two pools of root turnover that are influenced by another factor such as

root diameter. The use of herbicide may have caused all the fine roots to die simultaneously and hence fine root turnover may have been observed during phase I, before a slower turnover of the coarser roots in phase II. Isotopic methods that measure root turnover through coring generally underestimate or do not entirely capture fine root turnover due to difficulties in the extraction of fine roots (Eissenstat and Yanai 2002). This issue of not entirely capturing fine roots in isotopic methods could also explain the large variation in data (particularly in the unsprayed treatment, and in Chapter 4) as certain samplings may have better represented fine roots than others. With fine root production and turnover occurring continuously in living plants this could be a strong influence on isotope loss from roots. By spraying with herbicide, effectively all the fine roots would have been killed and turned over, and hence this may have been picked up by phase I.

The C input from roots to soil following herbicide application was roughly double that of the unsprayed pasture over the first 89 days after spraying (3238 and 1726kg C ha^{-1}, respectively). The C input of the unsprayed pasture was similar but greater to the C input measured in a dairy pasture by Saggar and Hedley (2001) (1320 kg C ha^{-1}). The increased C input under the sprayed pasture was not unreasonable considering all the plants were killed through the use of herbicide, and the measured root mass at the time of spraying accounted for approximately 3600 kg C ha^{-1}. This study confirmed the hypothesis that the use of herbicide during pasture renewal increased root turnover through plant death, and resulted in about twice the C input to the soil compared to the unsprayed pasture. The stability of this additional C inputs need to be further investigated, but these

results suggest that pasture renewal involving herbicide and direct drill of seeds may result in a short term increase in C input to soil.

The data measured in these three studies will improve the knowledge of root mass and C inputs to soil under pasture systems and will be of benefit to improve models of C dynamics in grazed pasture systems.

6.2 Recommendations for future work

The work in this project initially demonstrated that the more diverse pastures had the potential to increase soil C due to greater root mass, rooting depth and greater seasonal changes in root mass. However, this potential was constrained as both pasture swards had similar root turnover rates and C input. In addition, the use of herbicide during pasture renewal appeared to increase root turnover and provide a C input to soil about double that of the existing pasture sward through root death. However, this research also poses a number of other questions that need to be addressed in future research to improve our understanding of the quantity of C that could be stabilised in long term C pools within the soil through increasing root mass and rooting depth.

6.2.1 Improve the understanding of the root dynamics between monocultures and mixtures of species used in pastoral production.

While there was greater root mass and rooting depth under the moderately diverse pasture compared to the ryegrass-clover pasture, it was not clear whether this increase was due to an increase in species diversity or due to the presence of a particular species. This has been a relatively common question posed in ecological literature mainly for grassland experiments (Tilman et al. 2001; Tilman et al. 1996).

Investigating the root mass, rooting depth and root traits of monocultures compared to more diverse mixtures of species that are beneficial for pasture production would enable better quantification of these rooting dynamics. Better quantification of these root dynamics would also improve our understanding of the species or mixtures of species that would best provide the traits that are most

favourable for C sequestration and potentially other useful traits for productive purposes. This would be best achieved by establishing small plot trials with monocultures and various mixtures of pasture species with differing diversities similar to those used in grassland diversity trials (Tilman et al. 2001; Tilman et al. 1996).

Chapter 4 also noted difficulty in representing all the species within the moderately diverse pasture during that study. Therefore, a better assessment of the species contributing to the C input and root turnover under more diverse pastures is also important to understand but currently appropriately sensitive methodologies are not available. Methods such as pyrolysis GC-MS and ^{13}C NMR (Kögel-Knabner 2000; 2002) can be used to identify specific compounds within SOM from certain C sources. More recently, methods involving DNA stable isotope probing have been used to investigate the microbial populations that are assimilating labelled plant material in the soil (Bernard et al. 2007; Lee et al. 2011). DNA techniques might be further used to trace the contributions of specific plant species into SOM.

Assessing these root dynamics under pastures over a range of climates and soil factors will also improve our understanding on whether these more diverse pastures would consistently provide increased root mass and root depth.

6.2.2 Improve the understanding on the effect of root diameter and other root traits on root turnover and C input

Root diameter is thought to be a control on the root turnover, with smaller diameter roots generally having faster root turnovers (Gill et al. 2002; Gill and Jackson 2000). However, little is known on the effect of root diameter on root turnover in pasture species. The use of herbicide demonstrated two rates of root

turnover, which could represent roots of differing diameter turning over at different rates. Therefore, improving our understanding on the effect of diameter and other root traits (e.g. surface area, specific root length, and tissue quality) and how these influence root turnover and C input may enable key traits that enhance C sequestration to be identified. Identifying these key traits might also enable pasture species to be better chosen to maximise C inputs into soils that have lost C. This question would be difficult to answer in field studies, but the use of root scanning software and lab incubation or litterbag studies enable these questions to be addressed.

6.2.3 What proportion of the C input from roots into soil under pasture systems is stabilised into stable SOM pools

Increasing C inputs into soil does not necessarily mean that this C will become stabilised long term, as there are a wide range of C pools in soil with different stabilities (Christensen 1996; Post and Kwon 2000; Six et al. 2002b). While there is some scope to increase soil C under more diverse pastures that have greater root mass and rooting depth, this research was unable to identify differences in C input under these pastures in comparison to ryegrass-clover pastures. Therefore, it is important to further quantify whether these pastoral systems contribute C into different soil C fractions (e.g. POM, microaggregate fraction, silt and clay fraction) (Kong and Six 2010; Six et al. 2000) and assess the stability of this added C. Assessing whether a specific pasture species contributes more to certain C pools or whether traits of certain species enhance the stability of added C would also be of interest. Results of chapter 5 also demonstrated that a greater C input to soil occurs following herbicide use that would increase root turnover through root

death. However, the amount of this C that would remain in long-term stable pools is also unclear.

There is a need to further investigate the C inputs from roots under pastoral systems and quantify how much of this input is stabilised into stable soil C pools. This question could be addressed by using fractionation schemes such as those outlined by many studies (Kong et al. 2005; Six et al. 2002b; Six et al. 2000) whereby C inputs are traced into various soil pools. This type of fractionation scheme, when used following isotope labelling, would allow the proportion of C incorporated to various fractions to be directly measured. The various fractions also broadly reflect various stabilities of that C pool. However, it is important to note that measuring the C input in soils with high background C may be difficult with ^{13}C.

6.2.4 Is microbial activity influenced by using more diverse pasture systems and does this improve C stabilisation in soils

Plant roots can influence the microbial populations of soil by the growth, activity and release of organic compounds from roots (Paterson 2003). Furthermore, microbial activity and fungi can influence the C sequestration of soils through interactions of microbial biomass and microbial by-products with soil properties such as clay minerals (Six et al. 2006). Therefore, a central question of how these diverse swards may impact microbial communities is:

Does the use of these mixed pasture systems alter the microbial communities compared to ryegrass-clover pastures, and if so does this change increase or decrease the C input and stabilisation to soil? This question could be addressed by investigating the microbial communities under various pasture systems. The analysis of PLFA's following isotope labelling and soil fractionation has been

used to investigate contributions of microbial activity to various soil C pools (Kong et al. 2011; Kong and Six 2012) and a similar method also using ^{13}C labelling may enable this question to be better understood.

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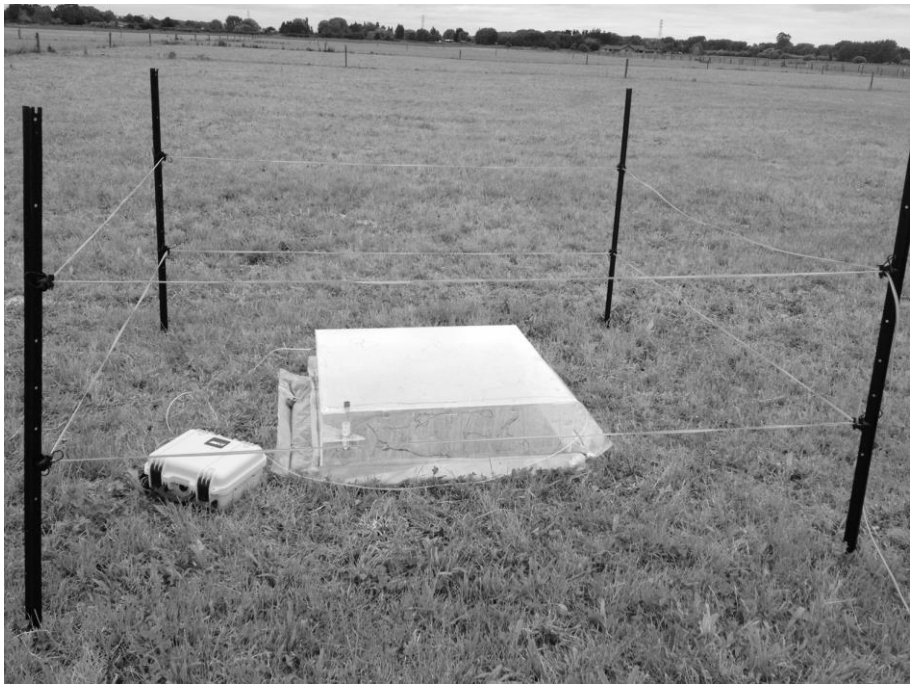
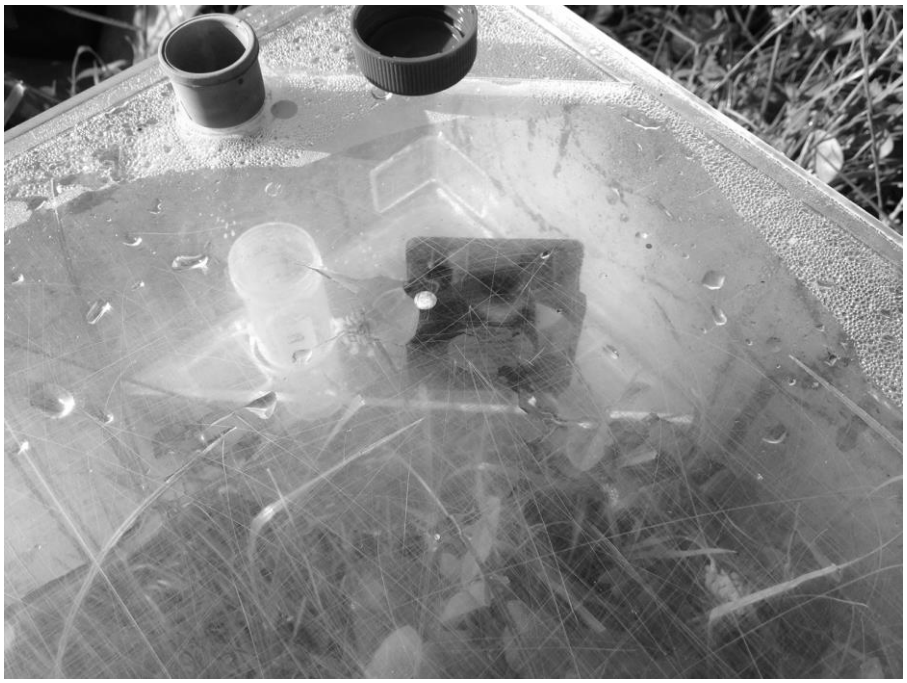
Appendices

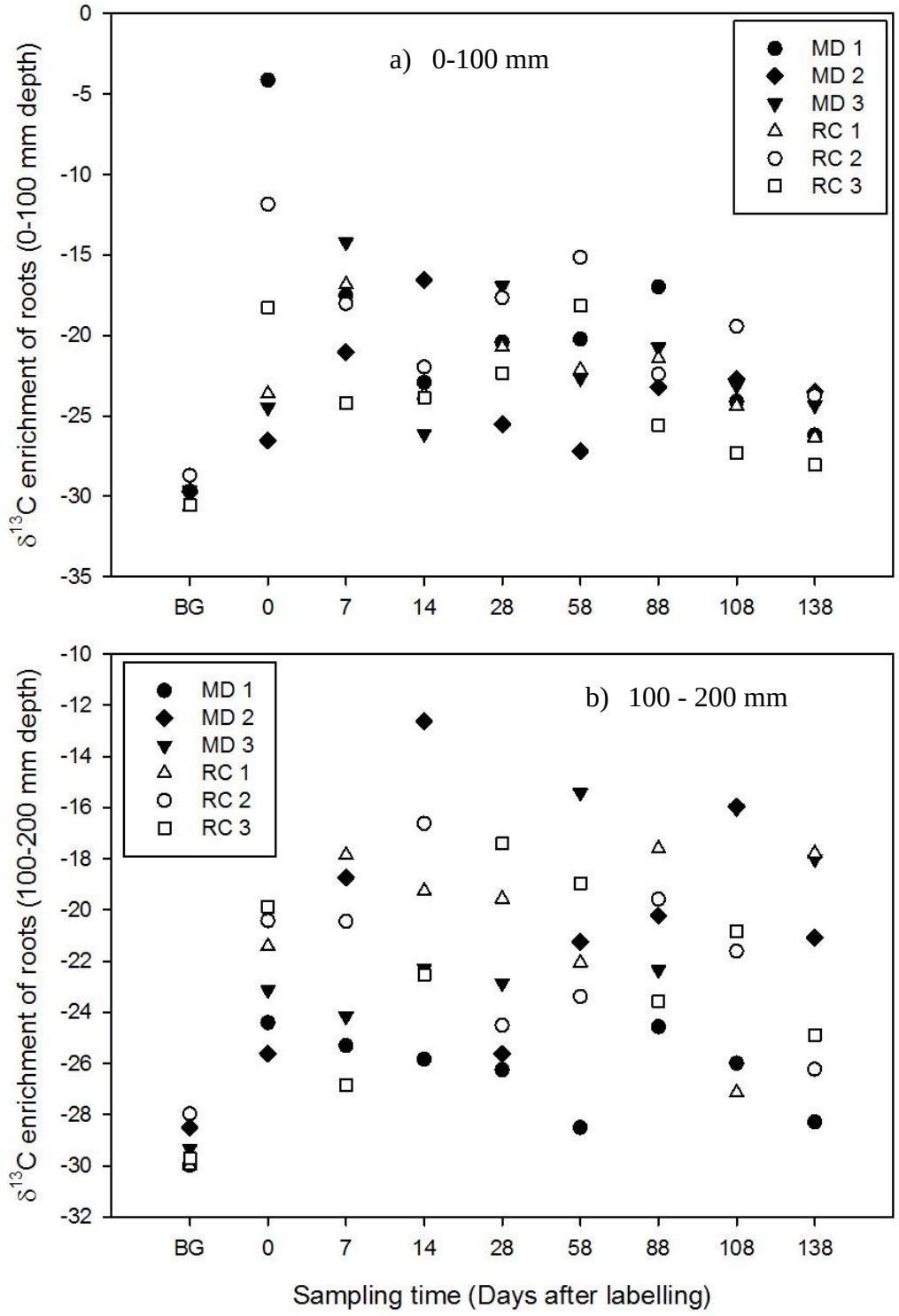
Appendix 1 – Experiment two (Chapter 4) supplementary data and photos



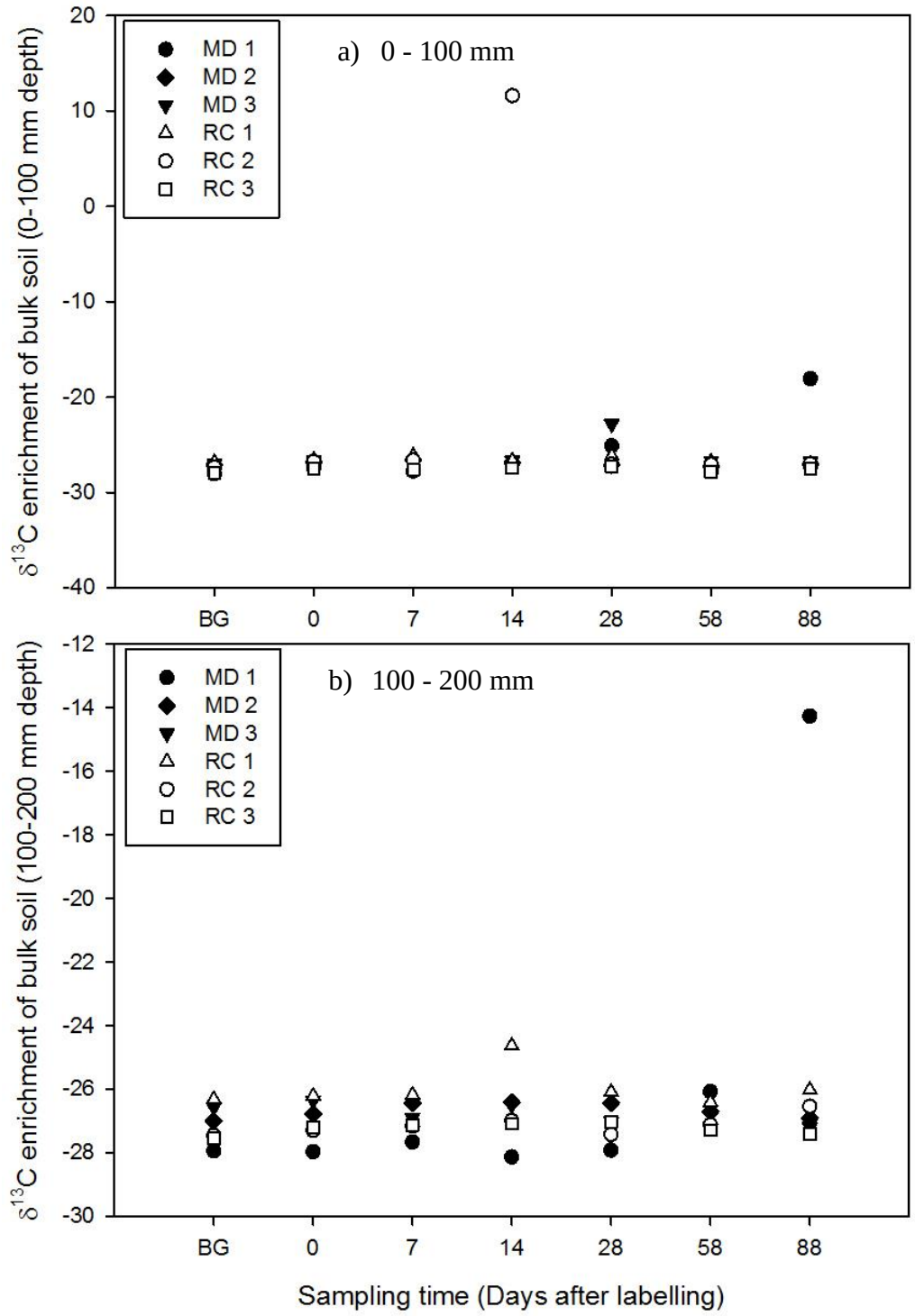
Appendix 1a Perspex chamber ($1m^2$) set-up of one plot in experiment two. Chamber, infrared gas analyser and fenced plot are displayed.



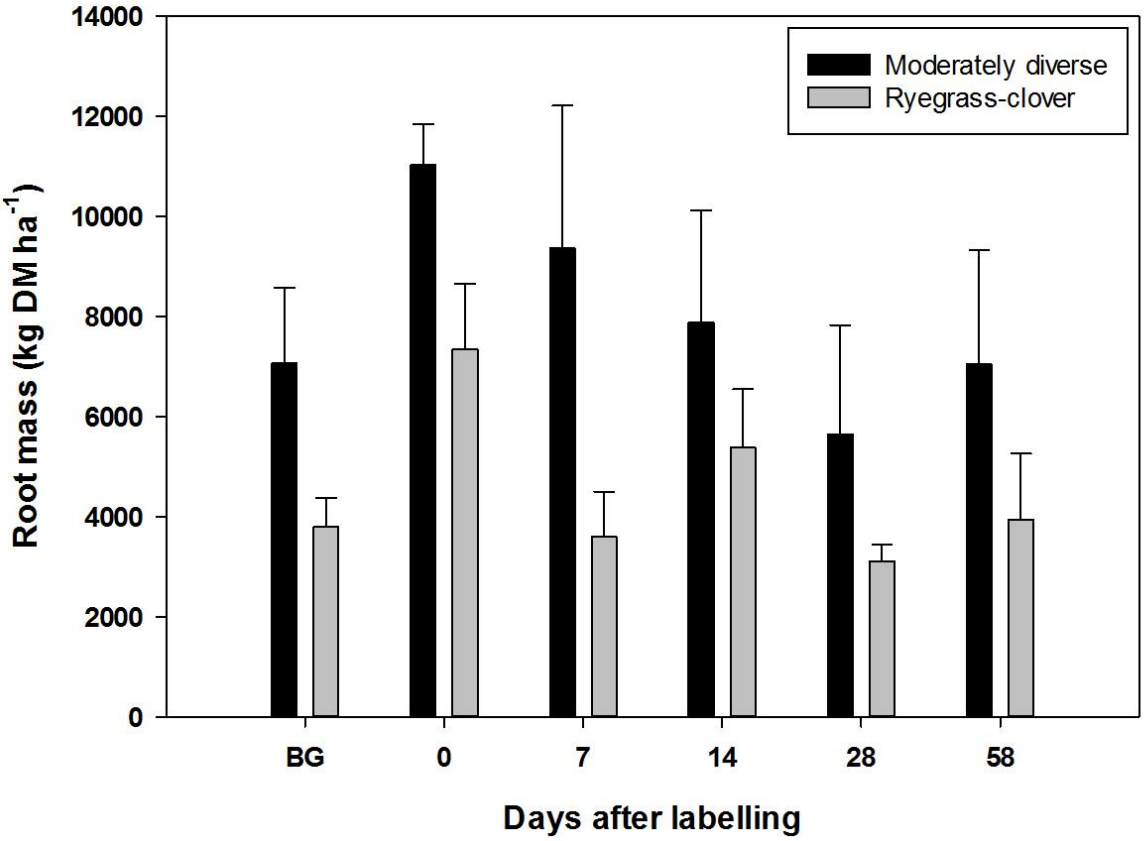
Appendix 1b Delivery system for $^{13}CO_2$ within chamber. Sample tube containing ^{13}C labelled carbonate, fan, and rubber septum where H_2SO_4 was injected into the carbonate solution.



Appendix 1c Isotope enrichment ($\delta^{13}C$) of roots (0 – 100 mm, 100 – 200 mm depth) under moderately diverse (MD) and ryegrass-clover (RC) pasture after isotopic pulse labelling (Chapter 4). BG refers to background sampling before any isotope labelling. MD 1-3 and RC 1-3 refer to the 3 replicate plots of each pasture.

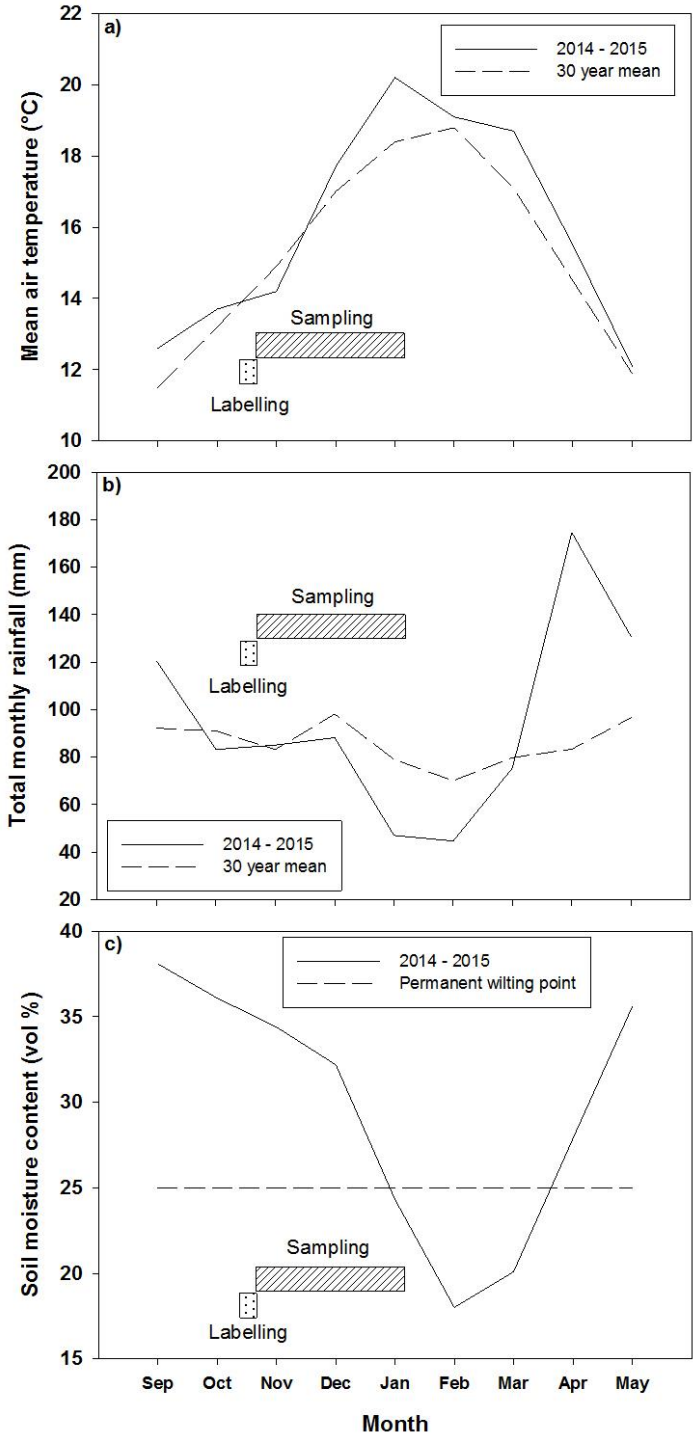


Appendix 1d Isotope enrichment ($\delta^{13}C$) of bulk soil (0 – 100 mm, 100 – 200 mm depth) under moderately diverse (MD) and ryegrass-clover (RC) pasture after isotopic pulse labelling (Chapter 4). BG refers to background sampling before any isotope labelling. MD 1-3 and RC 1-3 refer to the 3 replicate plots of each pasture.

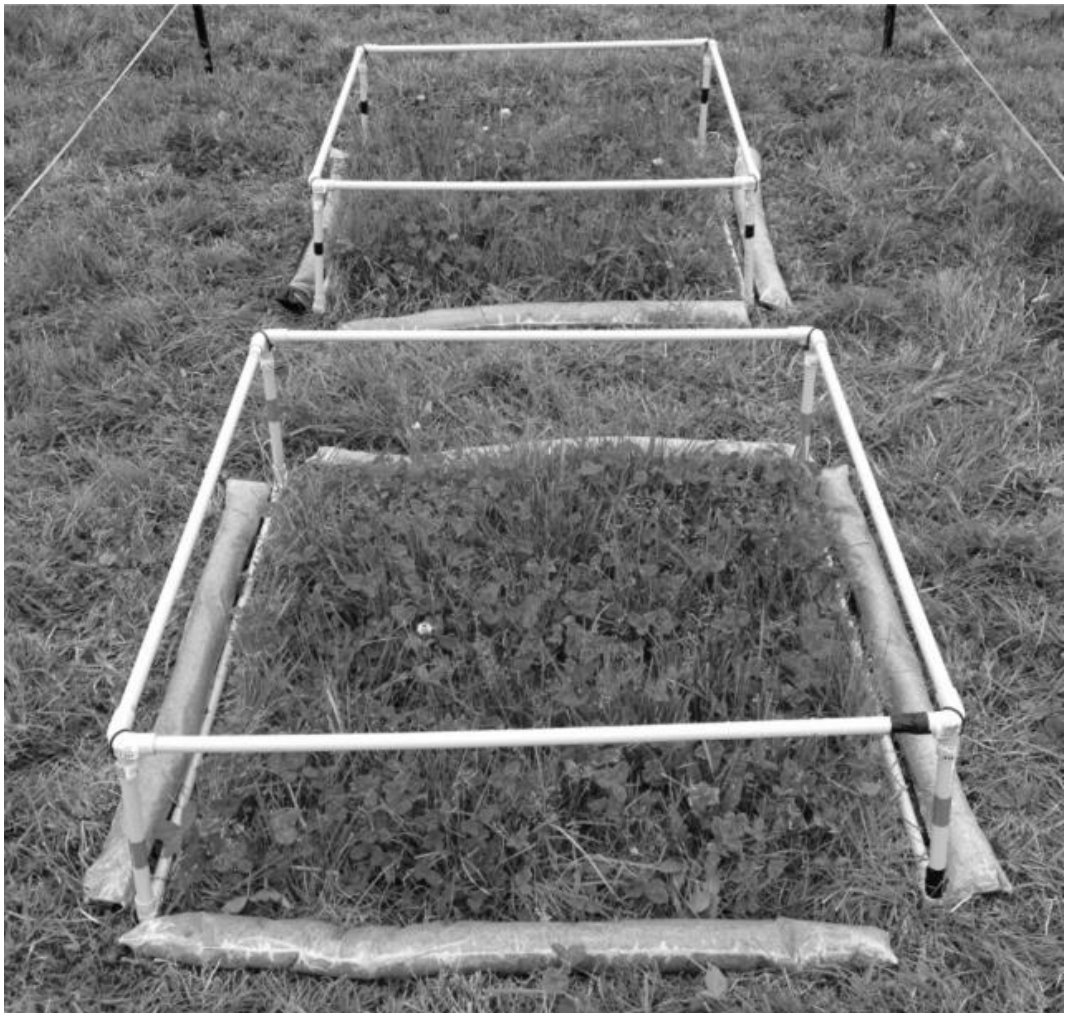


Appendix 1e Root mass (kg DM ha^{-1}) measurements between moderately diverse and ryegrass-clover pastures before (BG, background) and during each sampling (Chapter 4), n=3.

Appendix 2 – Experiment 3 (Chapter 5) supplementary data and photos



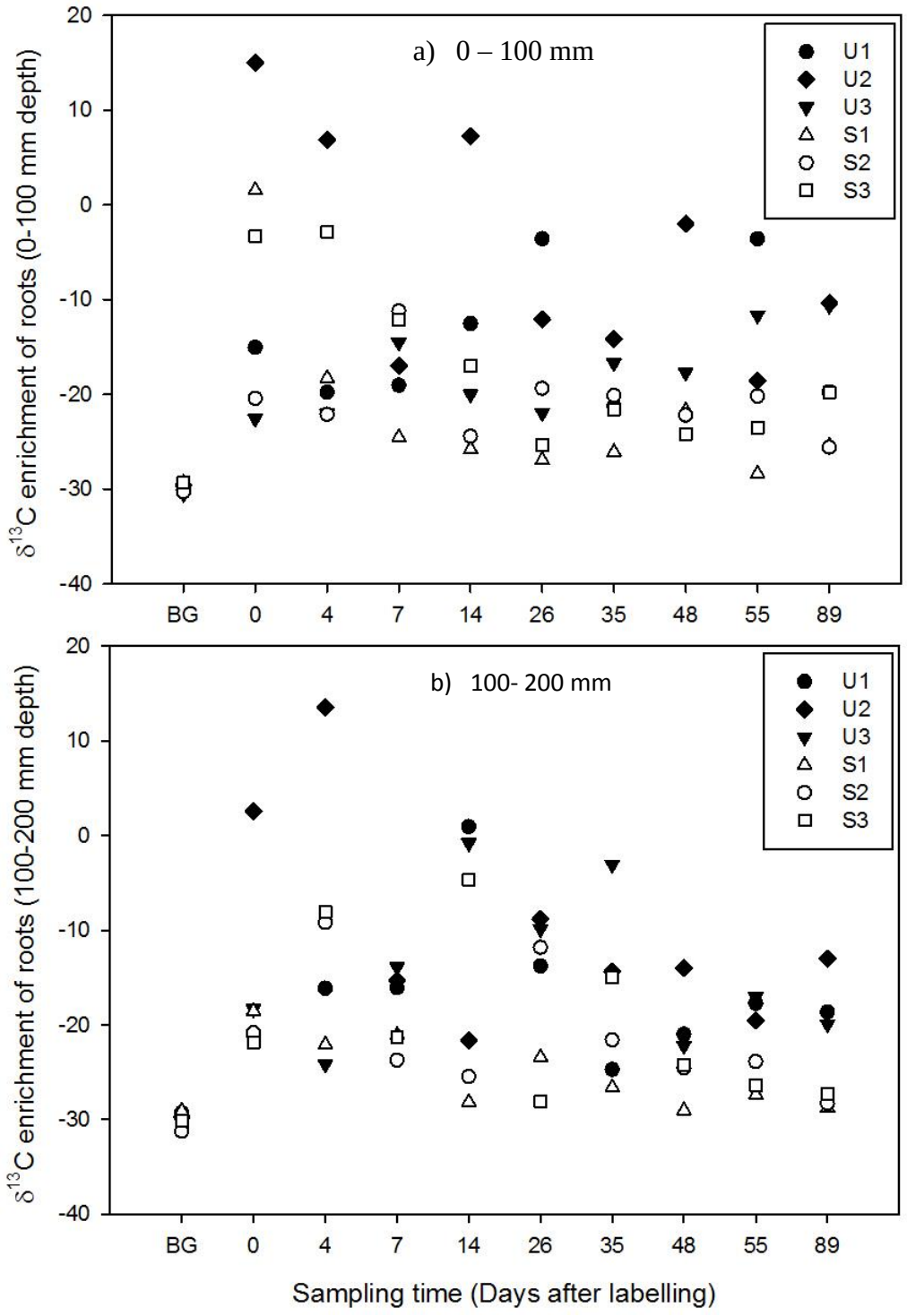
Appendix 2a a) Mean air temperature (°C), b) Total monthly rainfall (mm), c) Volumetric moisture content, between September 2014 to May 2015 and compared to the 30-year mean (for temperature and rainfall). Data were obtained from a NIWA climatological weather station within 6 km of the site. Labelling and sampling periods are shown. Horizontal line on c) is the permanent wilting point.



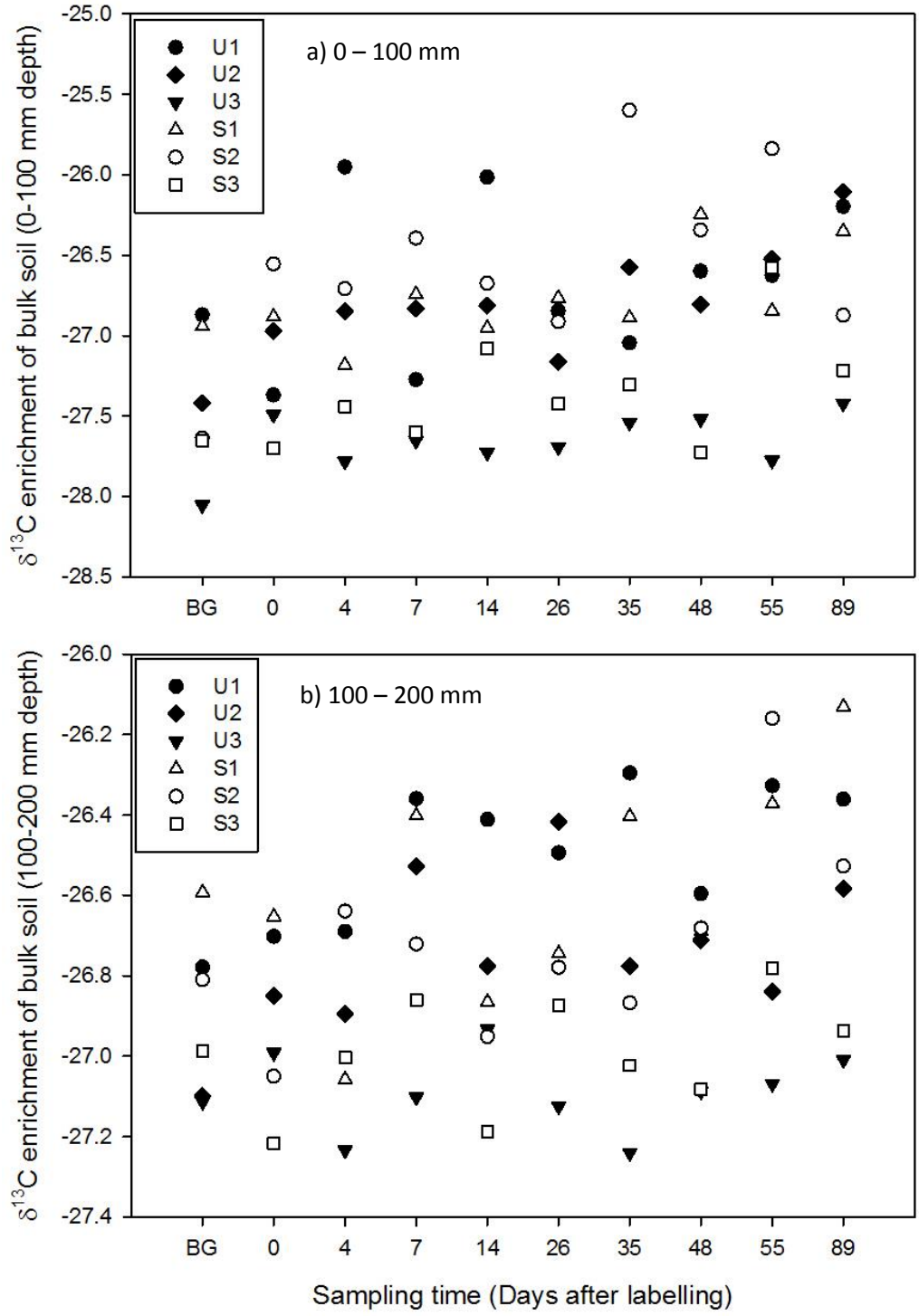
Appendix 2b Framing of chambers for one pair of plots in experiment 3. One of each pair was sprayed with herbicide following isotope labelling.

Appendix 2c Chamber set-up following installation of plastic sheeting over framing and injection of acid to evolve CO_2. Sandbags were placed around base of plastic sheeting to seal to ground.

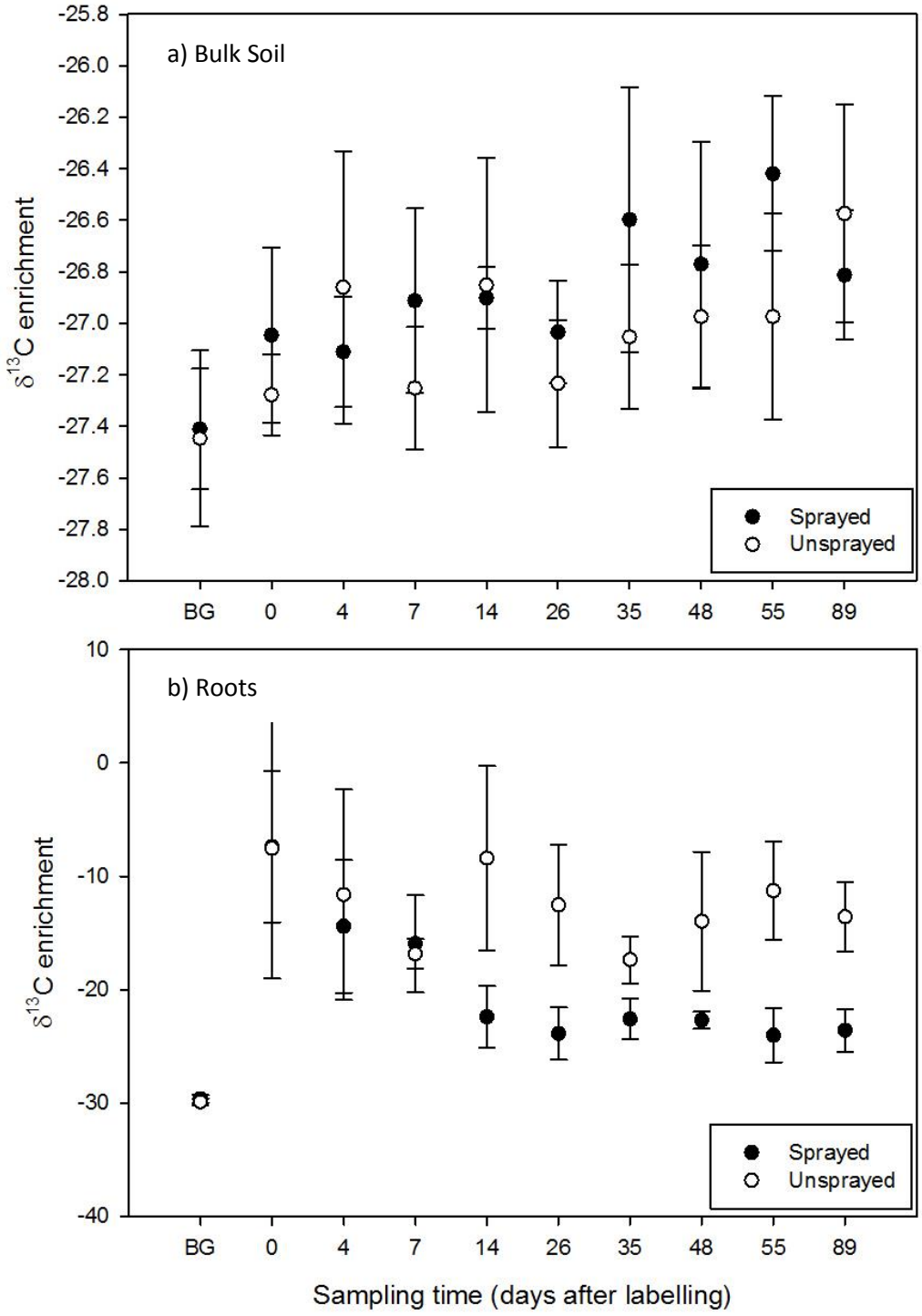
Appendix 2d Chamber set up with two Perspex chamber used in experiment two. Two Perspex chambers and four additional chambers construct with a frame and plastic sheeting were used during experiment three.



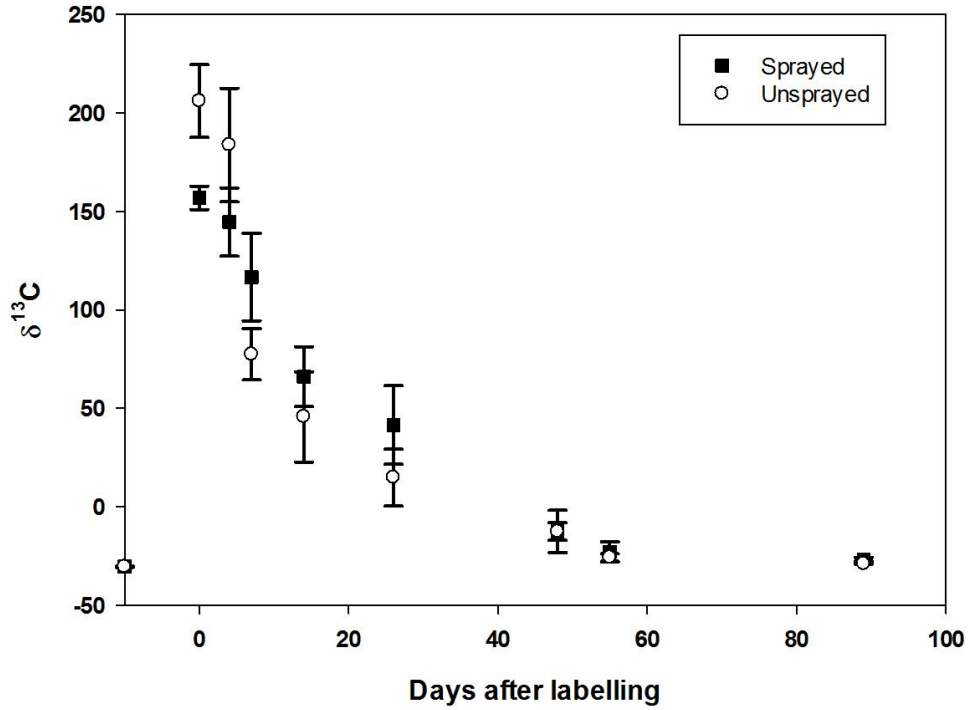
Appendix 2e Isotope enrichment ($\delta^{13}C$) of roots under unsprayed (U1-3) and sprayed ryegrass-clover (S1-3) pasture after isotopic pulse labelling (Chapter 5) for a) 0 – 100 mm and b) 100 – 200 mm depth. BG refers to background sampling before any isotope labelling. Numbers 1-3 represent the replicate plots for each treatment.



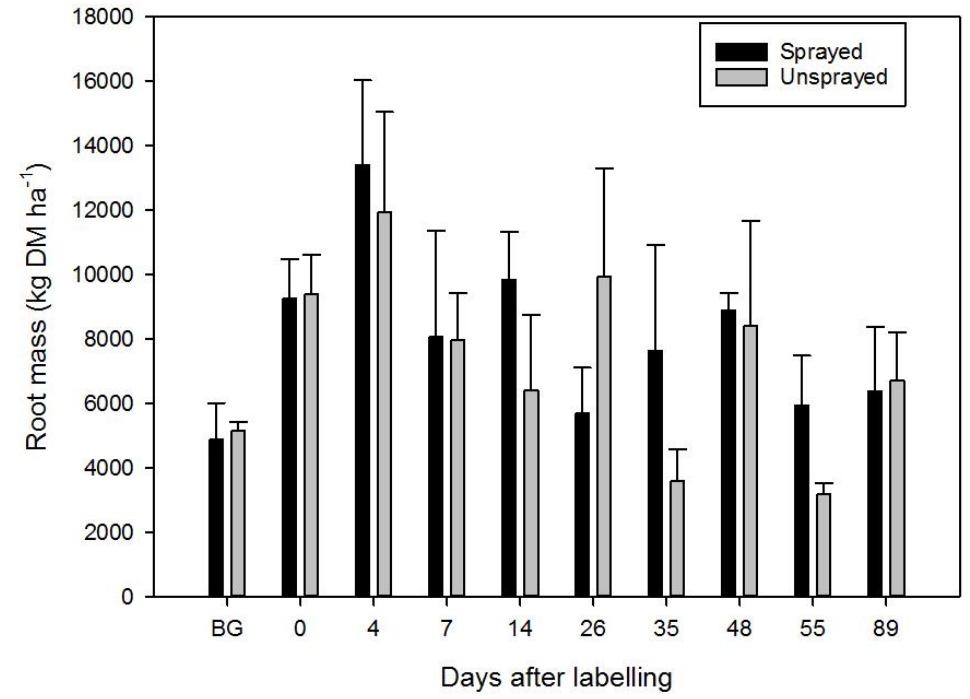
Appendix 2f Isotope enrichment ($\delta^{13}C$) of bulk soil (0 – 100 mm; 100 – 200 mm, depth) under unsprayed (U1-3) and sprayed ryegrass-clover (S1-3) pasture after isotopic pulse labelling (Chapter 5). BG refers to background sampling before any isotope labelling. Numbers 1-3 represent the replicate plots for each treatment.



Appendix 2g Average isotope enrichment ($\delta^{13}C$) for a) bulk soil and b) roots for sprayed and unsprayed ryegrass-clover pasture (0-100 mm depth, n=3). Error bars are 1 standard error. BG is the background sampling collected prior to any labelling. Data collected from isotope labelling outlined in Chapter 5.



Appendix 2h $\delta^{13}C$ of aboveground herbage samples with days after labelling for both sprayed and unsprayed treatments (Chapter 5). Sample taken before day 0 refers to background samples. Error bars represent 1 standard error, n=3.



Appendix 2i Root mass measurements of sprayed and unsprayed treatments throughout the sampling period (Chapter 5). BG refers to background samples. Error bars represent 1 standard error, n=3.

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Nature of contribution by PhD candidate	Collected and analysed field samples including data analysis and figures within this work. Wrote the thesis chapter/manuscript.
Extent of contribution by PhD candidate (%)	90%

CO-AUTHORS

Name	Nature of Contribution
Louis Schipper	Project conception; Collaboration on data analysis, interpretation and visualisation; provided substantial comments and editing on manuscript/chapter versions.
Daniel Laughlin	Collaboration on data analysis, interpretation and visualisation; provided substantial comments and editing on manuscript/chapter versions
Susanna Rutledge	Collaboration on data analysis, interpretation and visualisation; provided substantial comments and editing on manuscript/chapter versions
Mike Dodd	Collaboration on data analysis, interpretation and visualisation; provided substantial comments and editing on manuscript/chapter versions
Johan Six	Collaboration on data analysis, interpretation and visualisation; provided substantial comments and editing on manuscript/chapter versions

Certification by Co-Authors

The undersigned hereby certify that:

- the above statement correctly reflects the nature and extent of the PhD candidate's contribution to this work, and the nature of the contribution of each of the co-authors; and

Name	Signature	Date
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Daniel Laughlin		26/8/15
Susanna Rutledge	Susanna Rutledge	26/8/2015
Mike Dodd		18/8/2015
Johan Six		18/8/2015

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In the submitted thesis, Chapter 4 represents the second experimental piece of work titled "Root turnover and root C input to soil under moderately diverse and ryegrass-clover pastures". Currently, this work remains unpublished.

Nature of contribution by PhD candidate	Collected and analysed field samples including data analysis and figures within this work. Wrote the thesis chapter/manuscript.
Extent of contribution by PhD candidate (%)	90%

CO-AUTHORS

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Name	Signature	Date
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Daniel Laughlin		26/8/15
Mike Dodd		14/9/2015
Susanna Rutledge	Susanna Rutledge	26/8/2015
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In the submitted thesis, Chapter 5 represents the third experimental piece of work titled "Root turnover and C input under a perennial ryegrass and white clover pasture with and without pasture renewal involving herbicide". Currently, this work remains unpublished.

Nature of contribution by PhD candidate	Collected and analysed field samples including data analysis and figures within this work. Wrote the thesis chapter/manuscript.
Extent of contribution by PhD candidate (%)	90%

CO-AUTHORS

Name	Nature of Contribution
Louis Schipper	Project conception; Collaboration on data analysis, interpretation and visualisation; provided substantial comments and editing on manuscript/chapter versions.
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