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Irrigation effects on soil organic carbon under a ryegrass-white clover pasture on a Lismore stony silt loam soil

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

Irrigation effects on soil organic carbon under a ryegrass-white clover pasture on a Lismore stony silt loam soil

by

Carmen Rosa Medina Carmona

Long-term summer irrigation of temperate managed pastures has been reported to either increase or decrease soil organic carbon (SOC) stocks when compared with dryland systems. Understanding the short-term effects of irrigation on the assimilation, partitioning and storage of carbon (C) within the plant-soil system is important in order to identify key mechanisms that explain the observed differences in SOC responses to irrigation. Two continuous $^{13}CO_2$ pulse labelling experiments were performed to ^{13}C-labelled mesocosms established with ryegrass (*Lolium perenne* L.) and white clover (*Trifolium repens* L.) mixed pasture using a Lismore stony silt loam soil (Pallic Firm Brown soil).

In the first $^{13}CO_2$ pulse labelling experiment the pasture mesocosms were labelled over the summer period, under irrigated and dryland simulated conditions, to specifically measure the effects of irrigation on the net assimilation and partitioning of new photosynthate C within the above- and below-ground plant and soil components, including soil particle size fractions (> 250, 53–250, 20–53, 5–20 and < 5 µm). One day after the last $^{13}CO_2$ labelling event, a set of the ^{13}C-labelled irrigated and dryland mesocosms were destructively sampled, while the remaining mesocosms were maintained over an annual cycle under the same seasonal soil moisture conditions. Subsets of these mesocosms were then subsequently sampled at 12, 125, 237 and 349 days after the last $^{13}CO_2$ labelling event, in order to trace the fate of ^{13}C recovered at day 1, this phase of the experiment was identified as the ^{13}C chase period. The main objective of the ^{13}C chase period was to measure, over an annual pasture growth cycle, any legacy effect of summer irrigation on the re-distribution and short-term (349 days) persistence of this ^{13}C within the plant-soil system.

Chapter 3 presents data for mesocosms collected at the end of the irrigation period, 1 day after the last $^{13}CO_2$ labelling event, where summer irrigation increased the mass of ^{13}C partitioned into herbage by 16%, while reducing the quantity partitioned into root biomass in the 15–25 cm soil depth by 35%. However, under irrigation less new photosynthate C was observed in rhizosphere soil (0–15 cm depth), while more new photosynthate C was partitioned into the 53–250 µm and < 5 µm soil fractions, the fine particulate organic matter (POM) and mineral associated organic matter (MAOM or clay), respectively. Despite these differences, the net mass of new photosynthate C in the

whole soil (0–25 cm depth) was similar between treatments (2511 kg new C ha^{-1} dryland and 2509 kg new C ha^{-1} irrigated). Therefore, irrigation did not increase the net mass of new photosynthate C accumulated in the soil despite increasing above-ground pasture productivity.

Chapter 4 presents the results from the ^{13}C chase period for the first $^{13}CO_2$ labelling experiment. After 349 days, approximately 50% of the initial ^{13}C recovered in roots and rhizosphere soil remained in both, the previously irrigated and dryland treatments. There was no significant change in the ^{13}C recovered from non-rhizosphere soil (0–15 cm) or whole soil (15–25 cm) relative to day 1, but a greater partitioning of ^{13}C to the fine POM (53–250 μm) fraction was observed under irrigation. Despite higher recovery of ^{13}C in the clay (< 5 μm) fraction in the irrigated soil (0–15 cm) at day 1, the ^{13}C recovered in the clay fraction in the dryland soil increased over the autumn-winter period, yielding no difference in the clay fraction ^{13}C recovered over the remainder of the annual pasture production cycle. Irrigation did not affect the short-term (< 1 year) persistence of photosynthate C in roots or the soil compared to the previous dryland conditions, but did affect its spatial and temporal partitioning to below-ground plant and soil components over the annual pasture production cycle.

In the second $^{13}CO_2$ labelling experiment, pasture mesocosms were labelled under uniform soil moisture contents over the spring period. Then, 1 day after the last $^{13}CO_2$ event a set of ^{13}C-labelled mesocosms were destructively sampled as a baseline for the mass of ^{13}C partitioned into the above- and below-ground plant and soil components, including soil particle size fractions, before applying the irrigated and dryland treatments. These treatments occurred over 140 days during the subsequent summer-earlier autumn period. During the irrigation period, 2 destructive samplings were performed in order to measure the effect of summer irrigation on the re-distribution and persistence of the previously assimilated C partitioned within the plant-soil system. The remaining mesocosms were maintained under uniform seasonal soil moisture contents and subsequently sampled at 225 and 343 days after the last $^{13}CO_2$ labelling event, over the following winter and spring seasons. The objective for tracing the ^{13}C after the irrigation period was to assess potential legacy effects of irrigation on the previously assimilated C.

Chapter 5 presents the results from the second $^{13}CO_2$ labelling experiment where after 140 days, summer irrigation increased the losses of the previously assimilated ^{13}C in above- and below-ground pasture biomass, with the irrigated treatment producing approximately 3-fold more herbage dry matter but lower root biomass (2000 kg dry matter ha^{-1} lower) than the dryland treatment. The ^{13}C recoveries from root biomass (0–15 cm depth) in the irrigated treatment at 140 and 343 days were lower than in the dryland treatment by approximately 70% and 60%, respectively. Despite that irrigation reduced the persistence (increased the turnover) of ^{13}C previously assimilated in above-

ground (herbage) and below-ground (roots) plant biomass, it did not affect the storage and loss of ^{13}C in the soil after 1 year compared to dryland pasture conditions. However, 1 year after imposing the summer irrigation period, the dryland pasture soil retained more of the previously assimilated ^{13}C in root biomass and MAOM fraction (< 5 µm) compared to the irrigated soil. This study provides evidence that summer irrigation can increase the turnover of C in roots and MAOM that may have important implications for the longer-term SOC storage under intensified pastoral production systems.

This PhD study has demonstrated that summer irrigation applied to increase above-ground pasture productivity in managed ryegrass-white clover pastures had no demonstrable effects on the storage and loss of new photosynthate C partitioned into the soil over an annual production cycle when compared with dryland conditions. Therefore, based on these results it can be concluded that under the conditions of this research, summer irrigation had a neutral effect on the formation and short-term (< 1 year) storage of newly formed SOC when compared with dryland conditions. However, the study of soil C components or pools (e.g. rhizosphere soil and soil particle size fractions) demonstrated that irrigation affected the spatial and temporal partitioning of root derived-C in the soil, when compared with a dryland pasture system, by reducing the accumulation of new photosynthate C in the rhizosphere soil while increasing the accumulation of this new C in the fine POM (53–250 µm) and clay (< 5 µm) size fractions of the non-rhizosphere soil.

This re-distribution of root derived-C among the soil C components occurred relatively early in the irrigated pasture over the summer and autumn seasons, while in the dryland pasture the re-distribution occurred over the following autumn, winter and spring seasons. In addition, the results showed that with irrigation the root system became smaller and shallower, with the pasture biomass allocation to above-ground plant components being favoured. This effect persisted over an annual pasture growth cycle, especially during the autumn and spring seasons following the cessation of summer irrigation. These findings may have implications for the longer-term storage of SOC in dryland relative to irrigated pasture systems that were not tested in this study.

Further research is needed to improve our understanding on the mechanisms driving the different responses of SOC to irrigation with a particular focus on: (i) determining the causes for the higher root turnover under irrigated pastures and, (ii) evaluating how irrigation interacts with other pasture management practices (e.g. fertiliser, grazing) to affect the formation, function and storage (> 1 year) of SOC, including that associated with POM and MAOM in soils.

Keywords: Irrigation, pasture, photosynthate carbon, soil organic carbon, soil particle size fractions

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Chapter 1

Introduction

1.1 Agriculture and climate change

Natural and anthropogenic activities that alter the Earth's energy budget can induce climate change (IPCC, 2014). Anthropogenic greenhouse gas (GHG) emissions have risen since the pre-industrial period leading to an uptake of energy by the climate system resulting in global warming. This has resulted in an increased frequency, intensity and duration of extreme climate events (e.g. heat waves and droughts) that adversely impacted food security and terrestrial ecosystems (IPCC, 2018).

In New Zealand, the agriculture and energy sectors are the two largest contributors to New Zealand's gross GHG emissions footprint together contributing approximately 90% of emissions from 1990 to 2017 (Ministry for the Environment & Stats NZ, 2019b). The agriculture sector in New Zealand is dominated by continuously grazed pastures (dairy cattle and dry stock including sheep and beef cattle) which cover approximately 50% of the land surface (MacLeod and Moller, 2006) and contain approximately 80% of the total national soil organic carbon (SOC) stocks (Tate et al., 2005).

Soils hold the largest pool of C in the terrestrial ecosystems containing approximately twice as much C as the Earth's atmosphere (Lal, 2004b). Therefore, the balance between inputs and outputs of organic C (OC) from the soil has a critical influence on the atmospheric carbon dioxide (CO_2) concentration, and this has important implications for global climate change (Lal, 2004a; Lützow et al., 2006; Bradford et al., 2019). Land use and especially land-use change are considered the major drivers influencing the balance of (SOC) stocks and the global C cycle (Poeplau et al., 2011).

Following the global trend over the last few decades there has been an increase in the intensification of grazed pastures in New Zealand, in an effort to support the growing demand for meat, wool and especially dairy products (Thornton, 2010; Kastner et al., 2012). The intensification of grazed pastures has resulted from the increased use of synthetic fertilisers (e.g. nitrogen (N) often applied as urea) and/or the use of seasonal irrigation. The use of seasonal irrigation, particularly in the Canterbury and Otago regions, removes soil moisture stress and aims to maintain an optimum soil water content for pasture growth during warmer and drier seasons (Siebert et al., 2015).

However, intensification of pastoral farming with irrigation, has raised questions regarding water resources and environmental sustainability with respect to water quality, nutrient management and the maintenance of SOC and N stocks (Schipper et al., 2010; Mudge et al., 2017; Whitehead et al., 2018). Irrigation can alter C and N cycles by changing the balance between photosynthate inputs and

the rate of C and N mineralisation, that in turn can result in changes in the rates of release of CO_2 and nitrous oxide (N_2O) to the atmosphere (Trost et al., 2013).

Seasonal irrigation of grazed pasture results in increased above-ground dry matter production but tends to reduce root biomass, when compared with dryland pastures (Scott et al., 2012). However, the measured effect of irrigation on SOC stocks in these pasture systems is less well understood, with increases (Kelliher et al., 2015), neutral responses (Condron et al., 2014; Hunt et al., 2016; Moinet et al., 2017) and reductions (Mudge et al., 2017) in SOC stocks being observed. Therefore, understanding the effect of irrigation on the accumulation and turnover of OC in soil is important in order to understanding the net effect of intensification on SOC storage and loss under temperate grazed pastures, and hence, their role in contributing to or mitigating the atmospheric CO_2 concentration (Lal, 2016; Bünemann et al., 2018).

1.2 Thesis aims, objectives and hypotheses

The main aim of this PhD research was to investigate the effects of summer irrigation on the assimilation, partitioning and short-term persistence of photosynthate C within a pasture plant-soil system in New Zealand. The following specific objectives were proposed to address the main aim of this research:

1. To quantify the effects of irrigation versus dryland management on the net assimilation and partitioning of photosynthate C in the above- and below-ground components of the plant-soil system, including soil particle size fractions (Chapter 3).

2. To quantify the short-term persistence (over an annual cycle) and re-distribution of photosynthate C between above- and below-ground C components following a summer irrigation cycle where pasture had been ^{13}C labelled under irrigation or non-irrigated conditions prior to the start of the annual cycle (Chapter 4).

3. To quantify the effects of irrigation on the short-term persistence of photosynthate C previously assimilated and partitioned by the plant-soil system, when pasture had been ^{13}C labelled under similar soil moisture conditions, prior to the start of summer irrigation (Chapter 5).

Main hypotheses tested were:

1. Irrigation would increase the partitioning of new photosynthate C to above-ground plant components, while reducing its partitioning to root biomass. Reduced root mass under

irrigation would result in less photosynthate C partitioned and accumulated in the soil (Chapters 3).

2. The lower root biomass of summer irrigated pastures will result in less retention of previously fixed (photosynthate) C in the soil during the autumn through spring period compared to dryland management (Chapter 4).

3. Irrigation would increase the losses of the previously assimilated photosynthate C from the below-ground plant-soil components as a result of an enhanced decomposition of this photosynthate C (Chapter 5).

1.3 Thesis structure

This thesis is divided into 6 chapters. Chapters 3–5 are experimental work presented as papers for publication and, therefore, contain some repetition that is necessary for their publication in peer-reviewed journals.

- **Chapter 1:** an introduction that provides an overview of the thesis topic and presents the research objectives and associated hypotheses.

- **Chapter 2:** a literature review focussing on the importance of SOC stored under grazed pasture systems and reviews how environmental conditions and management practices can alter the transfer and accumulation of photosynthate C within the plant-soil system.

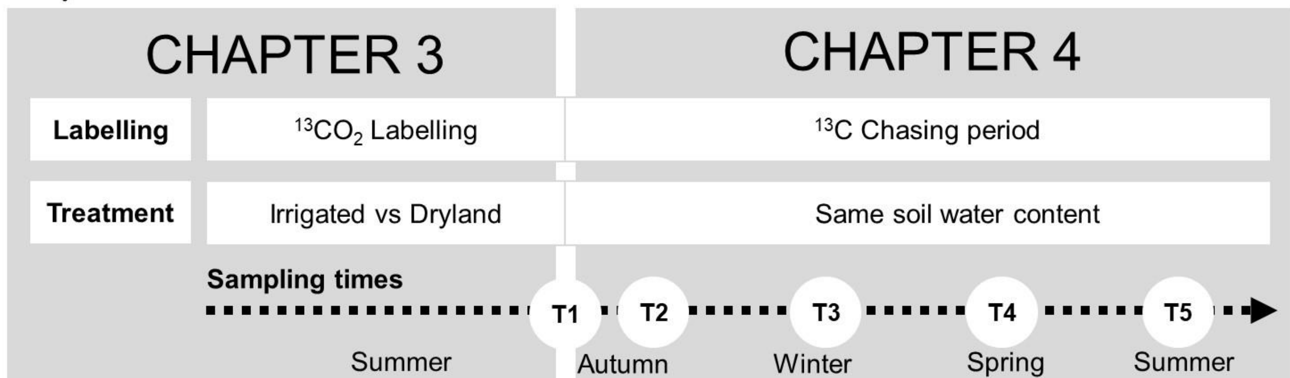
- **Chapter 3:** presents data of pasture production and partitioning of photosynthate C within the plant-soil system from the first $^{13}CO_2$ labelling experiment, where planted mesocosms were labelled under simulated irrigated and dryland conditions during summer-early autumn like conditions. This chapter is focused on results from the first destructive sampling (T1), one day after ^{13}C labelling ceased (Figure 1.1A). Chapter 3 addressed objective 1.

- **Chapter 4:** presents experimental work of the ^{13}C chase period for the first $^{13}CO_2$ labelling experiment, when mesocosms remaining after the first destructive sampling (T1) were brought to similar soil water content, and photosynthate C partitioned to plant and soil components was followed over an annual pasture growth cycle (Figure 1.1A). The results presented include data reporting changes in pasture production and SOC dynamics along with changes in ^{13}C recoveries. Chapter 4 addressed objective 2.

- **Chapter 5:** presents experimental work from the second $^{13}CO_2$ labelling experiment where planted mesocosms were labelled under the same soil water content during spring like conditions. Then summer irrigation was imposed one day after ^{13}C labelling ceased (Figure

1.1B). The results presented include data showing pasture production and changes in ^{13}C recoveries within the plant-soil system. Chapter 5 addressed objective 3.

- **Chapter 6:** presents a summary of the main results and conclusions of this research providing some broader implications of this work and suggesting where future research could be focused.



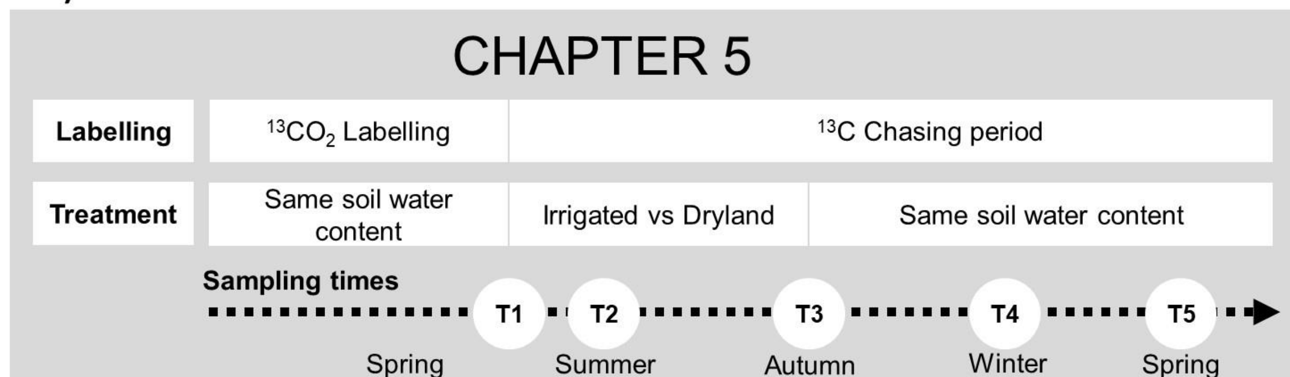


Figure 1.1. Schematic overview of the experimental design for A) Chapters 3–4, and B) Chapter 5.

Chapter 2

Literature Review

The main purpose of this literature review is to outline the importance of (i) soil organic carbon (SOC) stored within grassland agroecosystems, (ii) how agricultural management practices can alter the balance between inputs and outputs of organic carbon (OC) from the soil in these agroecosystems, and (iii) the implications of changes in SOC dynamics for global climate change.

First, a brief overview of the global carbon (C) cycle is presented, before the role of grassland SOC, and associated management practices (e.g. irrigation) affecting SOC are discussed. Then the current understanding of the mechanisms controlling OC inputs to soil via plant roots is discussed, especially with regards to rhizodeposition and root turnover. Finally, an overview of the methods used to study and characterise SOC is presented.

2.1 The global carbon cycle

Global C is partitioned into main five pools (Figure 2.1): oceanic C, geological C, soil C, atmospheric C, and biotic C (vegetation) (Lal, 2004a). The pool of C in terrestrial ecosystems is small relative to the oceans but contains approximately three times the mass of C compared to the atmosphere. The global soil C pool (2500 Pg) represents about 82% of the terrestrial C pool and is composed of 1550 Pg of SOC and 950 Pg of inorganic C (Lal, 2004a).

The terrestrial C cycle plays a major role influencing the climate through complex interactions that govern the fluxes of energy and mass through the atmosphere-plant-soil system (Govind and Kumari, 2014), and it is dominated by two main fluxes: photosynthesis (uptake of carbon dioxide (CO_2) from the atmosphere) and respiration (release of C to the atmosphere via plant, animal and soil microbial respiration) (Lal, 2004a).

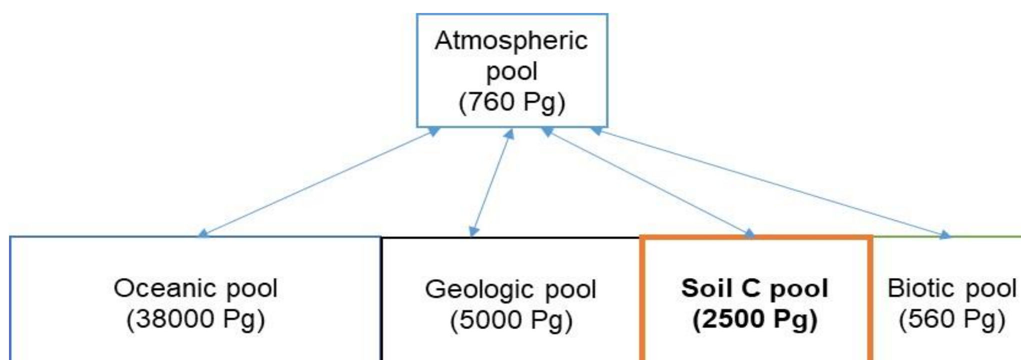


Figure 2.1. Principal global carbon (C) pools. Adapted from Lal (2004a). Pg = pentagram = 10^{15} g = 1 billion tonnes.

The greenhouse effect in the Earth's atmosphere results from the imbalance between the rate at which anthropogenic and natural sources emit greenhouse gases (GHG), primarily CO_2, methane (CH_4) and nitrous oxide (N_2O) and the rate at which the global C sinks offset these emissions to the atmosphere (Govind and Kumari, 2014). Anthropogenic activities responsible for releasing CO_2 and other GHG to the atmosphere include the burning of fossil fuel, deforestation, land use change, biomass burning, enteric fermentation, soil erosion and waste production (Boden et al., 2019).

Agricultural, forestry and other land uses are responsible for approximately one quarter of the anthropogenic global GHG emissions (IPCC, 2018) due in part to the significant area that agricultural lands cover, which is approximately 40% of the global land surface. However, sustainable land uses and agricultural practices that reduce emissions and remove GHG from the atmosphere can contribute to mitigating the impacts of climate warming on ecosystems and human societies (IPCC, 2018). Therefore, research to identify these sustainable land uses, and agricultural practices is essential for mitigating climate change.

Increasing or maintaining SOC in agricultural soils has been proposed as a potential strategy for mitigating global climate change and improving soil health and fertility (Chabbi et al., 2017; Poulton et al., 2018; Bradford et al., 2019). As noted above, soils contain a large reservoir of organic C (Figure 2.1), hence, soils are central to the global C cycle and have a direct impact on GHG emissions that impact on climate change (Staddon, 2004; Lützow et al., 2006).

2.2 The importance of soil organic carbon in grasslands

Grasslands, including grazed pastures cover approximately 70% of the world's agricultural land area (Soussana and Lüscher, 2007), and contain approximately 20% of the global SOC stocks (Stockmann et al., 2013). Therefore, small changes in grassland SOC stocks could significantly impact the atmospheric CO_2 concentration and the global C cycle (Conant et al., 2017; Whitehead et al., 2018). Grasslands are characterised by a high soil organic matter (SOM) concentration that is regarded as beneficial to soil health, due to the positive effects of SOM on soil aggregation, soil holding water capacity and nutrient cycling (Miller and Donahue, 1990; Conant et al., 2001).

The term SOM is typically used to refer to the organic constituents of soils (living, dead and partially decomposed plant and animal organic matter, < 2 mm diameter) that includes a wide range of primary elements (hydrogen, oxygen, nitrogen, phosphorus, calcium, etc.) in addition to C. It is generally agreed that SOM contains approximately 58% SOC (Stockmann et al., 2013). Research on the effects of agricultural management on SOC storage, particularly on grasslands managed for grazing has become imperative to identify strategies to increase, or maintain, SOC stocks in order to

both mitigate GHG emissions and improve the sustainability of agroecosystems for food security (Lützow et al., 2006; Lal, 2016; Whitehead et al., 2018).

2.2.1 Land management practices on soil organic carbon in grazed grasslands

Grassland SOC has been reported to be very sensitive to land management and land-use changes such as grazing, fertilisation and conversion from cropland, with either gains or losses of SOC resulting (Conant et al., 2001; Poeplau and Don, 2013). In a meta-analysis of the effects of agricultural practices on SOC in grasslands, Conant et al. (2017) concluded that improving grassland management practices by altering grazing intensity, fertilisation, introducing legumes, irrigation and converting from cropland to grassland, all increased SOC stocks (Figure 2.2).

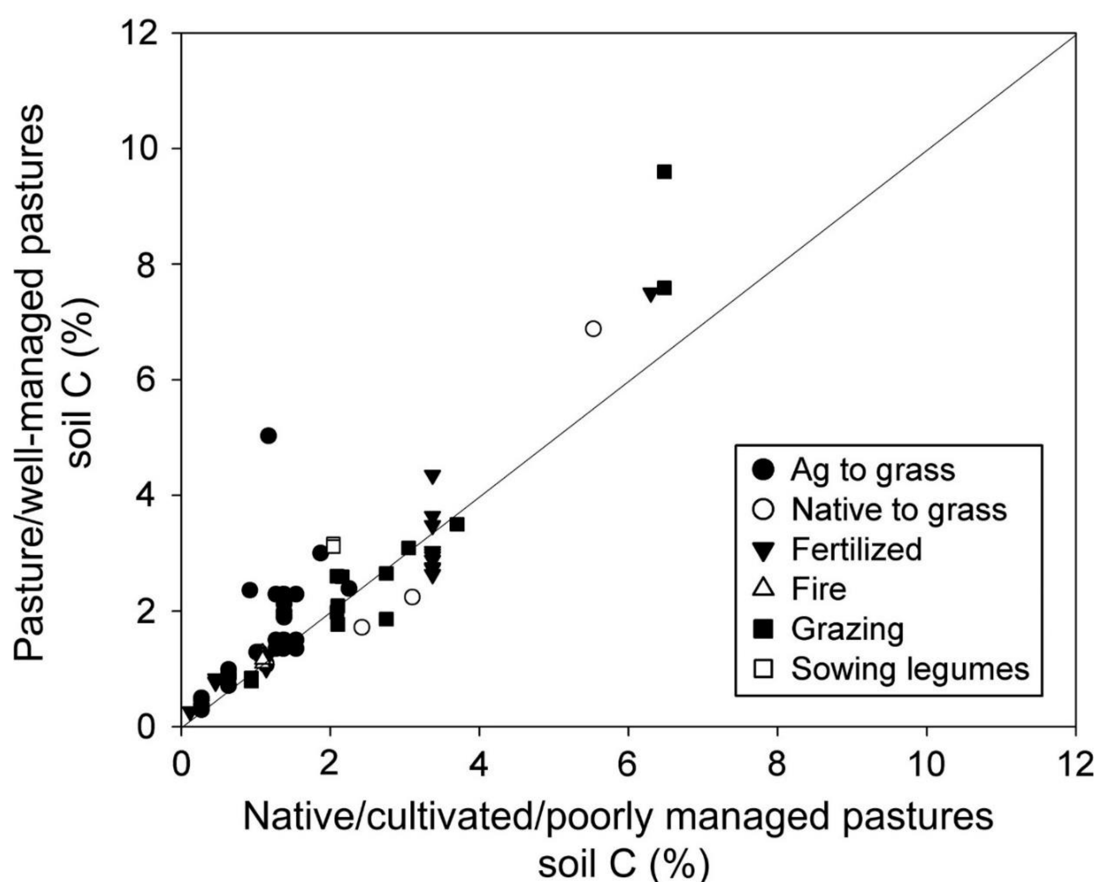


Figure 2.2. Soil C concentration for grasslands under improved (i.e., improved grazing, fertilized, sown with improved species, etc.) vs. unimproved management. Different symbols indicate different types of grassland management change. From Conant et al. (2017).

Therefore, research on measuring how agricultural management practices and environmental conditions affect the balance between inputs and outputs of OC from the soil, in conjunction with soil properties (e.g. structure and mineralogy), is needed to determine the potential for losses or accumulation of SOC in managed grassland systems (Conant et al., 2001; Kirschbaum et al., 2017; Whitehead et al., 2018; Frasier et al., 2019).

2.2.2 Grassland management in New Zealand

Previous studies in New Zealand have shown that grassland management such as increasing the fertility status of the soil and seasonal irrigation, can strongly affect the uptake and allocation of below-ground C (Saggar et al., 1997; Stewart and Metherell, 1999). Seasonal irrigation of temperate grasslands has been shown to increase above-ground pasture productivity during warmer and drier seasons, when water availability can limit pasture growth (Condron et al., 2014).

However, studies on the effects of irrigation on SOC stocks have produced contradictory results with increases (Kelliher et al., 2015), no effects (Condron et al., 2014; Hunt et al., 2016; Moinet et al., 2017) and decreases (Fraser et al., 2012; Condron et al., 2014; Mudge et al., 2017) in SOC stocks being reported compared with dryland (non-irrigated, rainfed) conditions. Therefore, it seems that there may be exceptions to the predictions made by some SOC models that increases in above-ground pasture production will result in increases of OC inputs and hence, increasing SOC content (Soussana et al., 2004; Smith et al., 2016), indicating that further research is required to address these differences.

New Zealand has followed global water use trends, with 51% of the fresh water that is allocated in the country now being used for irrigation (Ministry for the Environment & Stats NZ, 2017). The area of irrigated agricultural land in New Zealand almost doubled between 2002 and 2017, from 384,000 ha to 747,000 ha (94 percent increase) (Ministry for the Environment & Stats NZ, 2019a). While increases have been observed in nearly all New Zealand regions, this increase is largely driven by the area of irrigated land in Canterbury almost doubling (241,000ha to 478,000ha), with much of this expansion occurring on vulnerable shallow stony soils (Carrick et al., 2013).

These shallow stony soils are characterised by low soil water retention and rapid permeability, which are properties strongly correlated with increased nutrient leaching and low water storage capacity (Carrick et al., 2013; Cichota et al., 2016). Therefore, the use of irrigation and fertilisation has raised questions regarding water resources and environmental sustainability with respect to water quality, nutrient management and maintaining SOC and N stocks (Schipper et al., 2010; Mudge et al., 2017; Whitehead et al., 2018).

While the use of irrigation is predicted to increase to help secure global food production in response to greater climate change variability (Zhao et al., 2015), few studies have investigated the effects of irrigation on SOC, and most of these studies have focussed on cropping systems in arid or semi-arid climates (Gillabel et al., 2007; Denef et al., 2008; Apesteguía et al., 2015). Therefore, there is a lack of data available about how irrigation affects SOC under grazed pastures in temperate climates such

as New Zealand (Laubach and Hunt, 2018; Whitehead et al., 2018), where C fixation and organic C partitioning below-ground is expected to be high.

From a long-term field trial (60-year period) at Winchmore Research Station in New Zealand, with replicated irrigated and unirrigated (rainfed) pasture treatments, Kelliher et al. (2012) estimated that irrigation induced a 97% increase in the annual rate of C loss to the atmosphere compared with the unirrigated treatment. In contrast, when Moinet et al. (2017) applied an annual net ecosystem C balance, it was observed that both irrigated and dryland pastures were a net source of C.

Moreover, the specific role of irrigation in affecting SOC storage and loss is not well understood and the causal mechanisms responsible for these different responses in temperate grazed pastures appear to be complex (Whitehead et al., 2018). For example, it is not known whether the effects of irrigation on SOC stocks are primarily associated with differences in the partitioning of photosynthate C to above- and below-ground components of the plant-soil system, that affect SOC inputs, or to differences in the decomposition and turnover of C deposited below-ground, that will increase the outputs of C (Kelliher et al., 2012; Mudge et al., 2017). Therefore, studying the mechanisms associated with the responses of SOC under temperate managed grasslands to irrigation is crucial in order to assess SOC's role in contributing to or mitigating further increases in atmospheric CO_2 concentration.

2.3 Organic carbon inputs to soil

Plants connect the abiotic and biotic processes within the C cycle through photosynthesis that transforms atmospheric CO_2 into SOC (Cheng and Gershenson, 2007; Pausch and Kuzyakov, 2018). Approximately half of all photosynthate C assimilated by terrestrial vegetation is transferred to the soil, either as root and shoot litter after plant death, or C released by living roots referred as rhizodeposits, and the corresponding process called rhizodeposition (Jones et al., 2004; Cheng and Gershenson, 2007; Pausch and Kuzyakov, 2018). In addition, microbial derived-C is also acknowledged as an important input of OC for SOC formation and stabilisation (Kallenbach et al., 2015; Kallenbach et al., 2016; Kögel-Knabner, 2017). However, this review will focus on the contribution of root systems (Figure 2.3) since root derived-C inputs contribute more to SOC when compared with the contribution of OC inputs from above-ground litter (Kögel-Knabner, 2002; Rasse et al., 2005; Kong and Six, 2010).

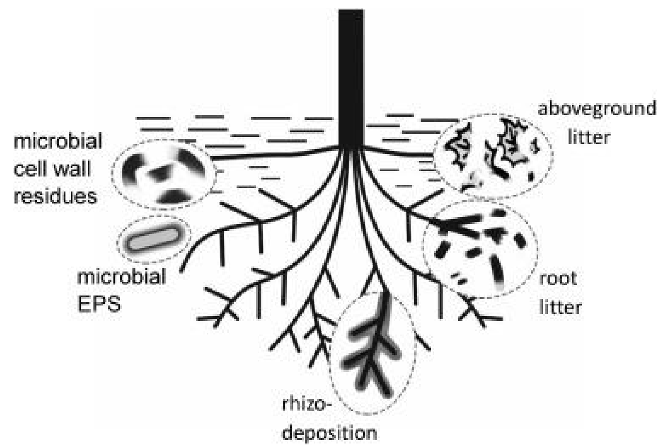


Figure 2.3. The input of OC to soils from above-ground litter and below-ground litter, consisting of roots and associated mycorrhiza, rhizodeposition, microbial extracellular polymeric substances (EPS) and microbial residues (necromass). From Kögel-Knabner (2017).

2.3.1 Rhizodeposition

Rhizodeposits have been classified based on their chemical composition, mode of release, or function, but they are all classically defined as organic compounds released by living roots, which are highly bioavailable, and occur as low-molecular weight compounds from root exudates of intact cells, lysates of sloughed-off cells and from mucilage (Whipps, 1990; Jones et al., 2009).

The transfer of C from rhizodeposition and the consequent proliferation of microorganisms associated with the physical presence of roots and nutrient uptake, modifies the chemical, physical and biological characteristics of the surrounding soil (Clark, 1949; Jones et al., 2004; Kuzyakov and Razavi, 2019). The soil volume affected by roots and the transfer of photosynthate C is referred as rhizosphere soil, which is considered one of the most important microbial hotspots regulating SOC cycling and, hence, the C fluxes in terrestrial ecosystems (Kuzyakov and Domanski, 2000; Pausch and Kuzyakov, 2018; Kuzyakov and Razavi, 2019).

However, quantifying the total input of OC from rhizodeposition (gross rhizodeposition) remains challenging due to the restricted zone of deposition around the root, fast microbial utilisation and decomposition, lower content of rhizodeposits compared to other organic compounds in soil, and the chemical similarity to organic substances that are released by soil microorganisms from the decomposition of SOM and plant litter (Kuzyakov and Domanski, 2000; Pausch and Kuzyakov, 2018). However, stable (^{13}C) and radioactive (^{14}C) isotopic labelling techniques and ^{13}C natural abundance methods provide approaches to overcome the challenges in separating root derived-C from SOM-derived C (Kuzyakov and Domanski, 2000; Kuzyakov and Siniakina, 2001; Pausch and Kuzyakov, 2018).

Net rhizodeposition is defined as the mass of OC that remains in the soil after microbial utilisation and partial decomposition to CO_2 (Pausch and Kuzyakov, 2018). Root derived-C is generally considered the main input of OC to soil (Rasse et al., 2005), contributing more to SOC stabilisation than inputs from shoot litter in the short term (Kong and Six, 2010). Farrar et al. (2003) summarised results from 95 ^{14}C-labelling experiments covering a wide range of plant species and reported that approximately 10% of the total photosynthate C transferred below-ground was recovered in the soil within the first 24 hours after isotopic labelling (Domanski et al., 2001; Jones et al., 2009; Kaiser et al., 2015). The transfer of photosynthate C from roots to soil (Figure 2.4), can occur directly via exudation, through symbiosis with mycorrhizal fungi or from root turnover. All these pathways stimulate microbial SOM decomposition and improve nutrient availability for plant growth (Jones et al., 2009; Kaiser et al., 2015; Liese et al., 2017; Vidal et al., 2018; Gorka et al., 2019).

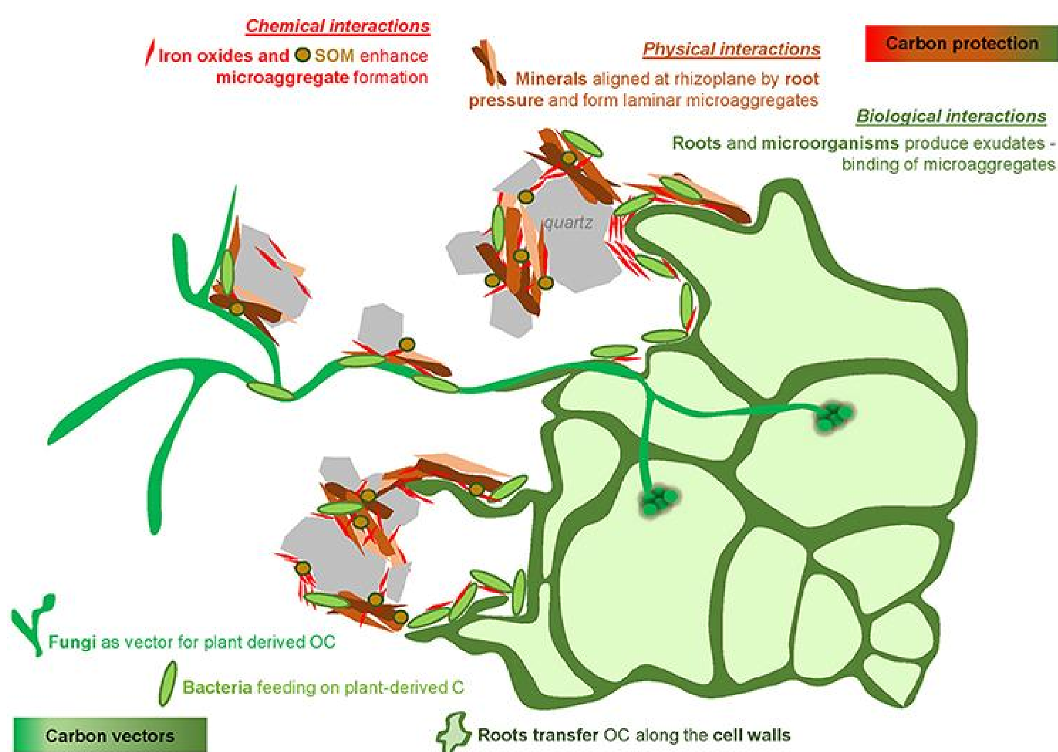


Figure 2.4. Conceptual model illustrating the complex interplay between vectors (roots, fungi, bacteria) leading to a transfer of plant-derived OC into root-free soil and stabilizing agents (iron oxides, root and microorganism products) which protect plant-derived OC in the rhizosphere from (Vidal et al., 2018).

Rhizodeposition depends on plant species, development stage, plant community composition and environmental conditions, e.g. drought (Figure 2.5) (Kuzyakov and Domanski, 2000; Jones et al., 2009; Sanaullah et al., 2012; Pausch and Kuzyakov, 2018). Other factors affecting the transfer of photosynthate C include leaf defoliation, herbivory activity, nutrient deficiency or toxicity, and the chemical, physical and biological properties of the surrounding soil (Farrar et al., 2003; Jones et al., 2004). Therefore, the transfer of photosynthate C into and through the plant to soil is regulated by

processes within the plants and soil in response to different stresses or agricultural practices, such as irrigation, by modifying soil water content that affects plant growth (Farrar et al., 2003; Jones et al., 2004).

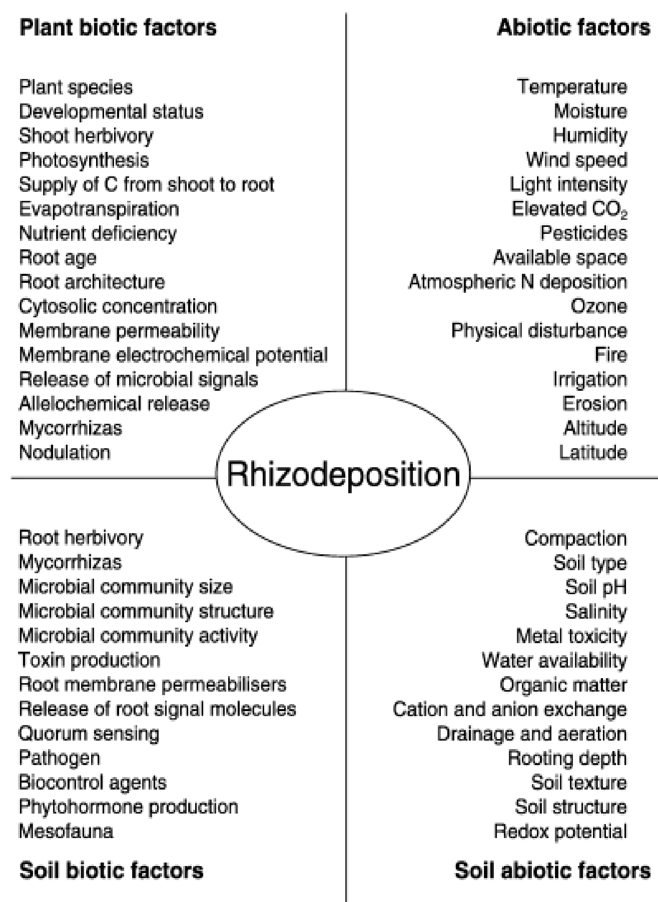


Figure 2.5. Schematic representation of the biotic and abiotic factors of plant and soil that influence rhizodeposition. From Jones et al. (2004).

Plants experiencing water stress have decreased photosynthetic activity due to stomatal closure that reduces transpiration (Peñuelas et al., 2004; Zhou et al., 2019). Water stress also decreases water and nutrient uptake per unit of root mass, which can result in enhanced allocation of photosynthate C to root biomass compared with shoots (Poorter and Nagel, 2000), while reducing the transfer of recent photosynthate C to soil food webs (Chomel et al., 2019). However, the magnitude of changes in photosynthate C transfer to soil will depend on the plant's nutrient status, the plant community composition (Poorter and Nagel, 2000; Sanaullah et al., 2011; Sanaullah et al., 2012) and environmental factors associated with seasonal variation (Saggar and Hedley, 2001).

Seasonal changes in the allocation of biomass to above- and below-ground plant components, hence, the partitioning of photosynthate C, is a response to variations of environmental conditions (soil water content, temperature and radiation) that serves to sustain maximum plant growth, in line with the 'functional equilibrium' theory (Lambers, 1983; Poorter and Nagel, 2000; Körner, 2015). The

‘functional equilibrium’ theory states that plants will shift their biomass allocation towards above- or below-ground organs, where a key resource for plant growth is limited (e.g. light, nutrients and water, etc.) as a strategy to improve the uptake of the limiting resource. Therefore, with enough water and nutrients plants would be expected to shift their biomass allocation towards above-ground components to enhance canopy structure that will in turn improve light interception (Wardlaw, 1990; Minchin et al., 1994).

However, despite a good body of knowledge on how water stress affects photosynthetic activity and photosynthate C allocation to roots, very few studies have quantified how water stress or its removal (e.g. with irrigation) affects the deposition and stability of organic C in soils.

2.3.2 Root turnover

Root turnover is considered a critical process in most terrestrial ecosystems for enabling nutrient cycling and C sequestration (Gill and Jackson, 2000; Eissenstat and Yanai, 2002). Broadly, root turnover is defined as the proportion of root mass that is produced and dies annually, assuming roots have a lifespan of 1 year (Eissenstat and Yanai, 2002). The production and maintenance of roots is potentially the largest sink for photosynthate C, and the death of roots is the primary input of OC and nutrient-rich organic substrates to below-ground food webs for the maintenance of SOC pools (Gill et al., 2002). In temperate grasslands approximately 24–87% of net primary production is allocated below-ground (Sims and Singh, 1978), with over 70% of the total root biomass found in the surface 15 cm of soil profile, which contains about 40% of the SOM measured in the top 100 cm of the soil profile (Gill et al., 1999).

Root death may provide C inputs to soil in several ways: 1) apoptotic root death where non-structural C and nutrients are translocated to other growing regions of the plant before death, 2) non-apoptotic root excision (e.g. physical separation of the root from the plant by tillage) where all the root C and nutrients enter the soil, and 3) non-apoptotic root damage (e.g. due to root disease or invertebrate feeding) where only some of the root C and nutrients may be returned to the soil (Gordon and Jackson, 2000; Jones et al., 2004).

In a meta-analysis of global patterns of root turnover for terrestrial ecosystems, Gill and Jackson (2000) reported that root turnover increased exponentially with mean annual temperature for forest and grasslands and decreased from tropical to high latitude systems for all plant functional groups. However, Gill and Jackson (2000) acknowledged that, despite global patterns of root turnover rates being observed between plant groups and different climates, the relatively high uncertainties of these measurements means that these patterns can not necessarily be used to predict root turnover globally in response to environmental perturbations such as climate change.

Saggar and Hedley (2001) using a $^{14}CO_2$ labelling technique reported that root turnover under an unirrigated dairy pasture in New Zealand varied seasonally, with a shorter turnover time in spring (93 days) and longer turnover time in autumn (160 days). These results indicate that seasonal variation in root growth and decomposition must be accounted when trying to precisely quantify root turnover. However, it has been also reported that seasonal patterns of pasture root growth are very site specific and can change depending on the pasture botanical composition, or year-to-year climate variability (Wedderburn et al., 2010; Dodd and Mackay, 2011).

Scott et al. (2012) calculated root turnover from a $^{13}CO_2$ labelling experiment on a long-term (approximately 60 year) irrigation trial in New Zealand, and observed that root turnover was generally faster under irrigated grazed pastures (438 days) when compared with unirrigated pastures (694 days). Assuming that OC inputs to soil from both rhizodeposition and root turnover are proportional to root mass, then research on the effects of a reduced root mass system reported under irrigated and fertilised grazed pastures (Scott et al., 2012) is required, as it could limit the potential to increase or maintain SOC in these grazed pastures through improving pasture rooting systems (McNally et al., 2015).

2.4 Characterisation of soil organic matter fractions

Fractionation of SOM has been widely used to gain a better understanding of SOM dynamics, and the wide range of fractionation methods applied reflects the diversity of mechanistic theories that exist to explain SOM formation (Cotrufo et al., 2015), turnover and stabilisation (Lehmann and Kleber, 2015). The aim of fractionation is to separate the complex continuum of SOM into components or fractions, each being relatively homogeneous in their characteristics or in their dynamics such as turnover rates and residence times in the soil (Chenu et al., 2015).

However, the distribution of SOM in fractions does not necessarily translate into a functional link, as isolated fractions or a combination of them can have similar turnover rates, residence times and respond similarly to perturbations (Poeplau et al., 2018). Therefore, biomarkers or tracers such as C isotopes (^{14}C and ^{13}C) are necessary to isolate "functional fractions" by either biological, chemical, or physical approaches.

In this review, there is an emphasis on physical fractionation approaches such as a separation of particle sizes that reflect well the interactions between organic and inorganic soil components involved in the turnover and stabilisation of SOM (Poeplau et al., 2018). However, a brief overview of biological and chemical fractionation methods is provided.

2.4.1 Biological

The dynamics of SOM can be approached by measuring the turnover rate of whole soil C using different methods, such as incubation and modelling the evolution of CO_2 (Chenu et al., 2015). Incubation of soil samples under controlled conditions is used to measure the mineralisation of SOM by CO_2 or mineral N accumulation. This method can also be used to biologically fractionate and characterise SOM by separating kinetic pools of C according to mineralisation curves. The rate of C mineralisation measured in the short-term (from a few days to a few weeks) is usually used as an indicator of general biological activity. However, with time and without inputs of fresh organic matter, the rate of C mineralisation declines as the most labile SOM is depleted.

2.4.2 Chemical

Chemical fractionation can be divided into extraction and hydrolysis, chemical destruction of the mineral phase, and oxidative degradation of organic C (Poeplau et al., 2018). Chemical fractionation is usually performed with water, alkali, acid, or organic solvents and attempts to isolate specific compounds, of different chemical recalcitrance, regarded as a crucial for SOM stability (Chenu et al., 2015). However, chemical recalcitrance may not be completely applicable to the study of SOC turnover and stabilisation processes, as there is evidence that the availability and accessibility of a substrate for decomposers appears to be more important for organic C turnover in soil than its chemical recalcitrance (Lehmann and Kleber, 2015).

Extraction with water is applied to separate dissolved organic C (DOC), which is a highly mobile and labile fraction of SOC (Chantigny et al., 2008). Water soluble C (WSC) and hot water-extractable C (HWC) are used as sensitive indicators of short-term changes in SOC caused by land-use and management practices in pastoral systems (Ghani et al., 2007). HWC, usually performed at 80˚C, tends to be closely related with soil microbial biomass and associated respiration, indicating that HWC is easily available for microbial decomposition (Gregorich et al., 2003). Recent studies in New Zealand have shown that HWC and SSA are good predictors of soils C mineralisation potential (Curtin et al., 2017; McNally et al., 2017).

Chemical destruction of the mineral phase is used to characterised or quantify complexed organic C, which is regarded as having a relative higher turnover time compared with uncomplexed organic C (Chenu et al., 2015). Chemical oxidation attempts to simulate a strong enzymatic decay and aims to separate oxidation-resistant associations that might be responsible for the stability of SOC (e.g. aluminium and iron oxide content). However, chemical oxidation is not the same as biological oxidation of SOC as they are driven by different SOM properties, which are not necessarily related with SOM's biological persistence.

2.4.3 Physical

Physical fractionation of SOM can help in understanding the mechanisms regulating SOM dynamics, including its persistence and function with changes in agricultural management practices (Grandy and Neff, 2008; Poeplau and Don, 2013; Cotrufo et al., 2019; Lavallee et al., 2020). Physical fractionation separates SOM into subsets according to physical criteria, such as size and/or density and is performed without or after various degrees of dispersion to break the aggregates structure (Puget et al., 2000). Physical fractionation procedures aim to minimize chemical alteration of SOM fractions and to separate primary soil particles, and primary or secondary organomineral associations (Figure 2.6).

Particle size fractionation relies on the idea that as SOM can be separated based on size, which reflects its state of decay, ranging from sand-sized, largely uncomplexed particulate organic matter to highly processed organic matter that bonds to fine-silt and clay mineral surfaces (Balesdent, 1996; Cotrufo et al., 2015; Lavallee et al., 2020). Particle density fractionation assumes that the association or disassociation of SOM with minerals affects SOM's turnover time. Both particle size and density fractionation were introduced to separate particulate organic matter (POM) from mineral associated organic matter (MAOM). However, some solubilisation of SOM takes place when separating POM and this can represent as much as 20% of total SOM when used to fractionate MAOM (Virto et al., 2008).

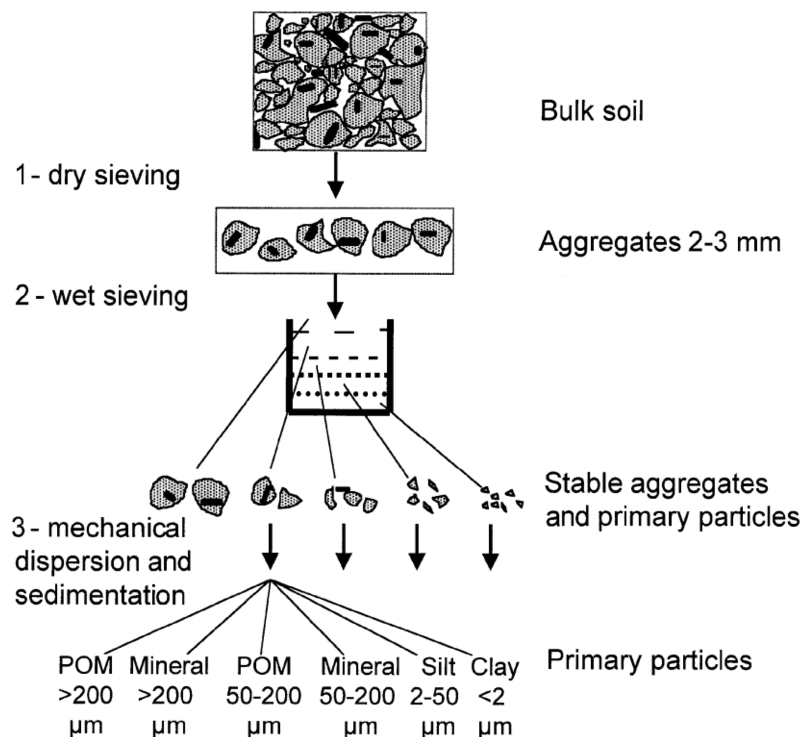


Figure 2.6. Schematic presentation of physical fractionation methods. Particulate organic matter is defined as POM. From Puget et al. (2000).

Aggregate size separation (by dry or wet sieving) is another approach to physical fractionation based on the premise that organic matter binds with mineral particles. This method can be used to separate OC responsible for aggregation (Puget et al., 1995). Moreover, aggregates of different sizes correspond to different microbial habitats and provide contrasting conditions for decomposition (Beare et al., 1994).

In a comprehensive comparison of methods to isolate OC fractions in temperate agricultural soils, Poeplau et al. (2018) reported that particle size class fractionation with density separation was the method that yielded the best outcome in terms of differentiating turnover rates between SOM fractions, while methods isolating different aggregate classes yielded the lowest range of differentiation, due to the fact that macroaggregates contain microaggregates and fine particles. However, aggregate fractionation methods are useful to study the effects on SOM dynamics and soil structure of agriculture practices such as tillage or land-use changes, especially in microaggregates that are proposed to act as a physical barrier between decomposers and substrates protecting SOM from rapid mineralisation (Six et al., 2002; Dungait et al., 2012).

Recently, it has been recommended that separating SOM into POM and MAOM fractions is the most efficient way to understand and predict the effects of land management practices on SOM and the impact towards increasing or maintaining SOC storage (Cotrufo et al., 2019; Lavallee et al., 2020). POM and MAOM are recognised as very contrasting forms of SOM in terms of their formation, persistence and functioning in soil (Figure 2.7). POM fractions (> 53 µm) are comprised of plant-derived compounds at different stages of decomposition, with short turnover and residence times in soil of < 10 years.

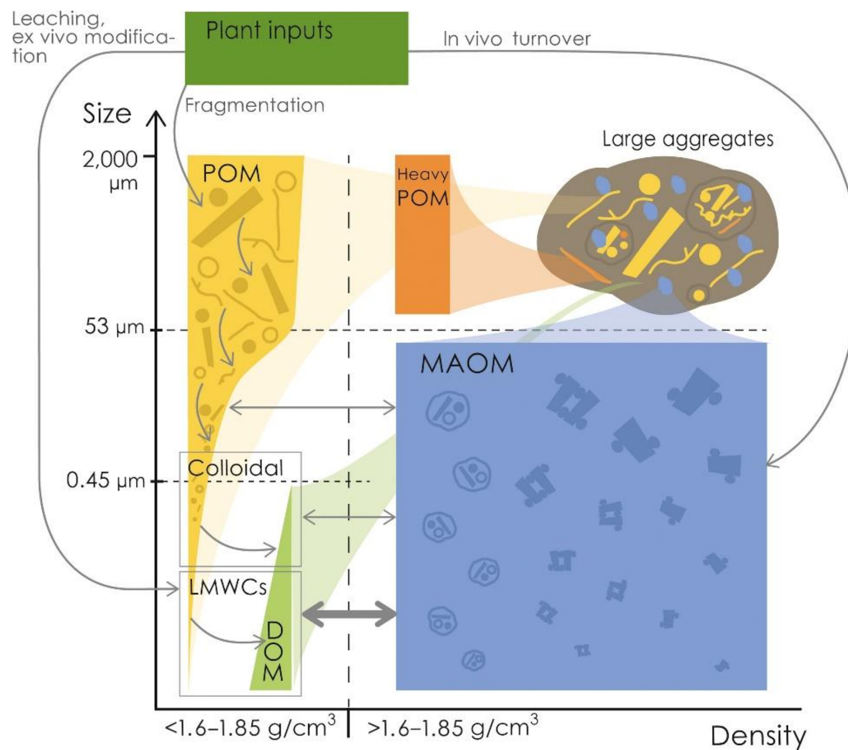


Figure 2.7. Conceptual representation of major soil organic matter (SOM) components discussed in Lavallee et al. (2020). These SOM components are physically defined based on size and density, shown on the y and x axes, respectively. The upper size limit specification for MAOM varies by region, from 20 to 63 µm, but 53 µm is show here for simplicity. Dissolved organic matter (DOM) is generally defined as <0.45 µm and water-extractable. Mineral-associated organic matter (MAOM) has multiple forms, including small particulate organic matter (POM) like structures encapsulated by minerals, organomineral clusters, and primary organomineral complexes. Large aggregates can contain all other components to varying degrees.

Moreover, POM fractions are also regarded as pivotal for the short-term storage of root derived-C (Balesdent, 1996; Kong and Six, 2010; Lavallee et al., 2020). While MAOM, is associated with the fine soil fractions (silt and clay) and comprises low molecular weight compounds that can be derived from plant and microbial activity, and are considered relatively more stable, with a longer turnover time > 10 years (Feller and Beare, 1997; Baldock and Skjemstad, 2000; Six et al., 2002; Lützow et al., 2006; Sokol et al., 2019).

2.5 Stabilisation of soil organic matter

Mechanisms for OC stabilisation in soils have received much interest recently due to their relevance in the global C cycle (Lützow et al., 2006). Stabilisation is defined as the protection of OM from mineralisation and involves processes or mechanisms that lead to prolonged turnover times in soil. Protection of SOM is not considered to equate to a permanent and complete removal of OC from mineralization, but rather to a reduced decomposition rate relative to similar unprotected SOM (Baldock and Skjemstad, 2000).

The degree of protection offered by each mechanism depends on the chemical and physical properties of the mineral matrix and the morphology and chemical structure of the SOM (Baldock and Skjemstad, 2000). The main mechanisms that contribute to SOM protection against decomposition in temperate soils have been proposed by Lützow et al. (2006): selective preservation due to recalcitrance, spatial inaccessibility of organic matter (OM), interactions with surfaces and metal ions, and substrate-driven biological rate limitation proposed by Ekschmitt et al. (2005).

2.5.1 Selective preservation

The process of selective preservation is related to the accumulation of recalcitrant molecules. The molecular characteristics of plant litter and rhizodeposits have been used to define the primary recalcitrance of organic matter inputs, whereas secondary recalcitrance refers to the recalcitrance of microbial products, humic polymers and charcoal (Lützow et al., 2006) . However, selective preservation due to the recalcitrant nature of SOM is not absolute as it has been shown that the mineralization of recalcitrant compounds may be enhanced through the addition of labile substrates such sugars (Marschner and Timonen, 2005). Therefore, SOM stability may be a function of substrate accessibility for decomposers rather than its chemical recalcitrance (Dungait et al., 2012). This explains the long-term stabilisation of potentially labile compounds found as “old” OC in temperate soils (Six et al., 2002).

2.5.2 Spatial inaccessibility

Spatial inaccessibility of SOM for decomposition by microbes and/or enzymes is caused by occlusion of SOM within aggregates or between layers of phyllosilicates, and by hydrophobicity and encapsulation in organic macromolecules (Lützow et al., 2006). SOM may be protected when it is occluded within aggregates because of its inaccessibility, or because the activities of microorganisms are limited by environmental factors such as oxygen diffusion and water availability (Six et al., 2002; Dungait et al., 2012).

Plant residues in decomposition or particulate organic matter (POM), and mucilage exuded from fungal hyphae and bacteria become absorbed to charged soil particles forming the core of stable microaggregates within macroaggregates (Vidal et al., 2018), contributing to aggregate stabilisation (Six et al., 2004). When soil is depleted of available POM the microbial activity diminishes and the aggregate stability declines due to a deficiency of binding agents (Vidal et al., 2018), and hence the contribution of occlusion to SOM protection declines (Six et al., 2002; Dungait et al., 2012). Thus, the maintenance of stable aggregates and their protective SOM function by occlusion depends in part on the turnover of SOM in aggregates to maintain the activity of soil microorganisms.

Several studies have indicated that C stabilisation is greater within free microaggregates compared to macroaggregates and that formation of microaggregates is crucial for soil C storage and its stabilisation in the long-term (Six et al., 1998; Gale et al., 2000). Beare et al. (1994) observed that C mineralisation increased when macroaggregates were disrupted, but this increase was only 1–2% of the C content of the macroaggregates supporting the concept of microaggregate formation within aggregates.

2.5.3 Interaction with mineral surfaces

The interaction between the fine mineral fraction and SOM is well stablished as a specific stabilization mechanism due to the protection of potentially labile organic matter against biological oxidation (Feller and Beare, 1997; Baldock and Skjemstad, 2000; Six et al., 2002), when low molecular weight compounds as microbial derived sugars, peptides and amino acids are adsorbed onto soil mineral particles.

SOM associated with soil mineral fractions (fine silt and clay) and multivalent cations is regarded to be relatively stable, with relative long turnover times (Balesdent, 1996). It also represents a large proportion of the total SOC and therefore, its measurement can be useful in determining stable organic C in soils (Baldock and Skjemstad, 2000).

Several studies have shown that there is a strong and positive relationship between the total SOC content and the relative mass of the fine mineral (e.g. clay) fractions (Hassink, 1997; Six et al., 2002; Homann et al., 2007). However, the relationship of SOC with the specific surface area (SSA) of minerals has been shown to be stronger than the relationship with the clay content (Saggar et al., 1996; Beare et al., 2014), and the SSA of Fe-oxides and Al- oxides may provide the most significant surface area for SOM to be absorbed (Lützow et al., 2006).

In New Zealand, several studies have reported that the clay content cannot be used to explain the variation in SOC content under permanent pastures (Percival et al., 2000; Beare et al., 2014), while Curtin et al. (2016) observed a relationship between stable C and the proportion of fine particles in Brown and Recent soils. Therefore, the SSA is currently considered to be a better indicator of a soil's capacity to stabilise SOM than the relative mass of the fine soil mineral fraction *per se* (Beare et al., 2014).

2.5.4 Substrate-driven biological limitations

Ekschmitt et al. (2005), suggested that SOM is stabilised by a complex of mechanisms that constrain decomposition rates, which are not necessarily a function of substrate quality or soil conditions, but rather a function of the soil biology and limits on its activity. Therefore, Ekschmitt et al. (2005)

concluded that under prevalent conditions of substrate limitations (e.g. organic molecules, water and oxygen) soil microorganisms are obligated to operate at low decompositions rates as their capacity to decompose is constrained energetically, and it may cost organisms as much to acquire energy from SOM as they gain from it. The ecological limitations inherent to soil microorganisms may play an important role within the complex of physical, chemical, and biological mechanisms of stabilisation of SOM, that in turn contribute to low decomposition rates and maintaining high SOC stocks in temperature soils.

2.6 Stable isotopes to study soil organic carbon dynamics

Isotopes refer to elements having the same number of protons, but different numbers of neutrons. Extra neutrons in the nucleus of an element generally impart only subtle chemical differences, however, isotopes of an element undergo chemical, biological, and physical reactions at slightly and consistently different rates, leading to isotopic fractionation whenever reactants are not exhausted (Fry, 2006). As a result, natural variations in isotopic abundance provide powerful insight into element dynamics, especially ones that cycle tightly with SOM such C (^{12}C, ^{13}C and ^{14}C) and N (^{15}N and ^{14}N) (Fry, 2006).

Labelling plants with C isotopes, at both natural abundance and artificially enriched levels has been widely used to study the transfer and dynamics of C within the plant-soil system (Saggar et al., 1997; Kuzyakov and Domanski, 2000; Studer et al., 2014). These studies using C isotope measurements have allowed major progress in measuring and understanding the dynamics of C in soil, as the partitioning and turnover of OC from roots in soil can be measured *in situ* and over decades, and can also be applied to bulk soil OC as well as to the physical and chemical fractions of SOC (Chenu et al., 2015). There are three C isotope labelling methods that are currently used to trace the transfer and partitioning of C from plants to the soil by exposure of the plants to isotopically labelled CO_2: natural abundance (NA), pulse labelling and continuous labelling (Kuzyakov and Domanski, 2000).

2.6.1 Natural abundance

The ^{13}C natural abundance (NA) method utilises known vegetation changes from C3 plants and C4 plants with contrasting photosynthetic pathways (Chenu et al., 2015). The plant compounds of these two photosynthetic types have contrasting ^{13}C to ^{12}C ratios, and despite that the microbial decomposition of these materials causes some isotopic fractionation, the resulting SOM still maintain similar isotopic $^{13}C/^{12}C$ ratios or isotopic signature of the parent vegetation (Balesdent et al., 1987; Skjemstad et al., 1990). Therefore, where a change of vegetation has occurred at a known date, the measurement of the change in the ^{13}C NA of SOC allows both the rate of loss of OC derived

from the initial vegetation and the rate of incorporation of C from the new vegetation to be determined (Figure 2.8).

Despite a narrow range of situations where the ^{13}C NA method can be applied, this method is valuable because is equivalent to a 'true' labelling *in situ* of the SOM (Balesdent et al., 1987; Skjemstad et al., 1990; Flessa et al., 2000; Shahbaz et al., 2018), is relatively simple and can distinguish between soil and plant OC decomposition, which cannot be distinguished using artificial enrichments (Cheng and Gershenson, 2007). However, the main disadvantage of the ^{13}C NA labelling is that it relies on change in vegetation from C4 to C3 or vice versa, which is uncommon under natural field conditions (Kuzyakov and Domanski, 2000).

In New Zealand for example, where the majority of pastures are based on perennial ryegrass and while clover, which are both C3 plants (MacLeod and Moller, 2006), the ^{13}C NA labelling method is inappropriate to measure the rates of formation and decomposition of SOC, as the difference in the isotopic signature between plant derived C and SOC is negligible (Werth and Kuzyakov, 2010).

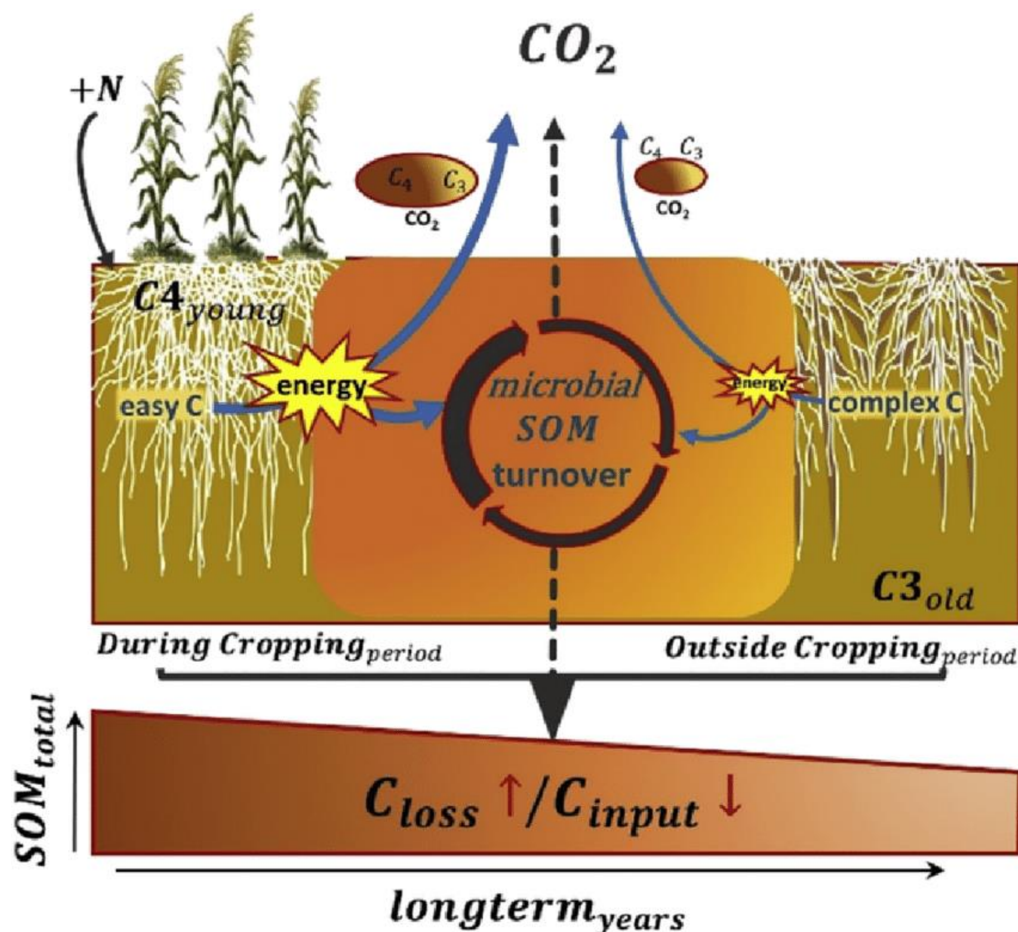


Figure 2.8. Schematic diagram showing decomposition of young (C4) and old C (C3) during or outside the cropping period and resulting changes in total long term soil organic matter (SOM) stabilisation, from Shahbaz et al. (2018).

2.6.2 Pulse labelling

In a pulse labelling experiment, highly ^{13}C enriched CO_2 (usually 99 atom% $^{13}CO_2$) is added to the plant-atmosphere system in a pulse during a short period of time, (e.g. hours), then the ^{13}C is traced in the plant-soil system over time. This method of ^{13}C labelling can be carried out as a one-off labelling event or over several pulse labelling events. The ^{13}C isotopic enrichment is calculated using the difference between ^{13}C enriched plant and/or soil C pools and the same C pools at natural abundance (NA) levels within the plant-soil system (Studer et al., 2014). This approach can be used to determine the dynamics of recently assimilated C fluxes in plant organs and rapidly turning over soil C pools or components, such as microbial biomass and DOC (Kuzyakov and Domanski, 2000; Studer et al., 2014). However, a meta-analysis comparing pulse and continuous labelling methods to quantify C inputs in rice paddy soils, Liu et al. (2019) reported that pulse labelling underestimates the total below-ground C inputs by approximately 15% when compared with continuous labelling (Figure 2.9), but provides detailed information about the dynamics and fate of rhizodeposits in the soil.

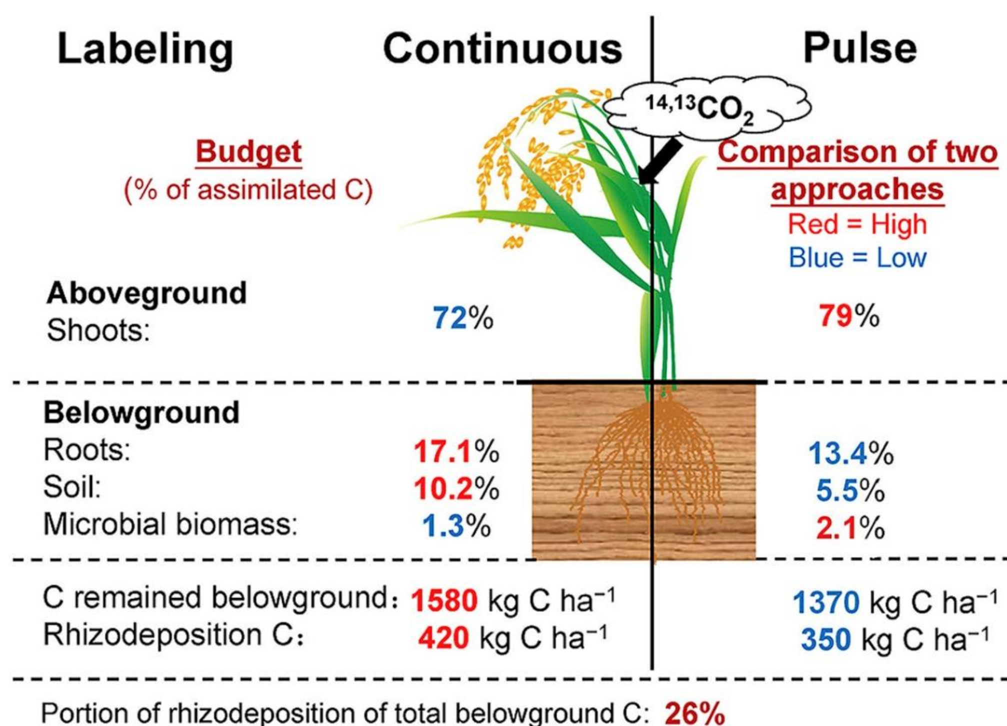


Figure 2.9. Schematic overview to compare carbon inputs, partitioning and rhizodeposition by rice into paddy soil between pulse and continuous isotopic labelling methods. From Liu et al. (2019).

2.6.3 Continuous labelling

In continuous ^{13}C labelling experiments, the plants are grown within chambers and continuously exposed to, usually, lower ^{13}C enrichments of CO_2 (generally < 10 atom% ^{13}C) or ^{13}C-depleted CO_2 over the whole experimental period with samples taken during and/or at the end of the labelling

(Esperschütz et al., 2009). This experimental methodology requires an expensive setup of equipment and ^{13}C labelled materials, but provides a more integrated C cycling result (due to the homogeneous labelling of C pools and fluxes), which allows a better estimation of photosynthate C partitioning within the plant-soil system. In addition, continuous labelling allows estimation of the mean transfer time through a compartment, including the short-term storage within the different components that the plant-soil can be divided into (Kuzyakov and Domanski, 2000; Kuzyakov and Schneckenberger, 2004; Studer et al., 2014).

2.6.4 Stable isotope notation

Methods involving stable C isotopes (^{12}C and ^{13}C) usually use the δ notation (pronounced delta) which represents the difference in ^{13}C values of a sample relative to the international reference standard PeeDee Belemnite (PDB) and has units of ‰ (per mill). The sign of the ^{13}C values indicates whether the sample has a higher or lower $^{13}C/^{12}C$ ratio than PDB and is expressed by Equation 2.1.

$$\delta^{13}C\ (‰) = \left[\left(\frac{R_{sample}}{R_{standard}}\right) - 1\right] \qquad [2.1]$$

Where R_{sample} and $R_{standard}$ are the $^{13}C/\,^{12}C$ abundance ratios of the sample and the standard PDB, respectively.

Organic C is generally depleted in ^{13}C as a result of biological isotope fractionation occurring primarily during the photosynthetic process. The isotopic composition of SOM largely reflects the photosynthetic pathway of the dominant species in the plant community. Typical values of $\delta^{13}C$ for SOM where the plant community mainly comprises C3 plant species is approximately -27‰ compared to -13‰ for plant communities dominated by C4 species (Boutton, 1991). These $\delta^{13}C$ values can be used to calculate OC inputs from a known source, for example, recent photosynthate C transfer from roots (source) to soil as shown in Equation 2.2.

$$f = \frac{^{13}C_{soil} - {}^{13}C_{soil\,NA}}{^{13}C_{roots} - {}^{13}C_{soil\,NA}} \qquad [2.2.]$$

Where $^{13}C_{soil}$ is the $\delta^{13}C$ (‰) value of the soil pool or component being measured, $^{13}C_{roots}$ is the $\delta^{13}C$ (‰) value of the sample root mass and $^{13}C_{soil\ NA}$ is $\delta^{13}C$ (‰) value of the soil sample at natural abundance (NA) level.

2.7 Summary

This literature review has covered the importance of terrestrial C pools, especially the role of grasslands SOC influencing the climate through complex interactions that govern the fluxes of C within the atmosphere-plant-soil system. This review has highlighted the need for identifying

pasture management practices that can balance the trade-off between food production and the impacts on the environment that includes maintaining or increasing SOC storage, outcomes that are crucial, not only for mitigating climate change but also to guarantee global food security and other important soil ecological functions.

While New Zealand is following a global trend in terms of the increased use of irrigation to support food production, the specific role of irrigation in altering SOC stocks under grazed pasture systems is not well understood and the causal mechanisms responsible for the different responses of SOC to irrigation appear to be complex. There is limited data available on studies investigating how irrigation affects the mechanisms controlling the balance between inputs and outputs of OC from the soil, such inputs from root derived-C via rhizodeposition and root turnover regarded as the main source of OC to maintain SOC storage under grasslands.

Furthermore, there are no data available for measuring the effects of irrigation on the uptake, partitioning and short-term persistence of photosynthate C within the entire pasture plant-soil system, and these are needed in order to understand the direct impacts of irrigation on SOC storage.

Chapter 3

Seasonal irrigation affects the partitioning of new photosynthate carbon in soil

A manuscript from this chapter has been published in the journal Soil Biology Biochemistry: Carmen R. Carmona, Timothy J. Clough, Samuel R. McNally, Michael H. Beare, Craig S. Tregurtha and John E. Hunt. Volume 143, April 2020, 107751. https://doi.org/10.1016/j.soilbio.2020.107751. This is referred to in later chapters as Carmona et al. (2020).

3.1 Abstract

Long-term irrigation of temperate pastures has been reported to either increase or decrease soil organic carbon (SOC) stocks when compared with dryland systems. Understanding the short-term effects of irrigation on the fixation and partitioning of carbon (C) to plant and soil components may be important to explaining the observed differences. Continuous $^{13}CO_2$ pulse labelling of ryegrass (*Lolium perenne L*.) and white clover (*Trifolium repens L.)* planted mesocosms was used to quantify the net accumulation and partitioning of new photosynthate C to above- and below-ground components of the plant-soil system, including soil particle size fractions: > 250 µm, 53–250 µm, 20–53 µm, 5–20 µm and < 5 µm, under simulated irrigation and dryland conditions.

After the $^{13}CO_2$ labelling, irrigation increased the quantity of ^{13}C partitioned into herbage by 16%, while reducing the quantity partitioned into roots in the 15–25 cm soil depth by 35%. However, less new photosynthate C was observed in rhizosphere soil (0–15 cm depth), while more new photosynthate C was partitioned into the 53–250 µm and < 5 µm soil fractions under irrigation. Despite these differences, the net amount of new photosynthate C in the whole soil (0–25 cm depth) was similar between treatments (2511 kg new C ha^{-1} dryland and 2509 kg new C ha^{-1} irrigated). Therefore, irrigation did not increase the net amount of new photosynthate C in the soil despite increased above-ground pasture productivity. Based on our results, we hypothesise that the recently reported losses of SOC from irrigated pastures may be driven by faster turnover of root-derived C, which may explain the increase in photosynthate C in the fine POM soil size fraction (53–250 µm), rather than a reduction in photosynthate C inputs to the soil.

3.2 Graphical abstract

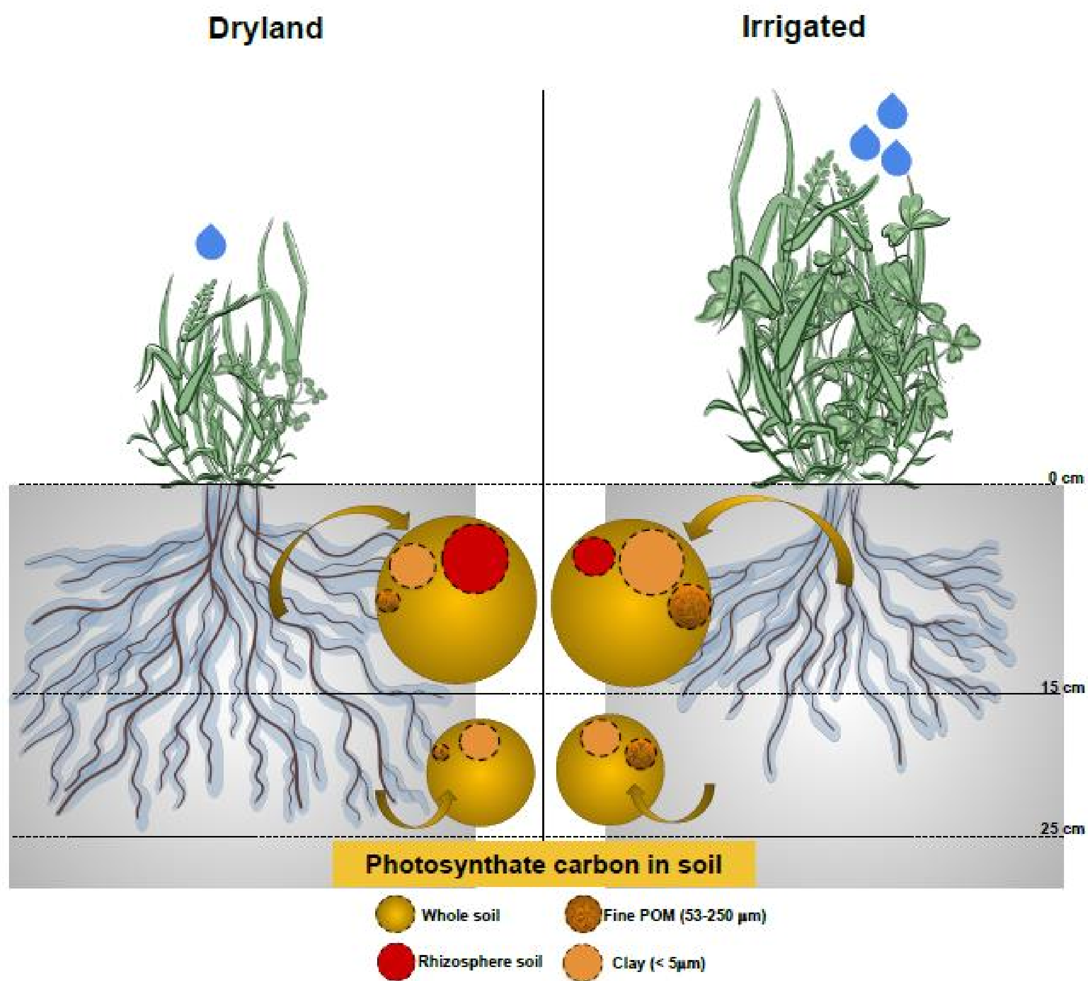


Figure 3.1. Graphical representation of the key results of Chapter 3: differences between the dryland and irrigated treatments on pasture biomass allocation and the partitioning of new photosynthate C within soil components.

Keywords: Irrigation, pasture, photosynthate carbon, soil carbon.

3.3 Introduction

Grasslands contain approximately 20% of the global soil C stocks (Stockmann et al., 2013), with 60% of the grassland SOC stock found in temperate climate regions (Ramesh et al., 2019). The potential for grassland soils to be a source or sink for C, depends on how management affects the fixation and partitioning of C to above- and below-ground components and the stability of C deposited in soils (Staddon, 2004; Ostle et al., 2007; Kong and Six, 2010; Horwath and Kuzyakov, 2018; Whitehead et al., 2018). Therefore, studying the responses of SOC under temperate grasslands to different management practices is crucial to assess their role in contributing to or mitigating further increases in atmospheric CO_2 concentrations.

Intensification of managed grasslands has increased globally in an effort to support the growing demand for meat, wool and dairy products (Thornton, 2010; Kastner et al., 2012), and includes the increased use of synthetic fertilisers and irrigation to enhance pasture production. However, the use of irrigation has raised questions regarding water resources and environmental sustainability, mainly with respect to water quality, nutrient management and maintaining soil C stocks (Mudge et al., 2017; Whitehead et al., 2018).

While the use of irrigation is predicted to increase to help secure global food production (Zhao et al., 2015), few studies have investigated the effects of irrigation on SOC, and most of these studies have focussed on cropping systems in arid or semi-arid climates (Gillabel et al., 2007; Denef et al., 2008; Apesteguía et al., 2015). There is a lack of data available about irrigation effects on SOC under grazed pastures in temperate climates (Laubach and Hunt, 2018; Whitehead et al., 2018) where C fixation and organic C partitioned below-ground are expected to be high.

Seasonal irrigation of temperate grasslands results in increased above-ground pasture productivity during warmer and drier seasons, when water availability can limit pasture growth (Condron et al., 2014), but the effects of irrigation on SOC stocks has produced contradictory results with increases (Kelliher et al., 2015), no effects (Condron et al., 2014; Hunt et al., 2016) and decreases (Fraser et al., 2012; Condron et al., 2014; Mudge et al., 2017) being reported.

The specific role of irrigation in altering SOC is not well understood and the causal mechanisms responsible for these different responses in temperate pastures appear to be complex (Whitehead et al., 2018). For example, it is not known whether the effects of irrigation on SOC stocks are primarily associated with differences in the partitioning of photosynthate C to above- and below-ground components of the plant-soil system, that affect SOC inputs, or with differences in the decomposition and turnover of C deposited below-ground (Kelliher et al., 2012; Mudge et al., 2017).

Plants transport photosynthate C to the soil via exudation from fine roots or through association with mycorrhizal fungi. Both exudates and mycorrhizae can stimulate microbial decomposition of SOC that in turn improves plant nutrient availability (Jones et al., 2004; Kaiser et al., 2015; Liese et al., 2017; Vidal et al., 2018; Gorka et al., 2019). Root exudates under grasslands, represent approximately 5% of the total photosynthate C inputs to soil (Pausch and Kuzyakov, 2018), and are a key driver of rhizosphere processes (Farrar et al., 2003; Jones et al., 2004). The partitioning of photosynthate C into and through the plant to soil is regulated by processes within the roots and the leaves in response to different stresses (Farrar et al., 2003; Jones et al., 2004).

Plants experiencing water stress have decreased photosynthetic activity due to stomatal closure that reduces transpiration (Peñuelas et al., 2004; Zhou et al., 2019). Water stress also decreases water

and nutrient uptake per unit of root mass, which can result in enhanced allocation of biomass to roots compared with shoots (Poorter and Nagel, 2000). The magnitude of this allocation change depends on the plant's nutrient status and on the plant community composition (Poorter and Nagel, 2000; Sanaullah et al., 2011; Sanaullah et al., 2012). Root derived-C is generally considered the main input of organic C to soil (Rasse et al., 2005) in the short term, contributing more to soil C stabilisation than inputs from above-ground biomass (Kong and Six, 2010).

To understand the mechanisms regulating SOC storage stabilisation and decomposition, SOC fractions must also be considered, as the form in which C is formed and stored in the soil will determine its persistence and functioning (Grandy and Neff, 2008; Poeplau et al., 2018; Cotrufo et al., 2019; Lavallee et al., 2020). For example, particulate organic C fractions (> 53 µm) are considered pivotal for the short-term storage of root derived-C and they have a relatively short turnover time (Balesdent, 1996; Lavallee et al., 2020). SOC associated with the fine soil fractions, silt and clay (5–20 µm and < 5 µm, respectively), are considered more stable with a relatively long turnover time (Feller and Beare, 1997; Baldock and Skjemstad, 2000; Six et al., 2002; Lützow et al., 2006; Poeplau et al., 2018; Lavallee et al., 2020).

The main objective of this study was to quantify the effects of irrigation on the partitioning of new photosynthate C to both above- and below-ground components of the plant-soil system, including soil particle size fractions, in a temperate perennial ryegrass and white clover pasture that was continuous-pulse labelled with $^{13}CO_2$.

We propose the following hypotheses to explain the effects of irrigation on the partitioning of photosynthate C above- and below-ground: *1)* where nutrients are not limiting, irrigation will increase the partitioning of new photosynthate C to above-ground plant components, while reducing the partitioning of new photosynthate C to root mass, and *2)* a reduction in root mass under irrigation will result in a lower net accumulation of new photosynthate C in soil.

3.4 Materials and Methods

3.4.1 Mesocosm establishment

A Lismore stony silt loam soil (Pallic Firm Brown soil [New Zealand]; Udic Ustochrept [USDA]) was collected (0–15 cm depth) from an established dryland Lucerne (*Medicago sativa* L.*)* pasture at the Ashley Dene Research and Development Farm (Lincoln, Canterbury, NZ;43°38'36"S, 172°21'21"E) in April 2016. The fine earth (stone free) soil compromised 53 ± 2% silt, 18 ± 1% clay and 29 ± 2% sand (± stdev). Gravimetric stone content, *in situ*, was 55 ± 6% (± stdev). The soil contained 4 g C kg^{-1} soil, with an *in situ* bulk density of 1.09 g cm^{-3}. Volumetric water content (VWC) at field capacity and permanent wilting point were 39% and 13%, respectively.

After soil collection, all stones were removed by sieving (6 mm) and the soil was air dried. The sieved soil and stones of a uniform size (14–20 mm diameter) were re-packed into polyvinyl chloride (PVC) mesocosms (*n* = 60, 15 cm diameter x 25 cm deep). The mesocosms had a gravimetric stone content of 50% (approximately 3500 g) and a soil fine earth bulk density of 1.10 g cm^{-3}. The re-packing of soil and stones was performed to be similar to the average stone content observed in the field.

Soil moisture (Em50 Decagon, ICT International, Armidale, Australia) and soil temperature (Tinytag, TGP 4104, Gemini Data Loggers, Chichester, UK) probes were inserted into a subset of mesocosms to monitor soil moisture (*n* = 20) and temperature (*n* = 8).

Mesocosms were sown in July 2016 with perennial ryegrass (*Lolium perenne* L. cv. Prospect AR37) and white clover (*Trifolium repens* L. cv. Weka) at rates of 20 and 5 kg ha^{-1}, respectively. During pasture establishment, the soil VWC of the mesocosms was maintained at approximately 20–32% (a winter-spring period VWC typical for this soil type) by periodic watering. Prior to initiating the $^{13}CO_2$ labelling, the mesocosms were housed inside an unheated glasshouse for 5 months (Jul 16–Dec 16) to allow the pasture to establish (Figure 3.2). Whenever the ryegrass reached the third leaf stage (Sean et al., 2016) the pasture was harvested to a residual height of 4 cm, which is a common grazing height used in New Zealand grazed pastures.

To ensure the pasture nutrient requirements were not limiting, mesocosms were periodically fertilised with nitrogen (N) (15 g L^{-1} urea solution, equivalent to 50 kg N ha^{-1}) and P-K-S (19 kg P ha^{-1}, 159 kg K ha^{-1} and 16 kg S ha^{-1}) in solution (Monaghan et al., 2007; Morton et al., 2017).

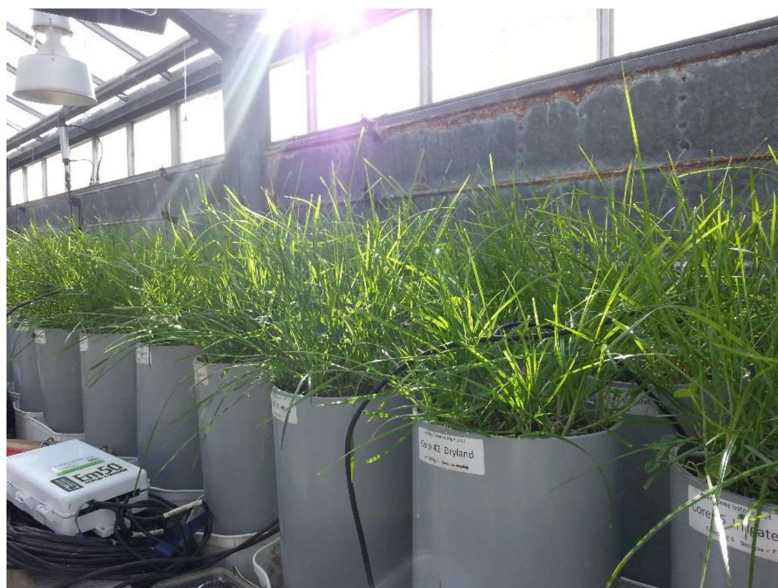


Figure 3.2. Ryegrass-white clover established mesocosms with connected Decagon soil moisture sensors.

3.4.2 Experimental design and treatment application

The mesocosms (*n* = 60) were randomly allocated to one of two treatments, a 'dryland treatment' where soil VWC was maintained between 13–28% and an 'irrigated treatment' with soil VWC maintained between 25–33%. The dryland and irrigated treatments simulated an unirrigated pasture and a typical summer irrigation system, respectively. The irrigated treatment was managed to maintain a small soil water deficit to avoid drainage and leaching. The soil VWC of all cores was monitored by regular weighing of each mesocosm (1–2 times per week). When required, water was applied manually using a volumetric dispenser. The soil water management treatments were imposed between 8 November 2016 and 22 March 2017.

After approximately 3 months of pasture establishment 48 mesocosms (*n* = 24 dryland and *n* = 24 irrigated) were placed into a sealed transparent (acrylic) plant growth chamber (2 m long × 1.2 m wide × 1 m high) in a split-plot design (*n* = 4 blocks). Environmental variables (temperature, relative humidity and photosynthetically active radiation (PAR)) were continuously monitored within the chamber. The temperature and relative humidity inside the chamber were maintained similar to the glasshouse conditions using a fan-forced heat exchanger.

A subset of mesocosms (*n* = 6 irrigated and *n* = 6 dryland) were maintained in a separate glasshouse under similar environmental conditions to the chamber to determine the natural abundance (NA) ^{13}C signatures of the plant-soil components.

3.4.3 $^{13}CO_2$ continuous-pulse labelling

Highly enriched $^{13}CO_2$ (approximately 36 atom% ^{13}C) was produced by injecting 50 mL of 0.6 M citric acid through a rubber septum of a 250-mL Schott bottle containing 5 g of ^{13}C-labelled sodium carbonate in aqueous solution ($Na_2{}^{13}CO_3$, 99 atom% ^{13}C, Sigma-Aldrich) and captured in a 3-L Tedlar® gasbag that was connected via a tube to the Schott bottle. The evolved $^{13}CO_2$ (approximately 1000 mL) was flushed through to the gasbag with ambient CO_2 (approximately 2000 mL) using a manual syringe connected to the Schott bottle and the gasbag.

The mesocosms in the chamber were continuously pulse labelled through daily additions (equivalent to 0.5 g $Na_2{}^{13}CO_3$) of $^{13}CO_2$ for 3 months over the summer period (Southern Hemisphere, 19 December 2016–22 March 2017) (Figure 3.3). Each morning a gasbag containing the $^{13}CO_2$ was connected to the plant growth chamber and CO_2 was delivered into the chamber using a precision air pump (Flow rate = 12.7 mL s^{-1} and injection time of approximately 57 s). The rare exceptions to daily labelling were when PAR was <50 μmol m^{-2} s^{-1} or the first day after dry matter cuts, as there was not enough leaf area, and the efficiency of photosynthesis was reduced.

Figure 3.3. Plant growth chamber for $^{13}CO_2$ labelling. The heat exchange unit can be seen under the chamber. The side wall panels are removable for easy access to the mesocosms. The addition of $^{13}CO_2$ and $^{12}CO_2$ into the chamber was controlled by a gas isotope analyser (Picarro).

The concentration of $^{12}CO_2$ and $^{13}CO_2$ within the chamber was monitored continuously and controlled using a system consisting of a data logger (Campbell Scientific CR1000, USA) connected to a cavity ring down spectrometer (Picarro G2210-*i,* Santa Clara, CA) (Figure 3.4). The atmosphere within the chamber was maintained at an ambient concentration of total CO_2 (approximately 400 ppm) using additions of food grade $^{12}CO_2$ from a cylinder connected to the chamber. When required the control system triggered a solenoid valve to make additions of $^{12}CO_2$. Each morning if the CO_2 concentration within the chamber exceeded 700 ppm, mainly due to plant and soil respiration at night, the chamber was opened manually to dump the excess CO_2 prior to the addition of $^{13}CO_2$.

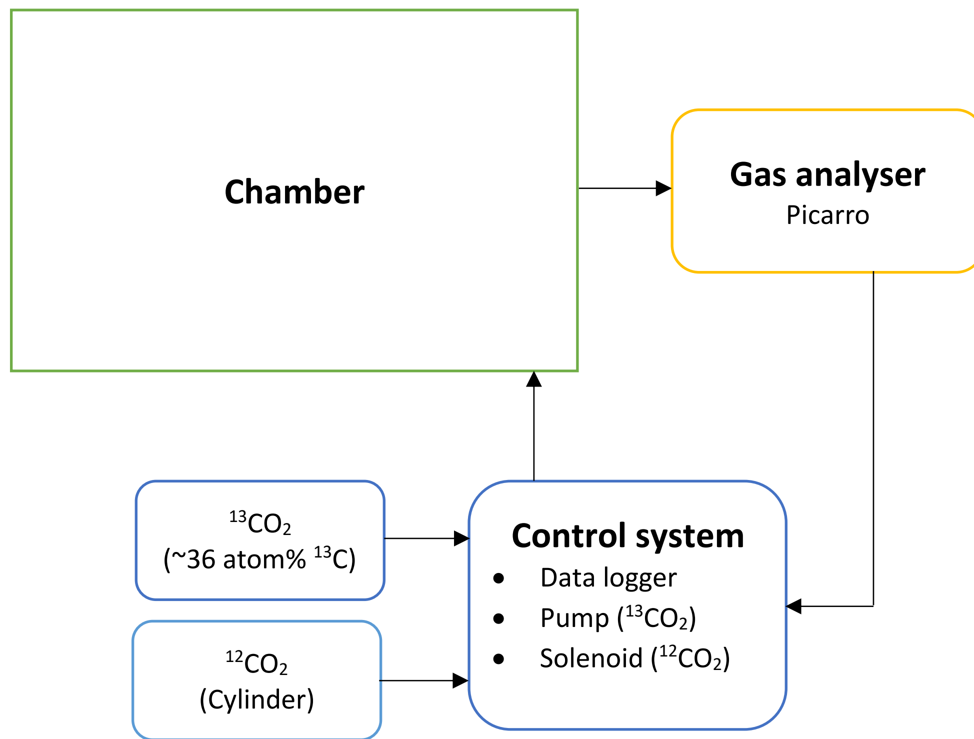


Figure 3.4. Schematic diagram of the control system for $^{13}CO_2$ labelling connected to the chamber and to the gas isotope analyser (Picarro).

3.4.4 Sampling and sample preparation

Destructive sampling of mesocosms occurred at five time points following the 3 months of ^{13}C labelling to reflect the net accumulation of new photosynthate C at the end of an irrigation phase (day 1) and to quantify the subsequent losses of that new C over an annual cycle (days 12, 125, 237 and 349). Here we report results from the first destructive sampling time (t = 1 day), where 22 mesocosms were sampled (n = 8 labelled mesocosms per treatment, n = 3 NA per treatment) at the cessation of the irrigation phase to determine the net accumulation and partitioning of C to above and below-ground components of the plant-soil system compared to dryland (non-irrigated) conditions. Results from 12–349 days after the last labelling event will be presented and discussed in a subsequent paper, as they address the persistence and loss of the C that accumulates under irrigation or dryland conditions. Herbage (leaves) was cut at height of 4 cm approximately above the soil surface. The remaining plant material (stem and stolon) was cut at the soil surface and is referred to as 'residual'.

The soil column was extracted from the PVC mesocosm and roots and soil were separated for two depths (0–15 cm and 15–25 cm). The roots were manually removed from the soil, and for the top depth the soil adhering to the roots was carefully shaken (approximately 6 times) and collected; hereafter referred to as 'rhizosphere soil' (Hafner and Kuzyakov, 2016). The soil remaining after

removal of both roots and rhizosphere was defined as 'non-rhizosphere soil'. In the 15–25 cm depth, rhizosphere soil was not separated from the roots, and the soil in this depth is subsequently called 'whole soil'.

Non-rhizosphere soil (0–15 cm) and whole soil (15–25 cm) were sieved (5.5 mm mesh) and soil subsamples were taken to determine the soil gravimetric water content (oven drying at 105°C for 24 h). A subsample of the 5.5 mm sieved soil was removed and passed through a second sieve (2 mm) before fine grinding to homogenise the soil samples prior to elemental and isotopic analysis. Rhizosphere soil (0–15 cm) was also sieved (2 mm). All sieved soil samples were air-dried at 25°C.

Root material collected from the sieves was combined with roots collected manually and homogenised prior to oven drying at 60°C (48 h). Dried root material was weighed, and a subsample was taken and washed with deionised water on a 250-µm sieve and dried again at 60°C (48 h). Subsamples of the 5-mm sieved, fresh non-rhizosphere soil (0–15 cm) and whole soil (15–25 cm) were taken and washed on a 250-µm sieve to separate roots. These roots were dried at 60°C (48 h) and weighed in order to determine any remaining root mass in the soil that was not removed manually, in order to calculate the total root mass (Equation 3.1). It is acknowledged that some very fine root material may have been lost through the 250-µm sieve.

The total root mass in kg (T_r) was calculated as follows:

$$Tr = r_m + \left[\left(\frac{r_w}{s_m}\right) \times T_s\right] \qquad [3.1]$$

Where r_m (kg) was the mass of roots manually collected during the sampling, r_w (kg) was the mass of root washed from the 5.5-mm sieved soils, s_m (kg) was the mass of soil subsample (5.5-mm sieved) and the T_s (kg) was the total mass of non-rhizosphere soil (0–15 cm) and whole soil (15–25 cm), 5.5-mm sieved. As the fine earth bulk density was similar between the treatments, the value of T_r (kg) was expressed in kg ha^{-1} using the surface area of the mesocosms (0.01767 m^2)

Prior to elemental and isotope analysis, dried samples of herbage, residual, roots, rhizosphere soil, non-rhizosphere soil and whole soil were ground (< 53 µm) using a bench top ring mill (BTRM, RockLabs, Auckland, NZ), taking care to avoid cross contamination by cleaning equipment between samples using deionised water and ethanol.

3.4.5 Soil particle size fractionation

Subsamples (30 g) of < 2 mm sieved air-dried samples of non-rhizosphere soil (0–15 cm, n = 8 per treatment) and whole soil (15–25 cm, n = 8 per treatment) were fractionated into five soil size fractions: > 250 µm (coarse particulate organic matter CPOM), 53–250 µm (fine POM), 20–53 µm

(silt), 5–20 µm (fine silt), and < 5 µm (clay) using a method similar to Qiu et al. (2010). Briefly, samples were dispersed in deionised water using an ultrasonic probe (BOSCH, UW 2200) for 60 s with sonication set at 60 J s^{-1} (Figure 3.5A). The dispersed soil suspension was passed through 250 µm and 53 µm sieves with the two POM fractions retained on the respective sieves. The < 53 µm material was separated into 20–53 µm, 5–20 µm and < 5 µm fractions by gravity, using their different rates of sedimentation and siphoning off the excess of water. A flocculent (calcium chloride dehydrate, $CaCl_2.2H_2O$) was added to the < 5 µm suspension to aid the sedimentation of the clay particles (Figure 3.5B). The mass of each of the five fractions was collected following oven drying at 60°C, before each fraction was homogenised using a mortar and pestle prior to analysis.

Figure 3.5. A) Ultrasonic probe for soil dispersion before the particle size fractionation, B) Soil size particle fractionation by gravimetric method using calcium chloride dehydrate as a flocculent to promote settling of clay soil particles (< 5 µm).

3.4.6 Isotopic composition after $^{13}CO_2$ labelling

Subsamples of the plant-soil components and the soil size fractions were analysed for their C concentration and isotopic C composition using an elemental analyser (Sercon, model GSL, Crewe, UK) coupled to a continuous flow isotopic ratio mass spectrometer (Sercon, model 20-22, Crewe, UK). The ^{13}C isotope composition ($\delta^{13}C$) of the samples was expressed in units of per mil (‰) (Equation 3.2).

$$\delta^{13}C\ (‰) = \left[\left(\frac{R_{sample}}{R_{standard}}\right) - 1\right] \qquad [3.2]$$

Where *R* = the $^{13}C/^{12}C$ ratio for either the sample or the standard (0.01118). The $\delta^{13}C$ values are expressed relative to Pee Dee Belemnite (PDB).

3.4.7 Partitioning of ^{13}C

The partitioning of ^{13}C between plant and soil components, and the soil particle size fractions, was calculated as the amount of ^{13}C recovered within these components, and soil size fractions, for each treatment (Equation 3.3–3.6). When applicable, values for the rhizosphere and non-rhizosphere soil in the 0–15 cm depth, were used to calculate a weighted average of $\delta^{13}C$ values and the mass of ^{13}C recovered expressed in units of mg ^{13}C m^{-2} for combined soil at the 0–15 cm depth.

The amount of C (mg C m^{-2}) in each plant-soil component or soil size fraction was calculated using Equation 3.3.

$$C = (TC/100) \times m \qquad [3.3]$$

Where *TC* is the total C (%) and *m* is the mass of each plant-soil component or soil size fraction per unit area (mg m^{-2}), using the surface area of the mesocosms (0.01767 m^2).

The fractional abundance (*F*) of the sample was calculated using Equation 3.4 modified from Stewart and Metherell (1999):

$$F = \frac{(\delta^{13}C+1000)}{[(\delta^{13}C+1000+(1000/R_{standard}))]} \qquad [3.4]$$

Where $R_{standard}$ = the $^{13}C/^{12}C$ ratio for the standard. The $\delta^{13}C$ values are expressed relative to Pee Dee Belemnite (PDB).

The atom% ^{13}C excess was calculated as the difference between the fractional abundance of the heavier isotope after labelling (F_a) and the fractional abundance at NA level (F_{NA}) using Equation 3.5.

$$atom\%\ ^{13}C_{excess} = (F_a - F_{NA}) \times 100 \qquad [3.5]$$

The amount of ^{13}C recovered expressed in mg m^{-2} ($^{13}C_{amount}$) within each plant-soil component or soil size fraction was calculated using Equation 3.6.

$$^{13}C_{amount} = \frac{atom\%\ ^{13}C_{excess}}{100} \times C \qquad [3.6]$$

3.4.8 The net accumulation of new photosynthate carbon

The net accumulation of new photosynthate C (kg ha^{-1}) in the soil components at the end of $^{13}CO_2$ labelling period was calculated using Equations 3.7–3.8 (Kong and Six, 2010):

The proportion (*f*) of new soil C derived from roots during the months of labelling was calculated using Equation 3.7.

$$f = \frac{^{13}C_{soil} - ^{13}C_{soil\,NA}}{^{13}C_{roots} - ^{13}C_{soil\,NA}} \qquad [3.7]$$

Where $^{13}C_{soil}$ is the δ^{13}C (‰) value of each soil component, $^{13}C_{roots}$ is the δ^{13}C (‰) value of the root mass and $^{13}C_{soil\,NA}$ is δ^{13}C (‰) value of the NA soil.

The amount of new C (kg ha^{-1}) was calculated using Equation 3.8.

$$New\ C = f * (TC/100) * s_m \qquad [3.8]$$

Where *TC* is the total C in each soil component expressed as a % and s_m is the soil mass expressed in units of kg ha^{-1}, based on the soil mass and mesocosms surface area.

The mass of ^{13}C in each fraction was expressed as a proportion of the total amount of ^{13}C recovered in each soil component (e.g. non-rhizosphere soil and whole soil). This proportion was applied to the mass of new C in each soil component derived from Equation 3.8.

3.5 Statistical and data analysis

Factorial analysis of variance (ANOVA) was run to compare the effects of dryland and irrigated treatments based on split plot design using GenStat (18th edition, Lawes Trust, Harpenden, UK). Plant-soil components and soil particle size fractions data were analysed with a model defined by treatment as fixed factor. Residual plots and the Shapiro-Wilk test were used to check the normality and the equal variance assumptions of ANOVA. When ANOVA assumptions were met without Log-transformed data, the Fisher's Protected Least Significant Difference test (LSD) with a significance level of 5% was used to determine the difference between the dryland and irrigated means. Where Log transformation of data was required, the Fisher's Protected Least Significant Ratio (LSR), with a significance level of 5% was used to assess differences of means between treatments as follow in Equation 3.9.

$$if\ \frac{a}{b} \geq LSR\ \therefore a \neq b \qquad [3.9]$$

Where *a* and *b* are the larger and smaller means, respectively and independent of the treatment, *LSR* is the Least Significant Ratio.

3.6 Results

3.6.1 Environmental conditions

Air temperatures in the plant growth chamber ranged from 12°C to 33°C with a mean temperature of 20°C, which was approximately 4°C higher than the 30-year mean outdoor air temperature (16 ±

3°C, ± stdev; Figure 3.6A) for Lincoln (NIWA, 2018). Soil temperature in the mesocosms (0–15 cm depth) during the labelling period ranged from 13°C to 30°C with a mean temperature of 20°C, which was 3°C higher than the 12-year mean for 0–30 cm soil depth under field conditions (17 ± 1°C, ± stdev; Figure 3.6B). Average soil temperatures were similar between dryland and irrigated treatments.

Mean soil VWCs during the labelling period were 21% and 29% for the dryland and irrigated treatments, respectively (Figure 3.6C). The soil VWC of the dryland treatment was wetter than the long-term average field conditions during this period (Figure 3.6C). However, the number of dry/wet cycles was comparable with the average number of >10 mm summer rainfall events (3–9) during the last 18-year (NIWA, 2018). During irrigation season, the soil VWC in the dryland mesocosms varied from a maximum of 28% to near wilting point (13%), while the irrigated treatment averaged 29% (range 34–24%, Figure 3.6D).

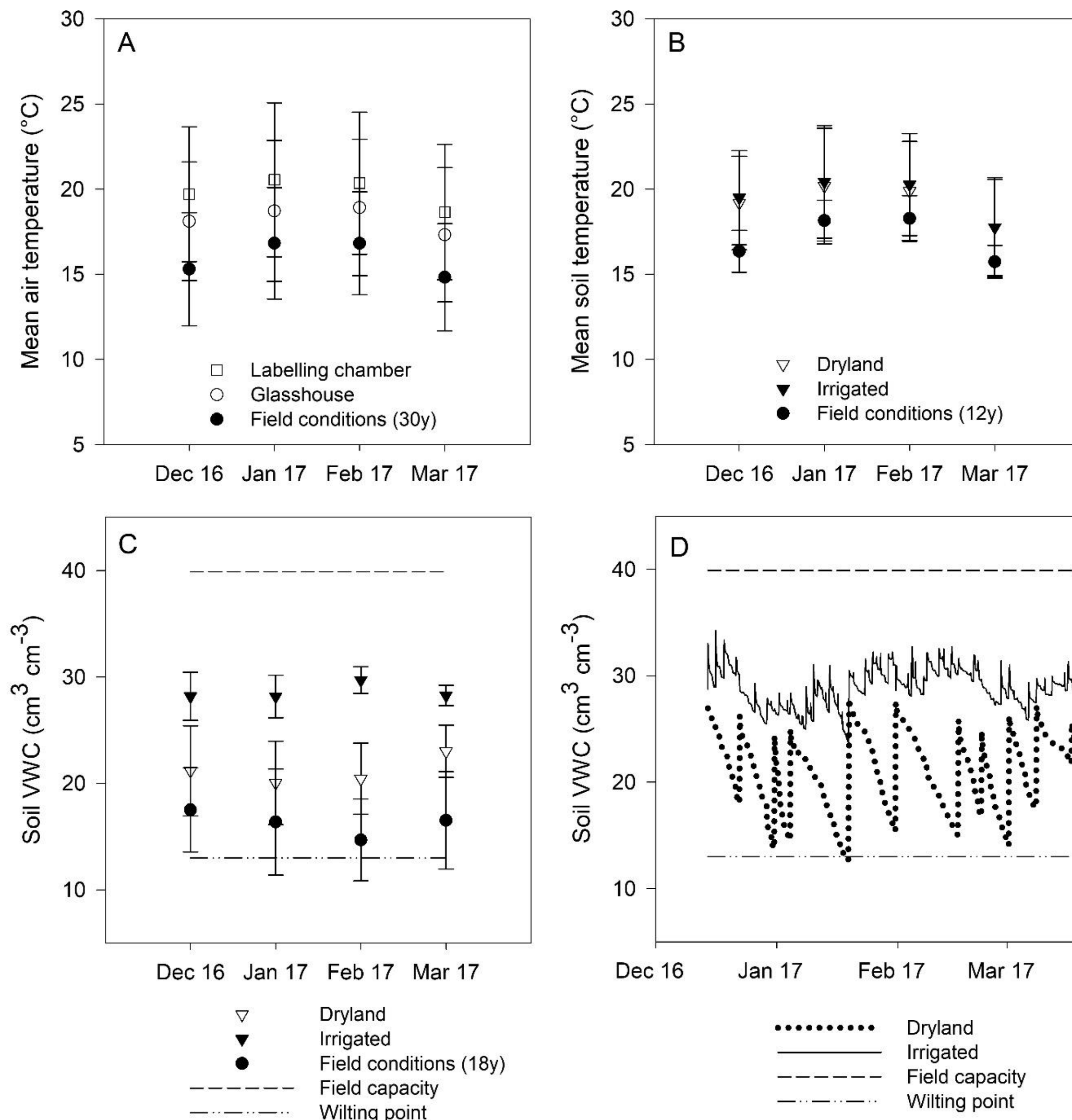


Figure 3.6. Monthly time series of mean values during the labelling period for A) Air temperature in the labelling chamber, glasshouse and the 30-year mean in field conditions, B) Soil temperature of the dryland and irrigated treatments, and the 12-year mean in field conditions, C), Soil volumetric water content (VWC) of the dryland and irrigated treatments, and the 18-year mean in field conditions D) Soil VWC of the dryland and irrigated treatments during the $^{13}CO_2$ labelling. Error bars are ± standard deviation (stdev) of the mean.

3.6.2 Plant production

Cumulative production of herbage during the labelling period was greater under the irrigated than the dryland treatment ($P < 0.001$; Table 3.1). A change in pasture composition was observed due to irrigation with an increase in white clover herbage production of approximately 16% compared with the dryland treatment (data not shown). The standing residual biomass (stem and clover stolon) was similar for both treatments ($P = 0.123$, Table 3.1). Approximately 80% of the total root mass was recovered from the 0–15 cm depth, and there was no significant difference in the mass of roots

recovered from the dryland and irrigated treatments at this depth (P = 0.116, Table 3.1). Root mass in the 15–25 cm depth was significantly higher under the dryland treatment (P = 0.035, Table 3.1). There was no difference between treatments in the sum of the total plant biomass (above-ground + standing root mass, Table 3.1).

Table 3.1. Herbage production, residual and root mass expressed as dry matter. Values for herbage are cumulative means ± standard error of mean (*n* = 24 per treatment) of the monthly production during the labelling period (Dec 2016–Mar 2017). Values for residual and roots are means ± standard error of mean (*n* = 8 per treatment) of the standing mass at 1 day after the last labelling event. The least significant difference (LSD) of means with significance level of 5%. LSD compares means between treatments is provided when P < 0.05.

Dry matter (kg ha^{-1})				
	Depth (cm)	**Dryland**	**Irrigated**	**LSD**
Herbage		6103 ± 38.6	8412 ± 140	533.4
Residual		2395 ± 135	2773 ± 234	
Total above-ground		8498 ± 140	11185 ± 273	961.8
	0–15	8541 ± 736	7220 ± 567	
Root mass	15–25	2653 ± 357	1689 ± 373	892
Total below-ground	0–25	11194 ± 818	8909 ± 679	2414
Total above and below-ground		19692 ± 830	20094 ± 732	

3.6.3 ^{13}C isotopic composition of plant-soil components

Following 3 months of $^{13}CO_2$ labelling, ^{13}C enrichment of herbage, residual and roots was greater than the soil components. There were no significant differences between treatments in $\delta^{13}C$ values of the combined herbage (ryegrass plus white clover) (P = 0.862, Table 3.2). Whereas the ^{13}C enrichment of clover herbage (969 ± 17.8 ‰ $\delta^{13}C$) was higher than the ryegrass (876 ± 9.11‰ $\delta^{13}C$) in the irrigation treatment (P < 0.018), there was no significant difference in the ^{13}C enrichment of clover and ryegrass herbage in the dryland treatment (P = 0.063). The residual plant material was more ^{13}C enriched under the dryland treatment (P < 0.001; Table 3.2). Root $\delta^{13}C$ values from the 0–15 cm depth were similar between treatments (P = 0.093, Table 3.2). Root $\delta^{13}C$ values from the 15–25 cm depth were higher under the dryland treatment (P = 0.040, Table 3.2). Rhizosphere soil ^{13}C enrichment did not differ between treatments (P = 0.617; Table 3.2), but had greater enrichment than the non-rhizosphere (0–15 cm depth, P < 0.001) and whole soil (15–25 cm depth, P < 0.001).

Table 3.2. The mean ± standard error of mean of $\delta^{13}C$ values for plant-soil components at natural abundance (NA, *n* = 6), and at 1 day after the last labelling event for the dryland and irrigated treatments (*n* = 8 per treatment). The least significant difference (LSD) of means with significance level of 5% compares means between treatments is provided when P < 0.05.

Isotopic composition $\delta^{13}C$ (‰)					
Component	**Depth (cm)**	**NA**	**Dryland**	**Irrigated**	**LSD**
Herbage		-28.6 ± 0.2	901 ± 8.37	903 ± 7.98	
Residual		-29.1 ± 0.1	518 ± 14.7	442 ± 19.1	34.2
Roots	0–15	-28.9 ± 0.2	104 ± 5.83	135 ± 14.7	
	15–25	-28.9 ± 0.1	76.5 ± 6.1	54.4 ± 7.6	20.9
Rhizosphere soil	0–15	-27.3 ± 0.2	-5.34 ± 1.84	-4.16 ± 2.56	
Non-rhizosphere soil	0–15	-27.5 ± 0.1	-22.1 ± 0.32	-20.9 ± 0.59	
Whole soil	0–15	-27.5 ± 0.1	-20.7 ± 0.37	-19.9 ± 0.56	
	15–25	-27.4 ± 0.02	-24.5 ± 0.25	-24.6 ± 0.36	

For the non-rhizosphere soil (0–15 cm depth), the greatest ^{13}C enrichment among soil size particle fractions was observed in the > 250 µm fraction (coarse POM) (P < 0.001, Table 3.3), although no significant difference was observed between the dryland and the irrigated treatment (P = 0.412, Table 3.3). The 53–250 µm (fine POM) fraction had greater ^{13}C enrichment in the irrigated treatment (P = 0.002, Table 3.3). Higher ^{13}C enrichment under irrigation was also observed in the 5–20 µm (fine silt) and < 5 µm fractions (P = 0.004 and P = 0.02, respectively). ^{13}C enrichment in the 20–53 µm fraction was similar between treatments (P = 0.108).

For the whole soil (15–25 cm depth), ^{13}C enrichment was greatest in the > 250 µm fraction (P < 0.001) with no differences between treatments (P = 0.592, Table 3.3). Irrigation had a significant effect only in the 53–250 µm fraction (P = 0.021, Table 3.3), with higher $\delta^{13}C$ values under the irrigated treatment. ^{13}C enrichment in the 20–53 µm, 5–20 µm and < 5 µm soil fractions was similar between treatments (P = 0.214, P = 0.096 and P = 0.493, respectively).

Table 3.3. The mean ± standard error of mean of δ^{13}C values for soil size fractions at natural abundance (NA, *n* = 4), and at 1 day after the last labelling event for the non-rhizosphere soil (0–15 cm depth, *n* = 8 per treatment) and the whole soil (15–25 cm depth, *n* = 8 per treatment). The least significant difference (LSD) of means with significance level of 5% compares means between the dryland and irrigated treatments is provided when P < 0.05.

Isotopic composition δ^{13}C (‰)					
Soil component	**Fraction (µm)**	**NA**	**Dryland**	**Irrigated**	**LSD**
Non-rhizosphere soil (0–15 cm depth)	> 250	-27.7 ± 0.12	8.29 ± 5.74	13.1 ± 3.09	
	53–250	- 28.5 ± 0.06	-23.0 ±0.55	-16.1 ±1.26	2.33
	20–53	-28.0 ± 0.06	-26.2 ±0.10	-25.6 ± 0.22	
	5–20	-28.1 ± 0.09	-26.5 ± 0.14	-25.5 ± 0.20	0.39
	< 5	-27.1 ± 0.03	-24.5 ± 0.17	-23.4 ± 0.21	0.74
Whole soil (15–25 cm depth)	> 250	-27.6 ± 0.4	-6.60 ± 3.89	-8.72 ± 2.72	
	53–250	-28.6 ± 0.09	-25.9 ±0.37	-23.4 ± 0.83	1.81
	20–53	-28.3 ± 0.06	-27.3 ± 0.07	-27.1 ± 0.15	
	5–20	-28.0 ± 0.03	-27.2 ±0.09	-26.9 ± 0.11	
	< 5	-27.1 ± 0.04	-25.5 ± 0.12	-25.7± 0.28	

3.6.4 Quantity of ^{13}C recovered

Plant-soil components

The total amount of ^{13}C retained within the plant-soil components was similar between the dryland treatment and for the irrigated treatment (Figure 3.7A). The total amount of ^{13}C retained in the plant and soil components, represented approximately 27% and 30% of the total ^{13}C added to the chamber during the labelling period for the dryland and irrigated treatments, respectively.

The quantity of ^{13}C recovered in herbage was greater under the irrigated treatment (P <0.001 and LSD > 55.7, Figure 3.7A), while for the residual plant material (stem and stolon) there was no significant difference between treatments (P = 0.123, Figure 3.7A). The sum of the above-ground plant components, for both treatments, represented approximately 77% of the total ^{13}C recovered in the whole plant-soil system (Figure 3.7A).

In the 0–15 cm depth, ^{13}C recovery in root mass accounted for approximately 10% of the total ^{13}C recovered per each treatment (Figure 3.7A) and the amount of ^{13}C recovered was not different between the dryland and the irrigated treatment (P = 0.382, Figure 3.7A). The amount of ^{13}C recovered in roots from the 15–25 cm depth represented approximately 2% of the total ^{13}C recovered and was higher under the dryland treatment (P = 0.045 and LSR > 1.89, Figure 3.7A).

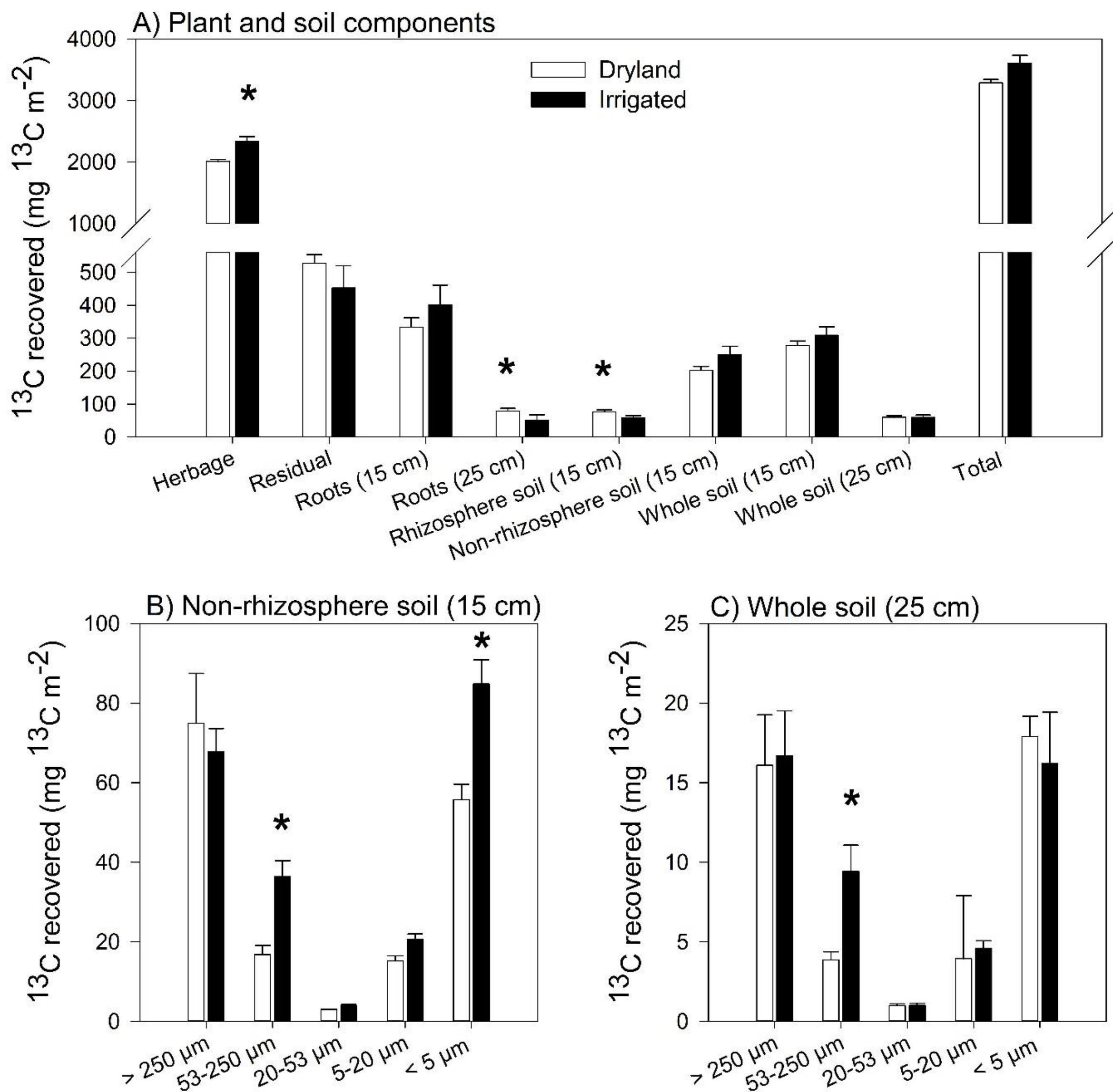


Figure 3.7. Quantity of ^{13}C recovered (mg ^{13}C m^{-2}) within A) plant-soil components, soil size particle fractions for B) non-rhizosphere soil in the 0–15 cm depth, and C) soil fractions for whole soil in the 15–25 cm depth, after 1 day of the last $^{13}CO_2$ labelling event. Values given as means and error bars represent 1 standard error of the mean. Asterisk symbol (*) represents statistical difference (P < 0.05) between the dryland and irrigated treatment. Note the break on the y-axes.

The total amount of ^{13}C in the rhizosphere was higher under the dryland treatment (P = 0.024 and LSR > 1.31, Figure 3.7A) and represented approximately 2% of the total ^{13}C recovered in the whole plant-soil system. There was no effect of irrigation on fine soil bulk density (0.81 ± 0.01 g cm^{-3} and 0.79 ± 0.01 g cm^{-3}, for the dryland (n = 8) and irrigated (n = 8) treatments, respectively). Hence, differences between treatments in the ^{13}C recovered in the rhizosphere soil could have been affected by differences in the mass of rhizosphere soil collected during the sampling as a direct effect of soil water content.

The quantity of ^{13}C recovered in the non-rhizosphere soil (0–15 cm depth) did not differ between treatments (P = 0.101, Figure 3.7A). Likewise, the quantity of ^{13}C in whole soil (0–15 cm depth) was similar between the dryland and irrigated treatments (P = 0.185, Figure 3.7A), and this amount of ^{13}C equated to approximately 8.5% of the total ^{13}C recovered in each treatment. The quantity of ^{13}C recovered in the whole soil for the 15–25 cm depth represented approximately 2% of the total ^{13}C recovered in each treatment, and this was again similar among treatments (P = 0.185, Figure 3.7A).

Soil size fraction

Nearly all of the original soil mass (98 ± 0.1%, n = 8 per treatment) was recovered in the particle size fractions, irrespective of treatment or soil sample. For the non-rhizosphere soil (0–15 cm depth), ^{13}C was mainly recovered in the > 250 µm and < 5 µm fractions (Figure 3.7B). However, significant differences between the dryland and irrigated treatments were only observed in the 53–250 µm and < 5 µm fractions, being higher under the irrigated treatment (P = 0.003 and LSR > 1.39, Figure 3.7B). In contrast to the > 53 µm and < 5 µm soil fractions, the 20–53 µm fraction contained the smallest quantity of ^{13}C recovered, with no significant differences between treatments (P = 0.003 and LSR < 1.39, Figure 3.7B).

The ^{13}C recovered in soil size fractions from the whole soil (15–25 cm depth) showed that ^{13}C recovery in the 53–250 µm fraction was higher under the irrigated treatment (P = 0.014 and LSR > 1.81, Figure 3.7C). Similar to the non-rhizosphere soil in the 0–15 cm depth, the quantity of ^{13}C recovered was also concentrated in the > 250 µm and < 5 µm fractions, but with no significant differences between treatments (P = 0.014 and LSR < 1.81, Figure 3.7C).

The net accumulation of new photosynthate C

There was less new photosynthate C accumulated in the rhizosphere soil (0–15 cm) of the irrigated treatment compared with the dryland treatment (P = 0.005, Figure 3.8A). The sum of the new photosynthate C accumulated in the rhizosphere and non-rhizosphere soil from the 0-15 cm depth (whole soil) was approximately 3-fold greater than the amount accumulated in the whole soil at 15–25 cm depth (P < 0.001, Figure 3.8A). However, there were no significant differences between the treatments in the amount of new C that accumulated in each depth for the whole soil (P = 0.961, Figure 3.8A). There was a significant interaction between treatment and depth that affected the distribution of new C in the soil profile, with approximately 70% of the total net amount of new C accumulated in the 0–15 cm depth (P = 0.05).

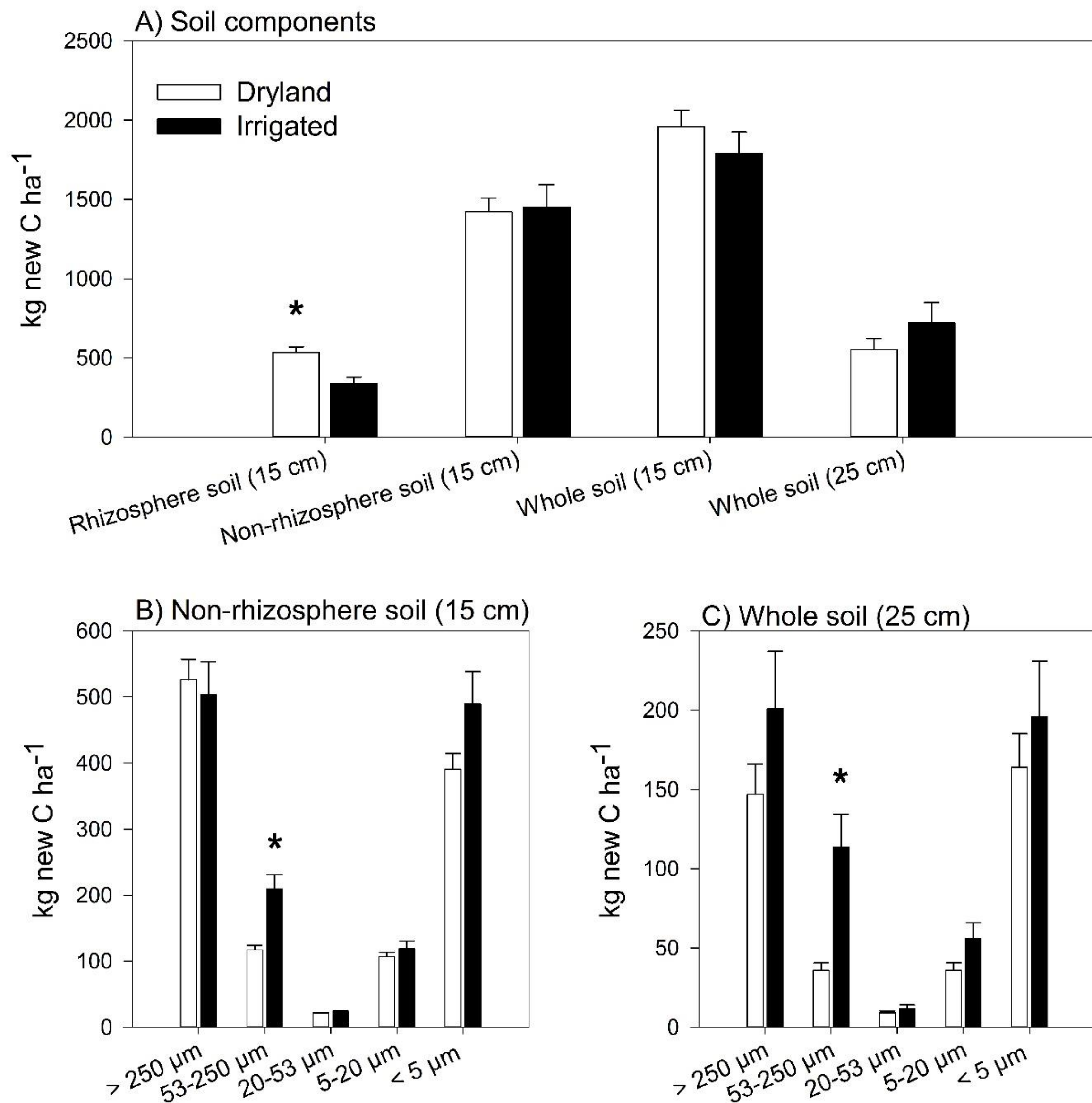


Figure 3.8. The net amount of photosynthate C (kg new C ha^{-1}) accumulated in A) the different soil components in the 0–15 cm depth and 15–25 cm depth, soil size particle fractions for the B) non-rhizosphere soil in the 0–15 cm depth and, C) soil fractions for whole soil in the 15–25 cm depth (c). Values given as means and error bars represent 1 standard error of the mean. Asterisk symbol (*) represents statistical difference (P < 0.05) between the dryland and irrigated treatment.

The accumulation of new C in size fractions from the non-rhizosphere soil (0–15 cm depth) was mainly in the > 250 µm and < 5 µm fractions, and represented approximately 80% of the total photosynthate C that was recovered among all fractions (Figure 3.8B). However, there were no significant differences between the dryland and irrigated treatments in the amount of new C accumulated within > 250 µm and < 5 µm fractions (P = 0.623 and P = 0.114, respectively). The net accumulation of new C in the 53–250 µm fraction was higher under the irrigated treatment (P < 0.001 and LSR = 1.30, Figure 3.8B). The amount of new C accumulated in the 20–53 µm and 5–20 µm was again similar between the treatments (P = 0.660 and P = 0.460, respectively). A similar trend was observed for the accumulation of new C within soil size fractions for the whole soil (15–25 cm

depth), where the amount of new C accumulated in the 53–250 µm fraction was higher under the irrigated treatment ($P < 0.001$ and LSR > 1.53, Figure 3.8C).

3.7 Discussion

3.7.1 Irrigation effects on production and allocation of plant biomass

Irrigation resulted in a substantial increase (approximately 38%) in herbage dry matter production over the 3-month (December-March) period of seasonal irrigation (Table 3.1). In contrast root biomass in the 0–25 cm soil depth was 20% higher under the dryland treatment. However, the difference in root mass was driven by a lower root mass allocation in the 15–25 cm depth under the irrigated treatment (approximately 36% less), while the root mass in the 0–15 cm depth was similar between treatments (Table 3.1). These differences in above- and below-ground biomass allocation due to irrigation are in agreement with other studies of temperate pastures on similar soil types as used in this study, where irrigation increased herbage production by approximately 10–28% (Stewart and Metherell, 1999; Scott et al., 2012) and decreased root mass by approximately 20% in the 0–20 cm soil depth (Scott et al., 2012).

3.7.2 Irrigation effects on the transfer of photosynthate C from plant to soil

Plant partitioning of ^{13}C

The observed differences in biomass production and its partitioning to herbage, residual and roots between the treatments were expected to be mirrored in the partitioning of ^{13}C between above- and below-ground plant components. Due to a greater dry matter production, herbage was the dominant sink for ^{13}C (mg ^{13}C m^{-2}), which was approximately 16% greater in the irrigated compared to the dryland treatment (Figure 3.7A).

The amount of ^{13}C partitioned to the residual plant component was similar between the treatments (Figure 3.7A). However, the higher ^{13}C enrichment observed under the dryland treatment (Table 3.2) suggests greater storage of photosynthate C, as the residual consisted of grass stems and clover stolons, which are plant organs that act as sinks for photosynthate C. Under water deficit conditions, plant stems store C until alleviation of water stress, where upon C can be reallocated to provide energy for herbage biomass recovery (Poorter and Nagel, 2000; Poorter et al., 2012).

The total amount of ^{13}C recovered in roots (approximately 14% per each treatment, Figure 3.7A) was within the typical range (10–21%) reported for roots of temperate pastures in previous labelling studies (Stewart and Metherell, 1999; Saggar and Hedley, 2001; Sanaullah et al., 2012). Under the irrigated treatment, roots in the 0–15 cm depth retained approximately 20% more photosynthate ^{13}C than the dryland treatment, however, due to a large variability under irrigation, this difference

was not statistically significant (Figure 3.7A). In contrast, ^{13}C recovered from roots in the 15–25 cm depth, was approximately 35% higher in the dryland treatment (Figure 3.7A).

For our first hypothesis (i.e., irrigation would increase the partitioning of new photosynthate C to above-ground plant components, while reducing its partitioning to roots), we aimed to test whether irrigation would change the partitioning of photosynthate C in the above- and below-ground plant components. Our results show that the observed patterns in plant biomass allocation to herbage, residual and roots between the treatments mirrored the partitioning of photosynthate C recovered in these plant components (Figure 3.7A). Irrigation significantly increased the amount of ^{13}C partitioned in herbage, while decreasing the amount partitioned in the deeper roots (15-25 cm depth). Under the conditions of this study, irrigation had a neutral effect on the amount of ^{13}C partitioned in the residual and upper roots (0–15 cm depth). Therefore, these results not only support our first hypothesis, but also demonstrate the functional differences between plant organs in response to environmental changes.

The contrasting effects on plant biomass and C partitioning (i.e. greater root mass in dryland) are consistent with the 'functional equilibrium' theory (Lambers, 1983; Poorter and Nagel, 2000), where plants experiencing water stress tend to reduce photosynthate allocation to above-ground biomass production and increase allocation to root proliferation in order to capture more of a limiting resource (Poorter and Nagel, 2000; Sanaullah et al., 2011; Sanaullah et al., 2012; Körner, 2015). Plants are able to adapt to water stress by developing larger and/or deeper root systems and by altering stomata closure to reduce evapotranspiration losses (Peñuelas et al., 2004; Zhou et al., 2019). In contrast, the greater allocation of photosynthate C to herbage and lower allocation of this C to root mass in the irrigated treatment is consistent with a plant strategy to maximise canopy structure and light interception under optimal environment conditions (Wardlaw, 1990; Mini

chin et al., 1994).

The net accumulation of new photosynthate C

There was no difference in the total amount of ^{13}C recovered in the whole soil (0–25 cm depth) between the irrigated and dryland treatments (Figure 3.7A), despite clear differences in above-ground dry matter production, root biomass and the partitioning of ^{13}C to plant components (Table 3.2 and Figure 3.7A). The total net amount of new photosynthate C recovered in the whole soil (0–25 cm depth) in each treatment, was approximately 10.5%, which compares well with the 5–10% range reported by Farrar et al. (2003) who summarised results from 95 ^{14}C-labelling experiments covering a wide range of plant species, and with other studies that reported that approximately 10% of the photosynthate C transferred below-ground is recovered in the soil within the first 24 hours

after isotopic labelling (Domanski et al., 2001; Jones et al., 2009; Kaiser et al., 2015). The transfer of plant photosynthates into the soil can occur within hours of photosynthetic fixation (Jones et al., 2004; Kaiser et al., 2015; Liese et al., 2017; Vidal et al., 2018; Gorka et al., 2019) and depends on plant species and plant community composition (Jones et al., 2009; Sanaullah et al., 2012; Pausch and Kuzyakov, 2018).

At cessation of the irrigation season (1 day after the last labelling event), we observed that the ^{13}C enrichment of white clover herbage was higher than ryegrass ^{13}C enrichment, but this difference was significant only under irrigation. However, our results do not allow us to isolate the contributions of clover and ryegrass to soil C allocation, so it is not clear to what extent differences in plant species composition may have contributed to any differences observed in ^{13}C enrichment of the soil. Never-the-less, the differences we observed in the relative contribution of clover and ryegrass to pasture composition under irrigation and dryland treatments are consistent with what has been reported under field conditions (Lucero et al., 2000). Studies on the effects of drought on C partitioning within plant-soil system, reported that partitioning patterns changed when plant species were grown in mixtures compared with monocultures (Sanaullah et al., 2011; Sanaullah et al., 2012). During drought, plants increase C partitioning to root biomass, but this increase was less pronounced when plant species were grown a in mixture, which significantly increased C partitioning into leaves, suggesting that plants growing in mixtures compete more strongly for light than for water and nutrients (Sanaullah et al., 2012).

The transfer of plant-derived C to soil can be direct via root exudation, or indirect, through the death and subsequent decomposition of roots (Jones et al., 2009). This transfer of C is often associated with processing by mycorrhizal fungi, bacteria and invertebrates (Ostle et al., 2007; Vidal et al., 2018; Chomel et al., 2019). The plant-derived C taken up by the soil biota is either respired, assimilated or released as metabolic by-products. Once in the soil, this organic C can form associations with mineral surfaces (e.g. iron oxides) and be stabilised, with most of the root-derived C sorbed to these surfaces being a by-product of biological decomposition rather than direct sorption of exudates (Kaiser et al., 2015; Vidal et al., 2018). A relative higher ^{13}C enrichment of the non-rhizosphere soil (0–15 cm depth) and whole soil (15–25 cm depth), indicate considerable transfer of ^{13}C from the rhizosphere to the wider soil (Table 3.2).

Our results do not represent the gross production of photosynthate C since plant and soil respiration were not measured, but it has been reported that under drought conditions total soil respiration (roots and soil) decreased and the partitioning of photosynthate C to dissolved organic carbon (DOC) increased when compared with optimum water conditions (Sanaullah et al., 2012). In this regard, irrigation may have increased the outputs of photosynthate C from the rhizosphere soil by

enhancing soil respiration. However, in this study the net amount of photosynthate C accumulated in the whole soil (0–25 cm depth) was similar between treatments (Figure 3.8A) suggesting that irrigation may have promoted a vertical pathway for the distribution of photosynthate C from topsoil to the subsoil.

For our second hypothesis, we aimed to test whether reduced root mass under irrigation would result in less photosynthate C inputs to soil and ultimately the accumulation of new photosynthate C in the soil, as previous studies have shown that in the short-term, roots make a greater contribution to soil C than above-ground biomass (Rasse et al., 2005; Kong and Six, 2010). While the results from the whole soil (0–25 cm depth) in this study do not support this second hypothesis, results from the rhizosphere soil do support it, as irrigation significantly reduced the net accumulation of photosynthate C in this soil component. This irrigation effect in the rhizosphere soil was 'diluted' by the large volume of non-rhizosphere soil. This finding supports the idea that soil processes measured at larger scales are occurring in soil hotspots, which usually compromise a small soil volume (Kuzyakov and Razavi, 2019).

Partitioning of photosynthate C among soil size fractions

Results from soil size particle fractions for non-rhizosphere soil (0–15 cm depth) and whole soil (15–25 cm depth), show that irrigation changed the partitioning of photosynthate C among soil fractions (Figure 3.7B, C and Figure 3.8B, C), especially in the 53–250 µm fraction (fine POM) and < 5 µm fraction, despite no significant differences observed in the net amount of photosynthate C accumulated in these soil components (Figure 3.7A and Figure 3.8A).

The higher amount of photosynthate C in the fine POM fraction (53–250 µm) from the non-rhizosphere soil (0–15 cm depth) and whole soil (15–25 cm depth) demonstrates that irrigation significantly enhanced C inputs to the soil in the form of uncomplexed particulate organic matter (Gregorich and Beare, 2008). Our results align well with those of Apesteguía et al. (2015) where the incorporation of maize-derived organic C in small macroaggregates and microaggregates (250–2000 µm and 50–250 µm) was only observed under irrigation.

The ^{13}C enrichment of the fine POM fraction in the non-rhizosphere soil (0–15 cm depth) and whole soil (15–25 cm depth) was higher than rhizosphere soil (Table 3.2 and Table 3.3), but less enriched than the root tissue at the time of sampling, suggesting that this relatively high ^{13}C enrichment could be derived from fragmented root material, as previous research indicates that the molecular signature of POM (> 53 µm) consists primarily of plant litter (Grandy and Neff, 2008). We did not separate roots mass by plant species, hence, it remains unknown if there were differences between white clover and ryegrass in root derived-C contributions to soil.

The higher recovery of ^{13}C in the fine POM fraction (Figure 3.7A) under the irrigated treatment suggests that root death and decomposition may have occurred faster compared with the dryland treatment. Root death may provide C inputs to soil in several ways: 1) apoptotic root death where non-structural C and nutrients are translocated to other growing regions of the plant before death, 2) non-apoptotic root excision (e.g. physical separation of the root from the plant by tillage) where all the root C and nutrients enter the soil, and 3) non-apoptotic root damage (e.g. due to root disease or invertebrate feeding) where only part of the root C and nutrients may be returned to the soil (Gordon and Jackson, 2000; Jones et al., 2004).

Irrigation has been reported to increase root turnover in temperate pastures (Saggar and Hedley, 2001; Scott et al., 2012), potentially due to an enhanced flow of C into soil food webs, in contrast to dry conditions were the flow of new photosynthate C into these food webs is reduced (Chomel et al., 2019). Fraser et al. (2012) found that there was a higher abundance of earthworms and herbivore soil mesofauna under a summer irrigated pasture compared with a dryland pasture. The higher ^{13}C recovery and accumulation of photosynthate C in the fine POM fraction (53–250 µm) under the irrigated treatment (Figure 3.7B,C and Figure 3.7B, C) may have resulted from greater activity of root-feeding mesofauna under irrigation compared to dryland conditions, leading to greater transfer of root fragments to the fine POM fraction.

The higher recovery of ^{13}C in the clay fraction (< 5 µm) of the non-rhizosphere soil in the 0–15 cm depth under irrigation (Figure 3.7B) may have resulted from the formation and stabilisation of C derived from microbial metabolites of new photosynthate C in association with the soil mineral matrix either by direct sorption of root exudates or via DOC–microbial pathway (Grandy and Neff, 2008; Cotrufo et al., 2015; Kallenbach et al., 2016; Vidal et al., 2018; Lavallee et al., 2020). The total amount of photosynthate C accumulated in the clay fraction (Figure 3.8B) was not significantly different between the treatments due to the large variability of the measurements observed under the irrigated treatment.

Further research on how continued irrigation, or the cessation of irrigation, affects the stability of new SOC associated with the fine POM and clay fractions is required, as increased SOC formation does not necessarily equate to high persistence in soil (Lützow et al., 2006; Cotrufo et al., 2015). SOC associated with the fine POM fraction (53–250 µm) is regarded as very sensitive to physical disturbances (Cotrufo et al., 2019; Lavallee et al., 2020), such as tillage and soil compaction.

3.7.3 Implications of temperate pasture irrigation for soil carbon storage and loss

Although irrigation resulted in greater herbage production and lower root biomass in the 15–25 cm soil depth compared with the dryland treatment, there was no difference in the net accumulation of

new photosynthate C in the whole soil (i.e. rhizosphere plus non-rhizosphere soil). Hence, our findings challenge the assumption that increases in above-ground plant production due to irrigation will increase SOC storage, as predicted by some SOC model simulations (Smith et al., 2016), and point towards the importance of understanding the scale at which soil processes occur (rhizosphere soil vs non-rhizosphere soil and bulk soil vs soil size fractions). The data provided in this study may be used to improve models and their resolution scale to predict more accurate SOC cycling under temperate pastures.

Based on the results from this study, we hypothesise that the causal mechanisms driving the reported losses of soil C from irrigated pastures (Fraser et al., 2012; Condron et al., 2014; Mudge et al., 2017) are associated with faster decomposition of plant-derived C under irrigation rather than a reduction in photosynthate C inputs to the soil. The current study does not report respiration or dissolved organic carbon losses, as the irrigated treatment was managed to avoid leaching, so the effect of irrigation on soil C losses, via leaching and respiration fluxes, needs to be determined. Future studies examining root and fine POM turnover rates along with these associated measures should be undertaken to gain a better understanding of the mechanisms regulating the effects of irrigation on soil C dynamics.

Furthermore, we highlight that increasing and/or maintaining soil C depends not merely on the balance between soil C inputs and outputs but also on the soil's capacity to stabilise organic C, climate conditions and synergies between different agricultural management practices for specific plant-soil system.

3.8 Conclusions

Irrigation did not increase the net amount of new photosynthate C that accumulated in the whole soil, despite increased above-ground pasture productivity, shallower and lower root biomass, and differences in the partitioning of new C to plant and soil fractions. We suggest that the previously reported losses of SOC from irrigated pastures may be driven by faster turnover of roots, which may explain the enhanced accumulation of photosynthate C in the fine POM soil size fraction (53–250 μm) rather than a reduction in photosynthate C inputs to the soil.

3.9 Acknowledgments

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Chapter 4

Fate of carbon fixed and partitioned to plant and soil fractions during summer irrigation over an annual pasture growth cycle

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4.1 Abstract

Soil organic carbon (SOC) is both a source and sink of atmospheric CO_2, with important implications for global climate change. Irrigation of grazed pastures has reportedly increased, reduced, or made no difference to SOC stocks relative to dryland management. This study examined, over an annual pasture growth cycle, the fate of photosynthate carbon (C) fixed and partitioned to plant and soil fractions during the previous summer under irrigated and dryland conditions. A continuous $^{13}CO_2$ pulse labelling method was used to label ryegrass (*Lolium perenne* L.) and white clover (*Trifolium repens* L.) mesocosms under simulated dryland and irrigated conditions. Plant and soil ^{13}C was traced over 349 days using destructive sampling on days 1, 12, 125, 237 and 349 post labelling, with all mesocosms maintained under uniform seasonal soil moisture contents, equivalent to a rainfed system.

After 349 days, approximately 50% of the initial ^{13}C recovered in roots and rhizosphere soil remained. There was no significant change in the ^{13}C recovered in non-rhizosphere soil (0–15 cm) or whole soil (15–25 cm) relative to day 1, but a greater partitioning of ^{13}C to the fine particulate organic matter (53–250 µm) fraction was observed under irrigation. Despite higher recovery of ^{13}C in the clay (<5 µm) fraction of irrigated soil (0–15 cm) at day 1, the ^{13}C recovered in the clay fraction of dryland soil increased over the autumn-winter period, yielding no difference in the clay fraction ^{13}C recovered over the remainder of annual pasture production cycle. Irrigation did not affect the short-term (1-year) persistence of photosynthate C in roots and bulk soil compared to dryland management, but did affect its spatial and temporal partitioning to below-ground plant-soil fractions over the annual pasture production cycle.

Key words: Irrigation, pasture, photosynthate C, particulate organic matter (POM)

4.2 Introduction

Increasing or maintaining soil organic carbon (SOC) has been proposed as an option to offset atmospheric carbon dioxide (CO_2) emissions, and therefore mitigate climate change (Chabbi et al., 2017; Minasny et al., 2017; Poulton et al., 2018; Bradford et al., 2019). However, land management, climate, soil properties (e.g. texture and mineralogy) and societal factors can influence the magnitude of organic carbon (OC) inputs to the soil and the potential for C accumulation (Bünemann et al., 2018; Poulton et al., 2018; Bradford et al., 2019; Frasier et al., 2019). Grassland SOC represents approximately 20% of the global terrestrial SOC stocks and small changes in these stocks, as consequence of land management practices, could significantly affect atmospheric CO_2 concentrations (Ostle et al., 2007; Kong and Six, 2010; Horwath and Kuzyakov, 2018; Whitehead et al., 2018).

Seasonal irrigation of grazed pasture systems increases above-ground dry matter production but typically results in lower root biomass, when compared with dryland pastures (Scott et al., 2012; Carmona et al., 2020). However, the measured effect of irrigation on SOC stocks in these pasture systems has given contrasting results, with increases (Kelliher et al., 2015), neutral responses (Condron et al., 2014; Hunt et al., 2016; Moinet et al., 2017) and reductions (Condron et al., 2014; Mudge et al., 2017) in SOC stocks being reported. Understanding the effect of irrigation on the accumulation and turnover of new photosynthate C is important to untangle the conflicting observations from these studies and to determine the net effect on SOC stocks (Lal, 2016; Bünemann et al., 2018).

Physical fractionation of SOC is an approach used to understand the mechanisms regulating SOC dynamics, including its persistence and function with changes in land management (Grandy and Neff, 2008; Poeplau et al., 2018; Cotrufo et al., 2019; Lavallee et al., 2020). Particulate organic matter (POM) and mineral associated organic matter (MAOM) fractions are recognised as two contrasting forms of soil organic matter (SOM) in terms of their formation, persistence and functioning in soil (Cotrufo et al., 2019; Lavallee et al., 2020). The POM fraction (> 53 µm) derived from plant compounds is considered to have a residence time in the soil typically < 10 years. Moreover, POM fractions are also regarded as pivotal for the short-term storage of root derived-C (Balesdent, 1996; Kong and Six, 2010; Cotrufo et al., 2015; Lavallee et al., 2020). The MAOM fraction (< 53 µm) associated with fine soil particle (i.e. silt and clay) is comprised of low molecular weight compounds derived from both, plant and microbial activity (Kallenbach et al., 2015; Kallenbach et al., 2016; Lavallee et al., 2020). This fine fraction, which can be divided into a number of other fractions, is considered to be stable with long residence time > 10 years (Feller and Beare, 1997; Baldock and Skjemstad, 2000; Six et al., 2002; Sokol et al., 2019).

The SOC within the POM fraction is also thought to be more vulnerable to loss by microbial decomposition than the SOC associated with MAOM fractions (Lavallee et al., 2020). Hence, physical soil disturbances such as tillage or soil compaction can potentially increase POM decomposition (Cotrufo et al., 2019; Lavallee et al., 2020). In contrast, MAOM is less vulnerable to losses by microbial decomposition but changes in soil chemistry can result in desorption of OC from the mineral soil matrix leading to rapid decomposition (Baldock and Skjemstad, 2000; Sokol et al., 2019).

Recent research has demonstrated that irrigation does not affect the net accumulation of new photosynthate C in bulk soil under pasture, but the partitioning within the different soil components is altered (Carmona et al., 2020). Irrigation resulted in a decrease in the partitioning of photosynthate C to rhizosphere soil while increasing the partitioning to the fine POM (53–250 µm) and clay (< 5 µm) soil particle size fractions, when compared to dryland pasture management (Carmona et al., 2020). This accumulation of photosynthate C in the fine POM fraction is in agreement with the results reported by Apesteguía et al. (2015), where irrigation of maize resulted in more OC preferentially partitioned to small soil macroaggregates and microaggregates (250–200 and 50–250 µm, respectively). However, increased SOC partitioning into the aggregates does not necessarily equate to long-term persistence of SOC (Lützow et al., 2006; Cotrufo et al., 2015).

Despite irrigation becoming increasingly common in agriculture (Zhou et al., 2016), there are very few studies that have investigated how irrigation, or its cessation, affects the storage of newly formed SOC and its partitioning among POM and MAOM fractions. Although Saggar and Hedley (2001) previously reported seasonal differences in the uptake and partitioning of photosynthate C to above- and below-ground plant and soil components in an unirrigated pasture, to our knowledge, no prior studies have considered seasonal effects on the persistence of recently fixed photosynthate C under an irrigated temperate pasture. Seasonal changes in the allocation of biomass to above- and below-ground plant components is a response to seasonal variation in environmental conditions (soil water content, temperature and radiation) that serves to sustain maximum plant growth, in line with the 'functional equilibrium' theory (Lambers, 1983; Poorter and Nagel, 2000).

The pronounced seasonal patterns observed in temperate pastures results in high growth rates in spring and the lower rates in winter (Anslow and Green, 1967; Saggar and Hedley, 2001; Dodd and Mackay, 2011). During spring soil water content, air and soil temperatures are optimal for pasture growth (Saggar and Hedley, 2001). The shorter sunlight hours in winter reduce photosynthesis, which lowers dry matter production and therefore, water use and uptake of nutrients (Poorter and Nagel, 2000).

Therefore, the main objective of this study was to quantify the short-term persistence (over an annual cycle) and partitioning of photosynthate C to pasture plant and soil components following a summer irrigation cycle. The following hypotheses are proposed to explain the short-term (< 1 year) persistence of photosynthate C previously partitioned to root biomass and soil under dryland and irrigated management during summer irrigation:

1. The lower total root biomass and greater concentration of roots near the soil surface of summer irrigated pastures will result in less retention of previously fixed (photosynthate) C in the soil during the autumn through spring period compared to dryland management.

2. Higher rates of above-ground dry matter production in the spring period will result in faster turnover of root C and higher accumulation of previously fixed (photosynthate) C in soil compared to the autumn and winter seasons.

3. The lower C storage reported for irrigated compared to dryland pasture soils can be explained by greater partitioning and faster turnover of C associated with the POM fractions.

4.3 Materials and Methods

The experimental setup, $^{13}CO_2$ continuous-pulse labelling method, treatment structure (dryland and irrigated) initially applied, along with the partitioning of new photosynthate C at the end of the irrigation period, have been previously reported in Carmona et al. (2020) (Chapter 3 in this thesis). However, relevant aspects to this study, which examines the short-term (< 1 year) persistence of photosynthate C that was assimilated by the plant-soil system during summer irrigation are outlined below.

4.3.1 Mesocosm establishment

A Lismore stony silt loam soil (Pallic Firm Brown soil [New Zealand]; Udic Ustochrept [USDA]) was collected (0–15 cm depth) from an established dryland Lucerne (*Medicago sativa* L.*)* pasture at the Ashley Dene Research and Development Farm (Lincoln, Canterbury, NZ;43°38’36”S, 172°21’21”E) in April 2016. The fine earth (stone free) soil compromised 53 ± 2% silt, 18 ± 1% clay and 29 ± 2% sand (± stdev). Gravimetric stone content, *in situ*, was 55 ± 6% (± stdev). The soil contained 4 g C kg^{-1} soil, with an *in situ* bulk density of 1.09 g cm^{-3}. Soil volumetric water content (VWC) at field capacity and permanent wilting point were 39% and 13%, respectively.

Soil and stones of a uniform size (14–20 mm diameter) were re-packed into polyvinyl chloride (PVC) mesocosms (*n* = 60, 15 cm diameter x 25 cm deep). The mesocosms had a gravimetric stone content of 50% (approximately 3500 g) and a soil fine earth bulk density of 1.10 g cm^{-3}. The re-packing of soil

and stones achieved the same average stone content and fine earth bulk density observed in the field. Soil moisture (Em50 Decagon, ICT International, Armidale, Australia) and soil temperature (Tinytag, TGP 4104, Gemini Data Loggers, Chichester, UK) probes were inserted into a subset of mesocosms to monitor soil moisture (n = 20) and temperature (n = 8).

Mesocosms were sown with perennial ryegrass (*Lolium perenne* L. cv. Prospect AR37) and white clover (*Trifolium repens* L. cv. Weka) at rates of 20 and 5 kg ha^{-1}, respectively. During pasture establishment, the soil VWC of the mesocosms was maintained at approximately 20–32% (a winter-spring period VWC typical for this soil type) by periodic watering. Prior to initiating the $^{13}CO_2$ labelling, the mesocosms were housed inside an unheated glasshouse for 5 months (Jul 16–Dec 16) to allow the pasture to establish. Whenever the ryegrass reached the third leaf stage (Sean et al., 2016) the pasture was harvested to a residual height of 4 cm, a common post-grazing pasture height. To ensure the pasture nutrient requirements were not limiting, mesocosms were periodically fertilised with nitrogen (N) (15 g L^{-1} urea solution, equivalent to 50 kg N ha^{-1}) and P-K-S (19 kg P ha^{-1}, 159 kg K ha^{-1} and 16 kg S ha^{-1}) in solution (Monaghan et al., 2007; Morton et al., 2017).

4.3.2 Experimental design and treatment application

The mesocosms (n = 60) randomly allocated to one of treatments (dryland or irrigated), were imposed between 8 November 2016 and 22 March 2017. The soil VWC of the dryland and irrigated treatments was maintained between 13–28% and 25–33%, respectively. These two irrigation treatments simulated an unirrigated pasture and a typical summer irrigation system, respectively. The irrigated treatment was managed to maintain a small soil water deficit to avoid drainage and leaching. The soil VWC of all cores was monitored by regular weighing of each mesocosm (1–2 times per week). When required, water was applied manually using a volumetric dispenser.

At the initiation of the irrigation treatments, a subset of mesocosms (n = 24 dryland and n = 24 irrigated) were placed into a sealed transparent (acrylic) plant growth chamber (2 m × 1.2 m × 1 m) in a randomised block design (n = 4 blocks), and continuously pulse labelled with daily additions of $^{13}CO_2$ (36 atom% ^{13}C) for 3 months over the summer period (Southern Hemisphere, 19 December 2016–22 March 2017).

The remaining mesocosms (n = 6 dryland and n = 6 irrigated) were not exposed to $^{13}CO_2$ and instead were maintained in a separate glasshouse under similar environmental conditions to the plant growth chamber. These mesocosms were used to determine the ^{13}C natural abundance (NA) values of the various plant-soil components. Further details on the design and execution of this experiment can be found in Carmona et al. (2020) (Chapter 3 in this thesis).

One day after the last $^{13}CO_2$ labelling event, designated here as T1, the first set of ^{13}C labelled (n = 8 dryland and n = 8 irrigated) and natural abundance (NA) (n = 3 dryland and n = 3 irrigated) mesocosms were destructively sampled as described below. The remaining mesocosms were removed from the plant growth chamber and placed inside an unheated glasshouse. Subsets of these mesocosms (n = 4 dryland and n = 4 irrigated per each sampling time) were destructively sampled at 12, 125, 237 and 349 days after the last ^{13}C labelling event, designated as T2 (autumn), T3 (winter), T4 (spring), and T5 (summer), respectively. The period after ^{13}C labelling, from T1 through T5 is referred to as the "^{13}C chase period" of the experiment.

During the ^{13}C chase period, the soil VWC was similar for the dryland and irrigated treatments and varied from 32–18% over winter and spring. In the following summer of the chase period, both the dryland and irrigated treatments were placed under a simulated irrigation regime, where the soil VWC was maintained between 25–29%. The soil VWC was monitored (hourly) using soil moisture probes (n = 10 dryland and n = 10 irrigated, Em50 Decagon, ICT International Australia), with weekly weighing of individual mesocosms. When required, water was applied manually using a volumetric dispenser. Soil temperature (0–15 cm soil depth) was also monitored using soil temperature probes (n = 4 dryland and n = irrigated, Tinytag, TGP4104, Gemini Data Loggers, Chichester, UK).

To ensure adequate pasture nutrient supply during the chase period, the mesocosms were periodically fertilised with nitrogen (N) (15 g L^{-1} urea solution, equivalent to 50 kg N ha^{-1}) and P-K-S (19 kg P ha^{-1}, 159 kg K ha^{-1} and 16 kg S ha^{-1}) in solution (Monaghan et al., 2007; Morton et al., 2017).

4.3.3 Sampling and sample preparation

At each sampling time (T1–T5) the above and below-ground plant components were collected by cutting the herbage 4 cm above the soil surface. The remaining stems and stolons were cut at ground level and are subsequently referred to as the 'residual'.

The below-ground plant and soil components were divided into two sample depths (0–15 cm and 15–25 cm). Roots were manually removed from the soil column by gently teasing apart the soil to collect the intact root system at each depth. Soil adhering to the roots (0–15 cm depth only) was carefully shaken (approximately 6 times) free and collected; hereafter referred to as 'rhizosphere soil' (Hafner and Kuzyakov, 2016). The soil remaining after removal of both roots and rhizosphere soil for the 0–15 cm depth is defined as 'non-rhizosphere soil'. In the 15–25 cm depth, rhizosphere soil was not separated from the roots, and the soil in this depth is referred to as 'whole soil'.

Non-rhizosphere soil (0–15 cm) and whole soil (15–25 cm) were sieved (5.5 mm mesh) and soil subsamples were taken to determine the soil gravimetric water content (oven drying at 105°C for 24

h). A subsample of the 5.5 mm sieved soil was also removed and passed through a second sieve (2 mm) before fine grinding to homogenise the soil samples, prior to elemental and isotopic analyses. Rhizosphere soil (0–15 cm) was also sieved (2 mm). All sieved soil samples were air-dried at 25°C.

Root material collected from the sieves was combined with roots collected manually, with the pooled root samples homogenised prior to oven drying at 60°C (48 h). Dried root material was weighed and a subsample was taken and washed with deionised water on a 250-µm sieve and dried again at 60°C (48 h). Subsamples of the 5-mm sieved, fresh non-rhizosphere soil (0–15 cm) and whole soil (15–25 cm) were taken and washed on a 250-µm sieve to separate roots. These roots were dried at 60°C (48 h) and weighed in order to determine any fine root mass remaining in the soil that was not removed manually, and to calculate the total root mass (Equation 4.1). It is acknowledged that a small quantity of very fine root material may have been lost through the 250-µm sieve.

The mass (kg) of the total root mass (T_r) was calculated as follows:

$$Tr = r_m + \left[\left(\frac{r_w}{s_m}\right) \times T_s\right] \qquad [4.1]$$

Where r_m (kg) is the mass of roots manually collected during sampling, r_w (kg) is the mass of root washed from the 5.5 mm sieved soils, s_m (kg) is the mass of soil subsample (5.5 mm sieved) and the T_s (kg) is the total mass of non-rhizosphere soil (0–15 cm depth) and whole soil (15–25 cm depth), 5.5 mm sieved. As the fine earth (after stones removal) bulk density was similar between the treatments, the value of T_r (kg) was expressed in units of kg ha^{-1} using the surface area of the mesocosms (0.01767 m^2). Prior to elemental and isotopic analyses, the dried herbage, residual, roots, rhizosphere soil, non-rhizosphere soil and whole soil samples were ground (< 53 µm) using a bench top ring mill (BTRM, RockLabs, Auckland, NZ), taking care to avoid cross contamination by cleaning equipment between samples with deionised water and ethanol.

4.3.4 Soil particle size fractionation

Subsamples (30 g) from the 2 mm sieved air-dried non-rhizosphere soil (0–15 cm depth, n = 4 per treatment) and whole soil (15–25 cm depth, n = 4 per treatment), from sampling times T1, T3 and T5, were fractionated into five soil size fractions: > 250 µm (coarse POM), 53–250 µm (fine POM), 20–53 µm (silt), 5–20 µm (fine silt), and < 5 µm (clay) using a method adapted from Qiu et al. (2010). Briefly, samples were dispersed in deionised water using an ultrasonic probe (BOSCH, UW 2200) for 60 s with sonication set at 60 J s^{-1}. The dispersed soil suspension was passed through 250-µm and 53-µm sieves with the two POM fractions retained on the respective sieves. The < 53 µm material was separated into 20–53 µm, 5–20 µm and < 5 µm fractions by gravity, using their different rates of

sedimentation and siphoning off the excess of water. A flocculent (calcium chloride dehydrate, $CaCl_2.2H_2O$) was added to the < 5 µm suspension to aid the sedimentation of the clay particles. The mass of each fraction was determined by oven drying at 60°C followed by grinding in a mortar and pestle prior to elemental and isotopic analysis.

4.3.5 Isotopic composition during the ^{13}C chase period

Subsamples of the plant-soil components and the soil size fractions were analysed for their C concentrations, and the isotopic C composition using an elemental analyser (Sercon, model GSL, Crewe, UK) coupled to a continuous flow isotopic ratio mass spectrometer (Sercon, model 20-22, Crewe, UK). The ^{13}C isotopic composition ($\delta^{13}C$) of the samples was expressed in units of per mil (‰) (Equation 4.2).

$$\delta^{13}C\ (‰) = \left[\left(\frac{R_{sample}}{R_{standard}}\right) - 1\right] \qquad [4.2]$$

Where *R* = the $^{13}C/^{12}C$ ratio for either the sample or the standard (0.01118) and with the $\delta^{13}C$ values hereafter expressed relative to Pee Dee Belemnite (PDB).

Partitioning of ^{13}C during the chase phase

The partitioning of ^{13}C between plant and soil components including soil particle size fractions, was calculated as the mass of ^{13}C recovered within these components for each treatment (Equation 4.3–4.6). When applicable, values for the rhizosphere and non-rhizosphere soil (0–15 cm) were used to calculate either a weighted average of $\delta^{13}C$ values or the mass of ^{13}C recovered expressed in units of mg ^{13}C m^{-2} for combined soil in the 0–15 cm depth.

The mass of C per unit area (mg C m^{-2}) within each plant-soil component was calculated using Equation 4.3.

$$C = (TC/100) \times m \qquad [4.3]$$

Where *TC* is the total C content expressed as a percentage (%) and *m* is the mass of the plant-soil component per unit area (mg m^{-2}), calculated using the surface area of the mesocosms (0.01767 m^2).

The fractional abundance (*F*) of the sample was calculated using Equation 4.4 modified from Stewart and Metherell (1999):

$$F = \frac{(\delta^{13}C + 1000)}{[(\delta^{13}C + 1000 + (1000/R_{standard}))]} \qquad [4.4]$$

Where $R_{standard}$ = the $^{13}C/^{12}C$ ratio for the standard (0.01118) and the $\delta^{13}C$ values are expressed relative to Pee Dee Belemnite (PDB).

Atom% ^{13}C excess was calculated as the difference between the fractional abundance of the heavier isotope after labelling (F_a) and the fractional abundance at NA level (F_{NA}) using Equation 4.5.

$$atom\%\ ^{13}C_{excess} = (F_a - F_{NA}) \times 100 \qquad [4.5]$$

The mass of ^{13}C recovered, and expressed in units of mg m^{-2} ($^{13}C_{amount}$), from each plant-soil component was calculated using Equation 4.6.

$$^{13}C_{amount} = \frac{atom\%\ ^{13}C_{excess}}{100} \times C \qquad [4.6]$$

4.3.6 Statistical analysis

Factorial analysis of variance (ANOVA) was run to compare the dryland and irrigated treatments using GenStat (18th edition, Lawes Trust, Harpenden, UK). The plant-soil components and soil size particle fraction data were analysed using repeated measurements, with a model defined by treatment and sampling time as fixed factors. Residual plots and Shapiro-Wilk test were used to check the normality and the equal variance assumptions of ANOVA. When ANOVA assumptions were met without Log-transformed data, the Fisher's Protected Least Significant Difference test (LSD) with a significance level of 5% was used to determine the difference of means between the dryland and irrigated treatments. When log-transformed data were required to meet ANOVA assumptions, the Fisher's Protected Least Significant Ratio (LSR), with a significance level of 5% was used to assess differences of means between treatments as follows in Equation 4.7.

$$if\ \frac{a}{b} \geq LSR\ \therefore a \neq b \qquad [4.7]$$

Where *a* and *b* are the larger and smaller means, respectively and independent of the treatments, *LSR* is the Least Significant Ratio.

4.4 Results

4.4.1 Environmental conditions during the ^{13}C chase period

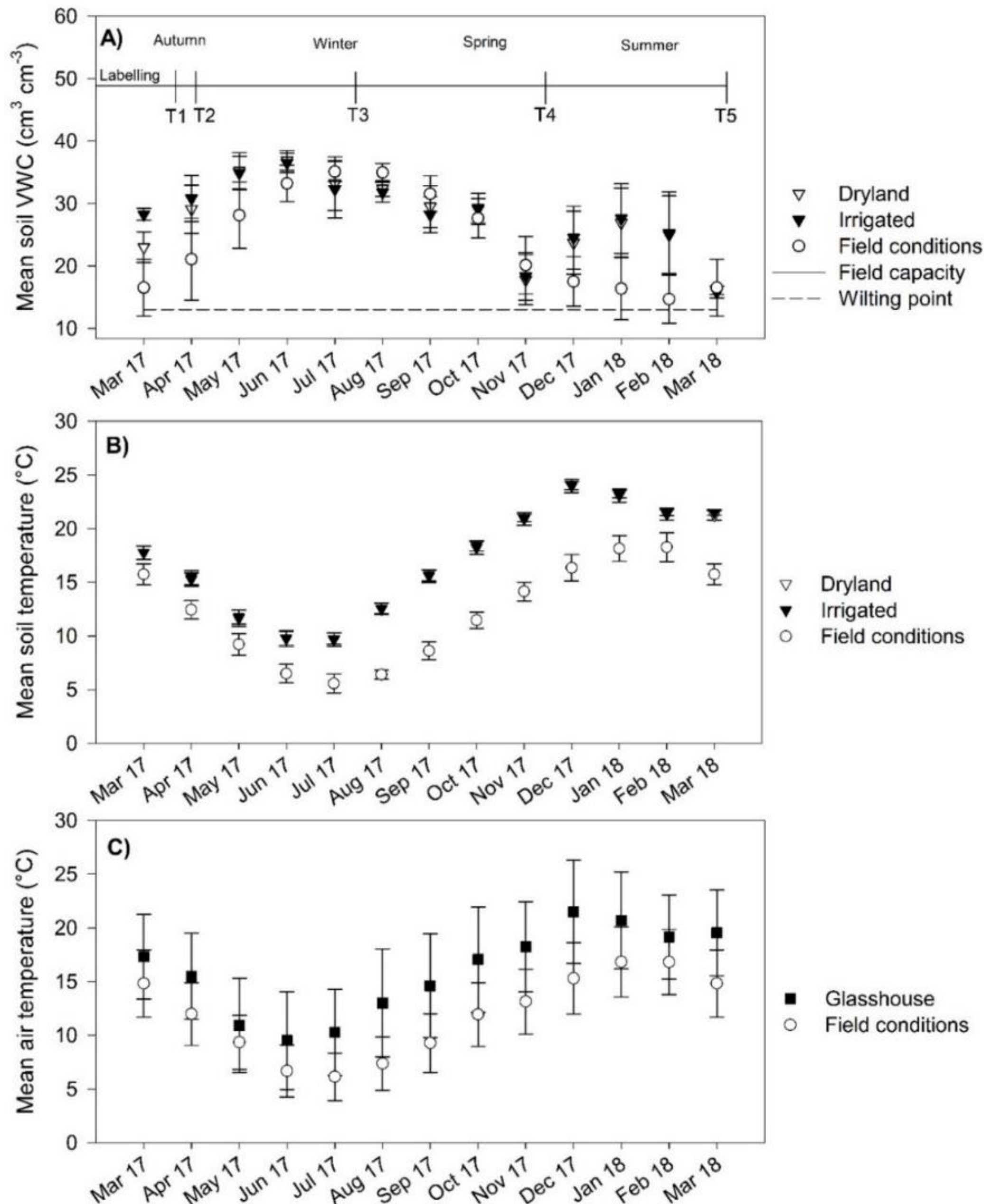

Figure 4.1. Monthly time series of mean environmental values over the ^{13}C chase period at sampling times T1, T2, T3, T4 and T5 (1, 12, 125, 237 and 349 days after the last $^{13}CO_2$ labelling event) for: a) Soil volumetric water content (VWC) for dryland and irrigated treatments, 18-year mean soil VWC under field conditions; b) Soil temperature for dryland and irrigated treatments at 0–15 cm depth, and 12-year mean soil temperature under field conditions at 0–30 cm depth; c) Air temperature in the glasshouse and 30-year mean air temperature under field conditions. Error bars represent the standard deviation of means (n = 8 for T1, per treatment and n = 4 for T2–T5, per treatment).

Soil volumetric water contents (VWC) for the dryland and irrigated treatments were similar to the long-term 18-year mean soil VWC under field conditions between sampling times T1–T4 (Mar 17–Nov 17), and at T5 (Figure 4.1A). However, the average soil VWCs for both treatments were higher than under field conditions between T4 and T5 (Dec 17–Feb 18). Although values were within the range of the 18-year mean soil VWC at this period (NIWA, 2018).

Soil temperatures in the mesocosms (0–15 cm depth) were similar between the dryland and irrigated treatments over the ^{13}C chase period (T1–T5, Figure 4.1B). However, average soil temperatures for both treatments between T3 and T4 (winter–spring period) were 6.3 ± 1.2°C (± stdev) higher than the 12-year mean soil temperature (0–30 cm depth) under field conditions.

Air temperatures in the glasshouse during the ^{13}C chase period were on average higher than the 30-year mean outdoor air temperature for Lincoln, especially between T3 and T4 (winter-spring period), when overnight air temperatures in the glasshouse were higher than outdoor air temperature (Figure 4.1C). During this period the minimum air temperature in the glasshouse was 7 ± 3.8°C (± stdev), while the 30-year mean of minimum outdoor air temperature was 5 ± 3.4°C (± stdev) (NIWA, 2018).

4.4.2 Pasture biomass allocation following summer irrigation

There were significant interaction effects of irrigation treatment (dryland vs irrigation) and time on herbage dry matter production, which varied over the ^{13}C chase period ($P < 0.001$, Figure 4.2). Daily herbage dry matter production was higher in the dryland than the irrigated treatment ($P = 0.001$ and LSD > 13.7) between T1 and T2, but there were no differences in daily production on subsequent harvest dates (Figure 4.2).

Significantly lower daily rates of herbage production (approximately 40–50 kg dry matter ha^{-1} day^{-1}) occurred during the winter months (June-August) ($P = 0.001$ and LSD > 13.7), prior to and just after the T3 (winter) sampling (Figure 4.2). The highest rates of daily herbage production occurred during the late spring and early summer months (November–December), on either side of the T4 (spring) sampling. Daily herbage production decreased in the later summer period between T4 and T5.

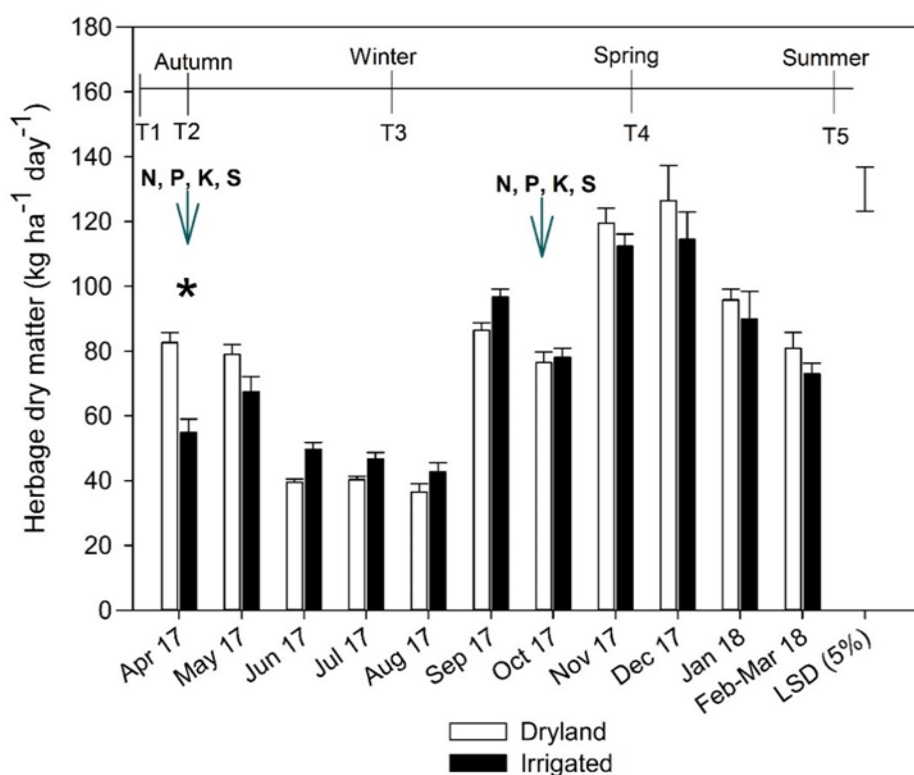

Figure 4.2. Mean daily herbage dry matter production rates (kg ha^{-1} day^{-1}) for dryland and irrigated treatments during the ^{13}C chase period. Destructive sampling times T1, T2, T3, T4 and T5 (1, 12, 125, 237 and 349 days after the last $^{13}CO_2$ labelling event) are indicated. Arrows indicate addition of nutrients: nitrogen (N), phosphorus (P), potassium (K) and sulphur (S). Error bars represent 1 standard error of the mean and LSD bar = 13.7 (the least significant difference of means between treatments) at 5% level of significance. Asterisk (*) denotes significant differences between treatments with P < 0.05.

The dry matter of residual plant material (stem and stolon) harvested between each sampling time (Supplementary Table 4.1) varied over the ^{13}C chase period (P < 0.001), but did not differ due to irrigation treatment (P = 0.341). Residual dry matter reached the highest value between T3 and T4 (spring), with 4022 ± 333 kg of dry matter ha^{-1} (± standard error of mean) for the dryland treatment and 4526 ± 447 kg of dry matter ha^{-1} for the irrigated treatment.

Root dry matter also varied over the ^{13}C chase period for both treatments in the 0–15 cm and 15–25 cm depths (P = 0.037 and P < 0.001, respectively; Figure 4.3A-B), with approximately 70% of the total root dry matter recovered from the 0–15 cm soil depth. In the 0–15 cm depth, root dry matter decreased under the irrigated treatment between sampling times T1 and T2 (P = 0.006, LSD1 = 1663 and LSD2–5 = 2351, Figure 4.3A), but at T3 no significant differences in root dry matter occurred between treatments (P = 0.214). At T4, root dry matter was again lower under the irrigated treatment (P = 0.022, Figure 4.3A). However, by T5, root dry matter had increased under the irrigated treatment, but there was no significant difference between treatments (P = 0.111).

In the 15–25 cm depth, root dry matter was higher under the dryland treatment at both T1 ($P = 0.035$ and LSD1 = 892) and T2 ($P = 0.046$ and LSD2–5 = 1262, Figure 4.3B) but did not differ between treatments at T3 ($P = 0.493$), T4 ($P = 0.182$) and T5 ($P = 0.559$). However, root dry matter at T5 was higher than at T1 for both treatments ($P < 0.001$, LSD2–5 = 1262, Figure 4.3B).

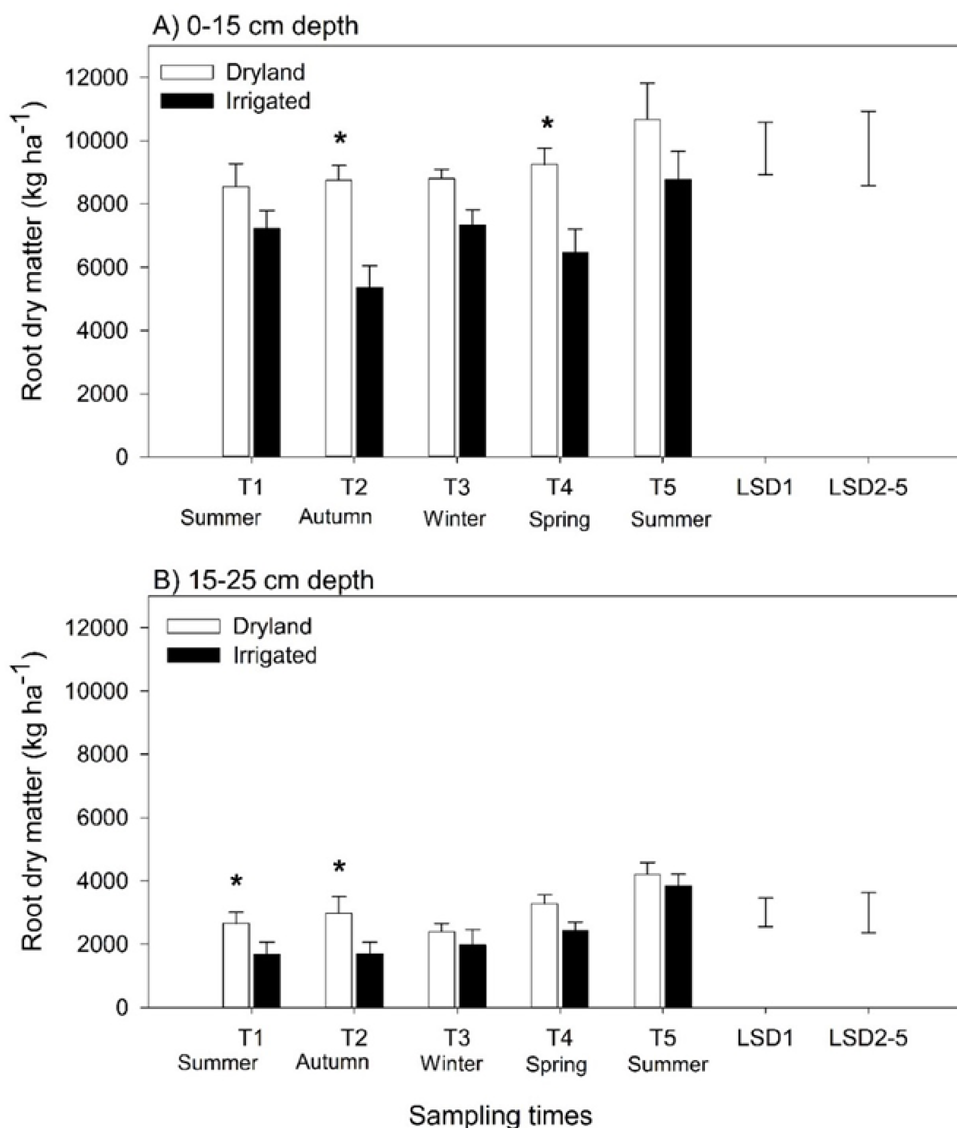

Figure 4.3. Mean values of root dry matter harvested at each sampling time (T1–T5) during the ^{13}C chase period for dryland and irrigated treatments in A) 0–15 cm depth and B) 15–25 cm depth. Errors bars represent 1 standard error of the means and LSD bars (the least significant differences of means between treatments) at 5% level of significance. LSD1 to compare means at T1 (n = 8 per treatment) and LSD2–5 to compare means between T2 and T5 (n = 4 per treatment). Asterisk (*) denotes significant differences with $P < 0.05$.

4.4.3 Quantity of ^{13}C recovered within the plant-soil system

The $\delta^{13}C$ values of the above- and below-ground plant-soil components, including soil particle size fractions, that were used to calculate ^{13}C recovery during the chase period (T1–T5) are shown in Supplementary data, Table 4.2–4.4.

The quantity of ^{13}C recovered in herbage at the end of the $^{13}CO_2$ labelling period (T1) was higher under the irrigated treatment compared with the dryland treatment ($P < 0.001$ and LSD1 = 55.73, Figure 4.4A). Recovery of ^{13}C recovered in herbage declined between T1 and T2, but with ^{13}C recovery higher under the dryland treatment ($P = 0.040$ and LSD2–5 = 78.81). Herbage remained ^{13}C enriched between T2 and T4 (237 days after last labelling event) with no effects due to treatment being observed ($P = 0.991$). At T5 (349 days after last labelling event), there was no significant ^{13}C enrichment in the herbage, as the $\delta^{13}C$ values were at natural abundance levels for the dryland (-28.9‰) and irrigated (-29.4‰) treatments (Supplementary Table 4.2).

The quantity of ^{13}C recovered in the residual declined over the ^{13}C chase period by approximately 80% from T1 to T5 ($P < 0.001$, LSD1 = 96.1, LSD2–5 = 134, Figure 4.4B), with no differences due to irrigation treatments ($P > 0.05$).

In the 0–15 cm depth, root ^{13}C recovery decreased over the ^{13}C chase period ($P < 0.001$, Figure 4.4C). At T1 there was no treatment effect ($P = 0.382$) after which a rapid decrease in the amount of ^{13}C recovered was observed at T2, with higher ^{13}C recovery in the dryland treatment ($P = 0.012$ and LSR =1.55). By T3, the ^{13}C recovered was similar between treatments ($P = 0.394$), due to an increase in the ^{13}C recovered in roots of the irrigated treatment between T2 and T3 ($P < 0.001$). At T4 the amount of ^{13}C recovered was not affected by treatment ($P = 0.073$) but there was a decrease in the recovery between T3 and T4 ($P < 0.001$). By T5, the ^{13}C recovered in roots from the 0–15 cm depth was approximately half of the initial amount recovered at T1, with no differences between treatments ($P = 0.563$). However, there was a significant decrease in ^{13}C recovered in the dryland treatment between T4 and T5 ($P < 0.001$).

In the 15–25 cm depth, the quantity of root ^{13}C recovered varied over the ^{13}C chase period ($P < 0.001$, Figure 4.4D). The ^{13}C recovered at T1 was higher under the dryland treatment ($P = 0.045$ and LSR1 = 1.89), but by T2 and T3 the ^{13}C recovered in these deeper roots was similar between treatments ($P = 0.328$ and $P = 0.329$, respectively). However, by T4 the amount of ^{13}C recovered had decreased in both treatments ($P < 0.001$), with lower ^{13}C recovery in roots of the irrigated treatment ($P = 0.003$, LSR2–5 = 2.457). As in the 0–15 cm soil depth, the quantity of ^{13}C recovered by T5 was approximately half of the initial amount recovered at T1, with no differences between treatments (P

= 0.482), but this represented a significant decrease in ^{13}C recovery in the dryland treatment relative to T4 (P <0.001).

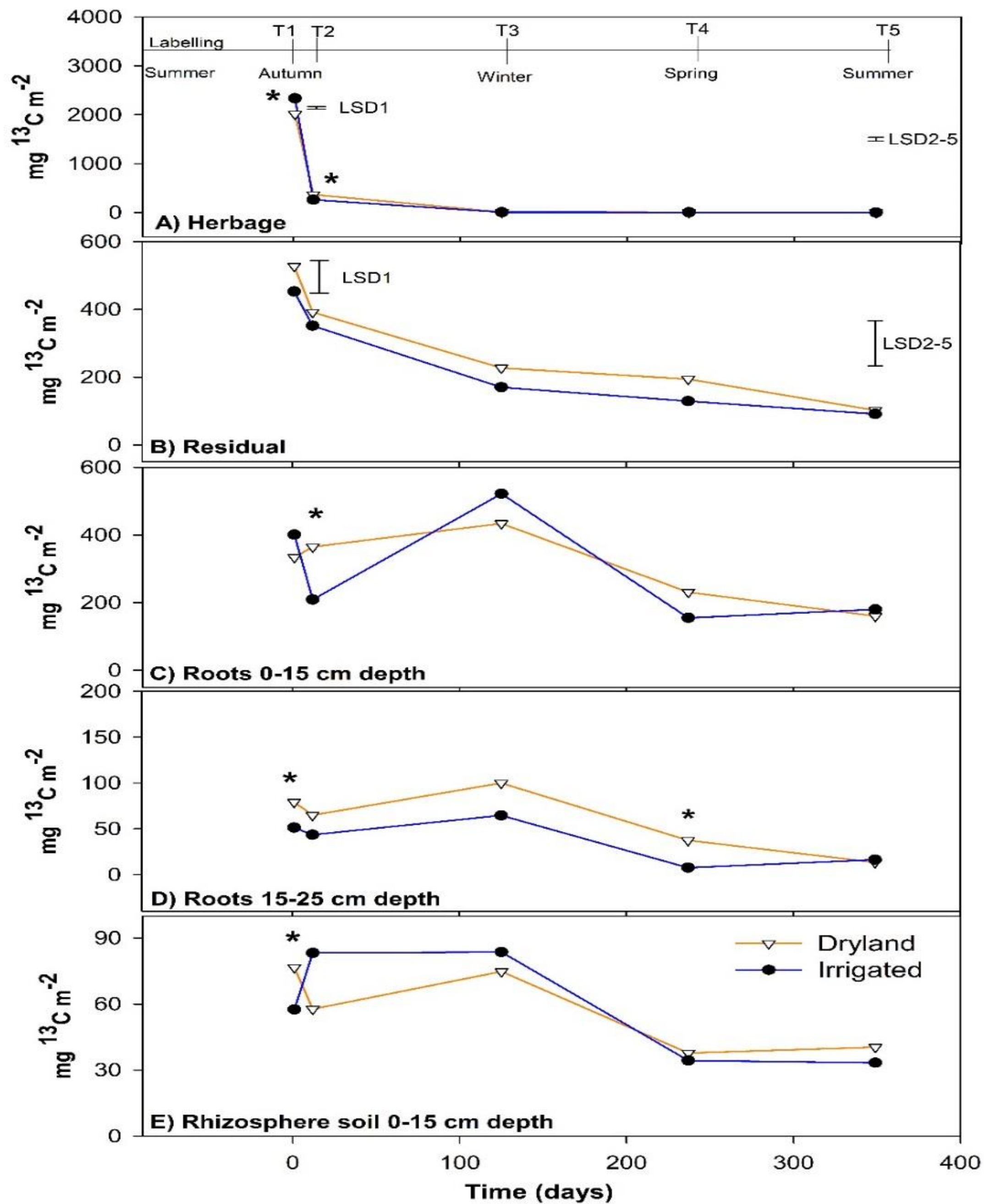

Figure 4.4. Quantity of ^{13}C (mg ^{13}C m^{-2}) recovered at sampling times: T1, T2, T3, T4 and T5 (1, 12, 125, 237 and 349 days after the last $^{13}CO_2$ labelling event) for dryland and irrigated treatments in: A) herbage, B) residual, C) roots in the 0-15 cm depth, D) roots in the 15–25 cm depth, and E) rhizosphere soil in the 0–15 cm depth. For herbage and residual, LSD bars, the least significant differences between pastures means at 5% level of significance. LSD1 to compare means at T1 (n = 8 per treatment) and LSD2–5 to compare means for T2–T5 (n = 4 per treatment). For roots and rhizosphere soil, LSR, the least significant ratio between treatment means at 5% level of significance. Asterisk (*) denotes significant differences with P < 0.05. Note differences in scale-y axes (mg ^{13}C m^{-2}) between the different plant and soil components.

The quantity of ^{13}C recovered in the rhizosphere soil (0–15 cm depth) varied over the ^{13}C chase period ($P < 0.001$, Figure 4.4E). There was a significant interaction between sampling time and treatments ($P = 0.024$ and LSR > 1.31), with lower ^{13}C recovery in the irrigated treatment compared to the dryland treatment at T1. By T2, ^{13}C recovery had increased for rhizosphere soil in the irrigated treatment, while the amount of ^{13}C recovered in the dryland decreased ($P < 0.001$ and LSR > 1.31, Figure 4.4E), though the differences between treatments was not statistically significant ($P = 0.056$ and LSR2–5 < 1.47).

Recovery of ^{13}C in rhizosphere soil (0–15 cm depth) at T3 was similar to T2, with no differences between treatments ($P = 0.538$) (Figure 4.4E). At T4 the quantity of ^{13}C recovered in rhizosphere soil decreased ($P < 0.001$), with no differences between treatments ($P = 0.934$). By T5, ^{13}C recovered in the rhizosphere soil (0–15 cm depth) was approximately half that initially recovered at T1, with no differences due to treatments ($P = 0.278$).

The quantity of ^{13}C recovered in non-rhizosphere soil (0–15 cm depth) did not vary over the ^{13}C chase period ($P = 0.426$, Figure 4.5A), with no differences due to treatment ($P > 0.05$). Similarly, the quantity of ^{13}C recovered in the combined soil (0–15 cm) and whole soil (15–25 cm), did not vary over the ^{13}C chase period ($P = 0.059$, Figure 4.5B–C), with no differences observed between treatments ($P > 0.05$).

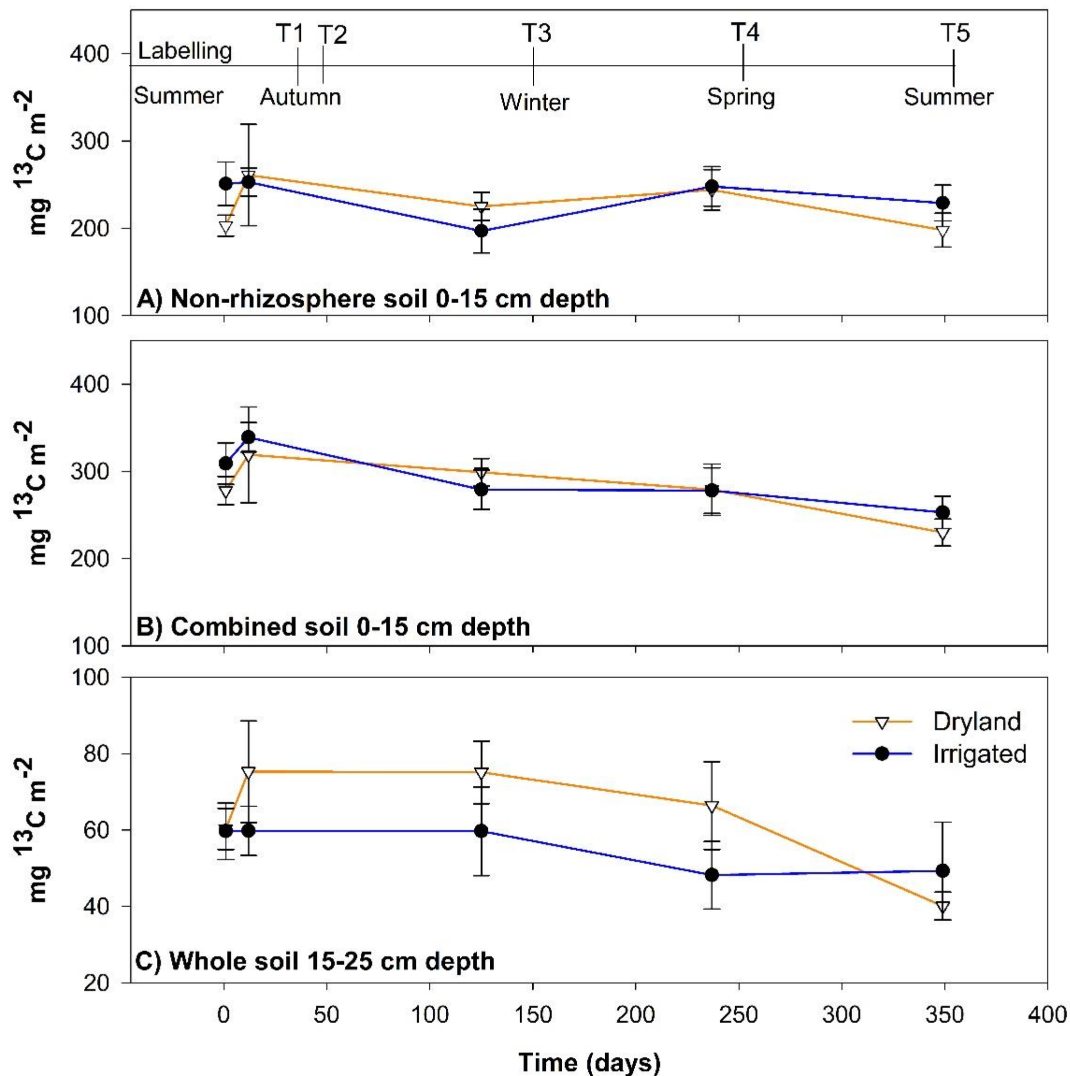

Figure 4.5. Quantity of ^{13}C (mg ^{13}C m^{-2}) recovered at sampling times: T1, T2, T3, T4 and T5 (1, 12, 125, 237 and 349 days after the last $^{13}CO_2$ labelling event) for dryland and irrigated treatments in: A) non-rhizosphere soil in the 0–15 cm depth, B) combined soil in the 0–15 cm depth, and C) whole soil in the 15–25 cm depth. LSD (the least significance difference between treatments means) are not provided as P > 0.05. Instead error bars are given and represent ± 1 standard error of the mean. At T1 (*n* = 8 per treatment) and T2–5 (*n* = 4 per treatment). Note differences in y-axes scale (mg ^{13}C m^{-2}) between the different soil components.

4.4.4 Quantity of ^{13}C recovered within soil size fractions

In the non-rhizosphere soil (0–15 cm depth), the quantity of ^{13}C recovered within the soil particle size fractions varied over the ^{13}C chase period for both treatments (P = 0.035, and LSRtime = 1.17, Figure 4.6). At T1, there was a significant interaction between ^{13}C recovery in soil fractions of non-rhizosphere soil (0–15 cm depth) and treatment (P = 0.003 and LSR = 1.39, Figure 4.6), with higher ^{13}C recovery in the 53–250 µm and < 5 µm fractions of the irrigated compared with the dryland treatment (LSR > 1.39). The ^{13}C recovery in > 250 µm, 20–53 µm and 5–20 µm fractions was similar between treatments (LSR < 1.39).

At T3, there was also a significant interaction between ^{13}C recovery in soil fractions of non-rhizosphere soil (0–15 cm) and treatment (P <0.001 and LSR = 1.39, Figure 4.6), with lower ^{13}C recovery in the > 250 µm fraction (LSR > 1.39, Figure 4.6A), due to a significant increase in the ^{13}C recovered in this fraction under the dryland treatment, relative to T1 (LSRtime > 1.17). The ^{13}C recovered in the 53–250 µm fraction was again higher under the irrigated treatment (LSR > 1.39, Figure 4.6B). By T3, ^{13}C recovery in the 5–20 µm fraction was reduced in both treatments relative to T1 (LSRtime > 1.17), but remained higher in the irrigated than the dryland treatment (LSR > 1.39, Figure 4.6D). At this sampling time, an increase in ^{13}C recovery in the < 5 µm fraction relative to T1 was also observed under the dryland treatment (LSRtime > 1.17). There were no significant effects of treatment or sample time on the ^{13}C recovery in the 20–53 µm fraction (LSR < 1.39 and LSRtime < 1.17, respectively, Figure 4.6C).

At T5, there was no significant interaction between ^{13}C recovery in soil fractions of non-rhizosphere soil (0–15 cm) and treatment (P = 0.178, Figure 4.6). However, ^{13}C recovery in the > 250 µm fraction significantly decreased under the dryland treatment relative to T3 (LSRtime > 1.17, Figure 4.6A), while under the irrigated treatment ^{13}C recovery in this fraction did not vary relative to T1 and T3 (LSRtime < 1.17). Recovery of ^{13}C in the 53–250 µm fraction did not vary in the irrigated treatment between T1 and T2, while the ^{13}C recovery increased in the dryland treatment (LSRtime > 1.17, Figure 4.6B) resulting in no treatment differences at T5 (P = 0.178). The quantity of ^{13}C recovered in the 20–53 µm fraction by T5, revealed an increase under the irrigated treatment (LSRtim$_e$ > 1.17), however, there was no difference due to treatment at this time (P = 0.178, Figure 4.6C). The ^{13}C recovered in the 5–20 µm fraction increased by T5 (LSRtime > 1.17), but there was no difference due to treatment (P = 0.178, Figure 4.6D). The quantity of ^{13}C recovered in the < 5 µm fraction at T5 was similar to the quantity recovered at T3, with no difference due to treatment (P = 0.178, Figure 4.6E).

In the whole soil (15–25 cm depth), the quantity of ^{13}C recovered within the soil particle size fractions also varied over the ^{13}C chase period (P = 0.048 and LSRtime = 1.39, Figure 4.7). At T1, there was a significant interaction between ^{13}C recovery in fractions of the whole soil (15–25 cm depth) and treatment (P = 0.014 and LSR = 1.81, Figure 4.7), with higher ^{13}C recovery in the 53–250 µm fraction under the irrigated treatment compared with the dryland treatment (LSR > 1.81, Figure 4.7B). Among the other soil size fractions for the whole soil (15–25 cm) the ^{13}C recovery was similar between treatments (LSR < 1.81, Figure 4.7).

At T3, there was a significant interaction between ^{13}C recovery in fractions of the whole soil (15–25 cm) and treatment (P = 0.013 and LSR = 1.81, Figure 4.7), with higher ^{13}C recovery in the < 5 µm fraction under the dryland treatment (LSR > 1.81, Figure 4.7E). There was a significant increase in the ^{13}C recovered in this fraction relative to T1 in both treatments (LSRtime > 1.39, Figure 4.7E). Despite

no significant differences between treatments in the quantity of ^{13}C recovered in the > 250 µm fraction (LSR < 1.81) at T3, the ^{13}C recovery in this fraction increased under the dryland treatment, relative to T1 (LSRtime > 1.39, Figure 4.7A). The ^{13}C recovery among the other fractions of the whole soil (15–25 cm) did not differ significantly at T3 compared with the quantity of ^{13}C recovered at T1 (LSRtime < 1.39), with no differences due to treatment (LSR < 1.81).

At T5, in contrast to non-rhizosphere soil (0–15 cm depth), there was also a significant interaction between ^{13}C recovery in soil fractions of the whole soil (15–25 cm) and treatment (P < 0.001 and LSR = 1.81, Figure 4.7), with higher ^{13}C recovery in the 53–250 µm and 20–53 µm fractions under the irrigated treatment (LSR > 1.81, Figure 4.7A–B). The quantity of ^{13}C recovered in the 5–20 µm fraction in both the irrigated and the dryland treatment at T5 had increased relative to T3 (LSRtime > 1.39), with no difference due to treatment (LSR < 1.81, Figure 4.7D). In contrast, ^{13}C recovery in the > 250 µm fraction decreased between T3 and T5 (LSRtime > 1.81), with no effects due to treatment (LSR < 1.81, Figure 4.7A). At T5, a decrease in ^{13}C recovery in the < 5 µm under the dryland treatment was observed, relative to the quantity recovered at T3 (LSRtime > 1.81), while ^{13}C recovery in this fraction under the irrigated treatment did not differ relative to T3 (LSRtime < 1.81), however, no difference due to treatment was observed (LSR < 1.81, Figure 4.7E).

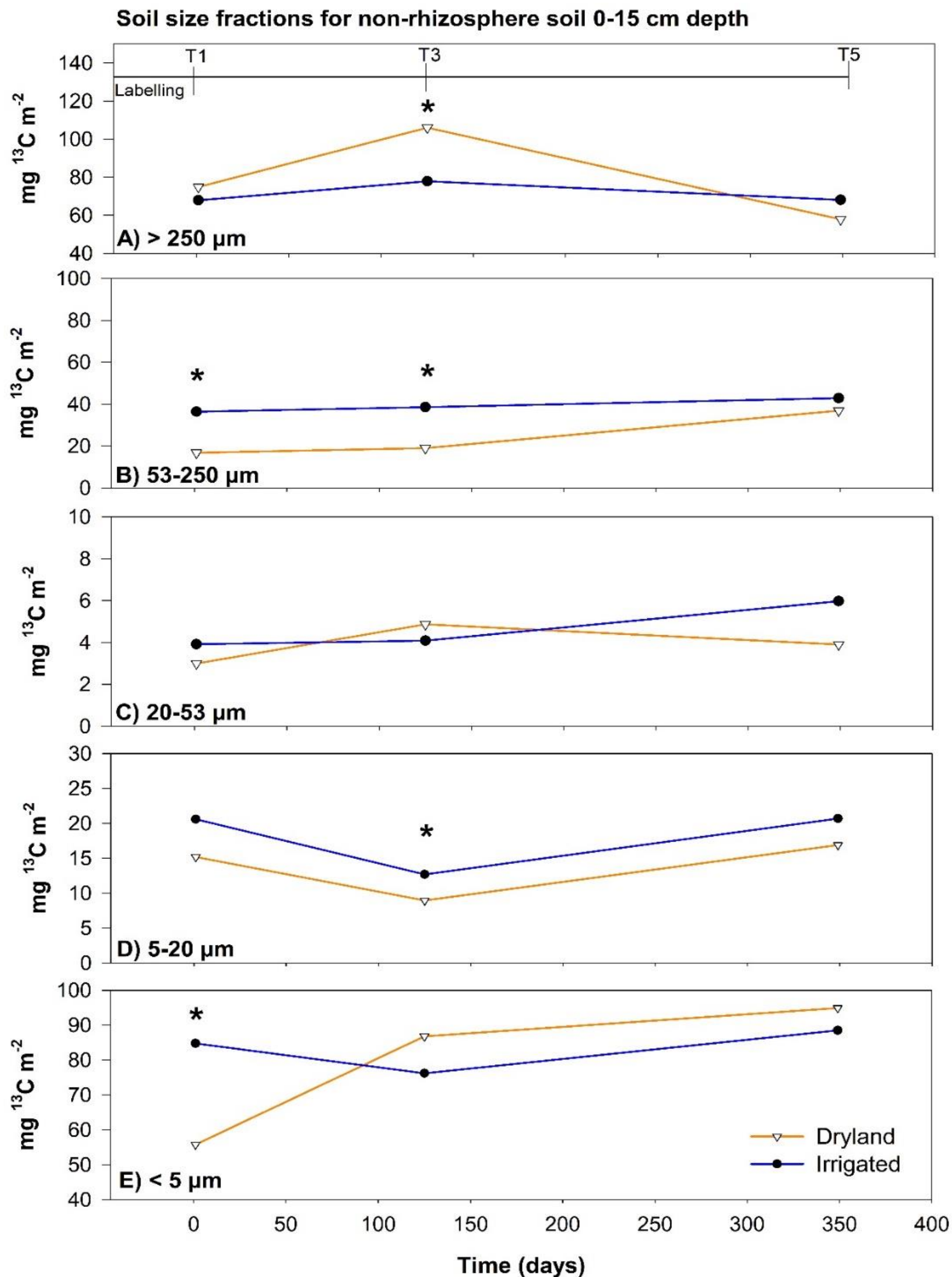

Figure 4.6. Quantity of ^{13}C recovered (mg ^{13}C m^{-2}) within soil size particle fractions of non-rhizosphere soil (0–15 cm depth) for the dryland and irrigated treatments: A) > 250 µm, B) 53–250 µm, C) 20–53 µm, D) 5–20 µm, and E) < 5 µm at sampling times: T1, T3 and T5 (1, 125 and 349 days after the last $^{13}CO_2$ labelling event). LSR (the least significant ratio between treatment means = 1.38) and LSR_{time} (the least significant ratio between means at different sampling times, at the same level of treatment = 1.17), at 5% level of significance. Asterisk (*) denotes significant differences between treatment means. Note differences in y-axes scale (mg ^{13}C m^{-2}) between the soil size fractions.

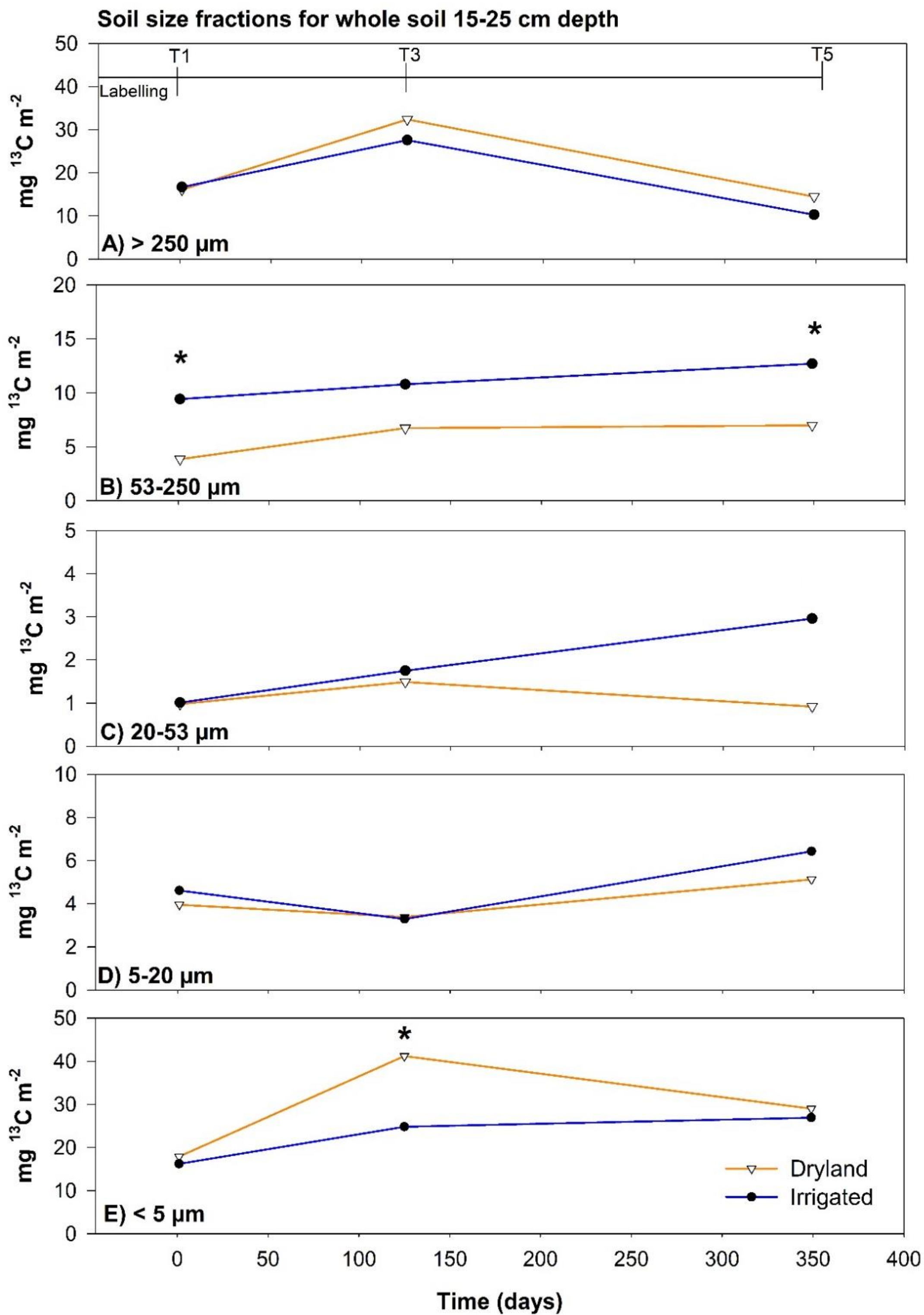

Figure 4.7. Quantity of ^{13}C recovered (mg ^{13}C m^{-2}) within soil size particle fractions of the whole soil (15–25 cm depth) for the dryland and irrigated treatments: A) > 250 µm, B) 53–250 µm, C) 20–53 µm, D) 5–20 µm, and E) < 5 µm at sampling times: T1, T3 and T5 (1, 125 and 349 days after the last $^{13}CO_2$ labelling event). LSR (the least significant ratio between treatment means = 1.81) and LSR_{time} (the least significant ratio between means at different sampling times, at the same level of treatment = 1.39), at 5% level of significance. Asterisk (*) denotes significant differences between pastures means. Note differences in y-axes scale (mg ^{13}C m^{-2}) between the soil size fractions.

4.5 Discussion

4.5.1 Legacy effects of summer irrigation on pasture biomass allocation

The pasture growth of both the dryland and irrigated treatments following the labelling phase, was similar to seasonal patterns observed under field conditions; i.e. higher biomass production in spring and the lower production in winter (Saggar and Hedley, 2001; Dodd and Mackay, 2011) (Figure 4.2 and Figure 4.3). However, root biomass was less sensitive to seasonal variation than the above-ground plant components (Figure 4.3A–B). Seasonal patterns of pasture root growth are very site specific and can change depending on pasture botanical composition or year-to-year climate variability (Wedderburn et al., 2010; Dodd and Mackay, 2011). The variability in soil temperatures in this study (i.e. warmer and a smaller amplitude) compared to that of field conditions (Figure 4.1B) may explain the lack of seasonal change in root growth, although improved canopy structure for light competition between ryegrass and white clover plants could have also affected root growth by allocating more biomass to above-ground plant components (Wardlaw, 1990; Minchin et al., 1994).

While summer irrigation increased pasture dry matter production there appeared to be a change in biomass allocation during the subsequent autumn and spring seasons (Figure 4.2 and Figure 4.3). Higher pasture production occurred in autumn under the dryland treatment (at T2) when compared to the irrigated treatment, and this may have been due to a flush of nutrient availability, especially nitrogen (N), when the dryland treatment was rewetted. Irrigation during summer has previously been shown to increase above-ground pasture productivity (Scott et al., 2012; Moinet et al., 2017; Carmona et al., 2020) and the uptake of nutrients such as N (Moinet et al., 2017) and phosphorus (P), which cycle within organic matter (Rumpel et al., 2015).

Following the application of fertiliser in autumn, the herbage productivity in the irrigated treatment increased, supporting the assumption that pasture growth was nutrient limited in this treatment at T2 (Figure 4.2). The following spring after the irrigation period (at T4), the lower root biomass in the irrigated treatment compared to the dryland treatment suggests that there may have been a legacy effect of irrigation that reduced root production. This is consistent with the findings of Wedderburn et al. (2010) who reported that in ryegrass pasture root counts in spring were related to the root counts in autumn, as a well-developed root system during autumn will determine a plant's ability to search for nutrients and water when environmental conditions are favourable for growth after winter.

4.5.2 The short-term persistence of photosynthate C partitioned to the plant-soil system under summer irrigation

Above-ground plant components

The sharp decline in ^{13}C recovery from herbage by T2 (Figure 4.4A) in the dryland and irrigated treatments most likely occurred as a consequence of ^{13}C dilution due to active pasture growth (under natural abundance conditions), and from the transfer of photosynthate C to other plant organs (Meuriot et al., 2018), and to the soil (Farrar et al., 2003; Jones et al., 2004). However, the dilution of ^{13}C from herbage was greater in the irrigated compared with the dryland treatment by T2. Losses of photosynthate C from herbage can also occur via biomass removal during grazing but during the period between T1–T2 herbage was not harvested. Therefore, we suggest that the losses of ^{13}C in both treatments were primarily through leaf respiration and transfer to other plant-soil components.

Despite the rapid loss of ^{13}C from the herbage, there did not seem to be a significant accumulation of ^{13}C in the residual. Therefore, the residual was not a store for this photosynthate C between T1 and T2 (Figure 4.4B). The residual plant material in this study included ryegrass leaf meristems, stems and clover stolons, which are all considered to be sink organs for carbohydrate reserves used to sustain plant growth and respiration when photosynthesis is limited following defoliation (White, 1973; Meuriot et al., 2018) or deficient light, water and nutrients supply (Poorter and Nagel, 2000). However, the concurrent loss of ^{13}C from the residual following the labelling period, did not correspond to an increase in ^{13}C recovered in the herbage at each defoliation event. Therefore, this reallocation of ^{13}C may have been less important for herbage growth during the autumn and winter period (T2–T3) when productivity was lower.

By winter (at T3, Figure 4.4B), losses of ^{13}C from residual in both treatments accounted for more than 50% of ^{13}C stored in the residual at the end of the irrigation period. During winter in temperate pastures, losses of C due to respiration from the plant-soil system increase, while less photosynthate C is partitioned to the below-ground plant-soil components (Saggar and Hedley, 2001). Plant and soil respiration was not measured in this study, but it is likely that the decline in ^{13}C recovery from the residual was due to plant respiration processes involved in plant biomass maintenance rather than growth, as during winter pasture growth is limited (Saggar and Hedley, 2001; Dodd and Mackay, 2011).

The decline in ^{13}C from the residual during spring (at T4, Figure 4.4B) continued due to plant growth and respiration losses with no significant differences between treatments. However, ^{13}C enrichment expressed in $\delta^{13}C$ values was significant lower in the irrigated treatment compared with the dryland treatment (Supplementary Table 4.2) indicating that in relative terms, the persistence of ^{13}C in

residual was lower in the irrigated treatment. This lower persistence of ^{13}C in the residual may be related to a higher investment of photosynthate C by plants in rebuilding a root system that was restricted due to irrigation during summer. However, at the end of this study (at T5) there was no difference either in ^{13}C enrichment or mass of ^{13}C recovered between treatments, with approximately 80% of ^{13}C initially partitioned to the residual being lost from the plant-soil system.

Below-ground plant-soil components

There was a sharp decline in ^{13}C recovery of roots (0–15 cm depth) in the irrigated treatment between T1 and T2, indicating that root turnover and transfer of root C to soil occurred faster than in the dryland treatment (Figure 4.4C). Two weeks after the cessation of labelling (T2), the root biomass (0–15 cm) in the irrigated treatment also declined, while the ^{13}C recovery in the rhizosphere soil (0–15 cm, Figure 4.4E) increased. This result provides evidence that the photosynthate C lost from the roots was transferred to the 0–15 cm rhizosphere soil. This increase in ^{13}C recovered in the rhizosphere soil accounted for approximately 13% of ^{13}C lost from the roots between T1 and T2, which is in close agreement to the 5–10% range of photosynthate C partitioned to soil reported by Farrar et al. (2003). Photosynthate C transfer from plant to soil can occur via root exudation, through symbiosis with mycorrhizal fungi and through root turnover, all of which are pathways that stimulate microbial SOM decomposition and improve nutrient availability for plant growth (Jones et al., 2009; Kaiser et al., 2015; Liese et al., 2017; Vidal et al., 2018; Gorka et al., 2019). Therefore, the nutrient limitation in the irrigated treatment at the end of the irrigation season may have triggered this increase in ^{13}C transfer from roots to rhizosphere soil to compensate for lower nutrient availability.

In contrast, the ^{13}C recovered in the roots (0–15 cm depth) of the dryland treatment did not change between T1 and T2, but there was a significant reduction (approximately 25%) in the ^{13}C recovered in the rhizosphere soil (0–15 cm depth) compared to the initial mass of ^{13}C recovered at T1. However, the ^{13}C recovered in non-rhizosphere soil increased by approximately 29% under the dryland treatment relative to T1, though this was not statistically significant (Figure 4.5A). This result indicates that once the water deficit was alleviated in the dryland treatment in autumn, rhizosphere processes increased allowing for a higher transfer of photosynthate C to the wider non-rhizosphere soil. These results are supported by Chomel et al. (2019), who reported that under drought conditions the transfer of recent photosynthate C to soil food webs is reduced. The fact that ^{13}C recovery in rhizosphere soil (0–15 cm depth) was higher in the dryland treatment at the end of the labelling indicates that the soil water deficit favoured the accumulation of photosynthate C in rhizosphere soil, while in the irrigated treatment this C was readily mineralised or transferred to non-rhizosphere soil. Therefore, there is evidence that summer irrigation affects the temporal partitioning of photosynthate C from plant to rhizosphere soil and to the wider non-rhizosphere soil compared with dryland management. Furthermore, these results also highlight the role of plant and

rhizosphere processes regulating SOC and nutrients cycling in response to different environmental conditions affecting plant growth.

The ^{13}C recovered in winter (T3), also supports the idea that plants control the partitioning of photosynthate C not only to different plants organs but also to the soil, to optimise growth under given environmental conditions (Poorter and Nagel, 2000). During winter photosynthesis efficiency is reduced, hence, pasture growth is also reduced (Saggar and Hedley, 2001), decreasing water and nutrients uptake (Poorter and Nagel, 2000). In that sense, the transfer of photosynthate C from above-ground plant components to roots and soil is expected to be lower in winter. However, the ^{13}C recovered from roots in the 0–15 cm soil increased significantly in the irrigated treatment relative to the mass of ^{13}C recovered by T2 (Figure 4.4C). This result indicates that a transfer of photosynthate C from above-ground components (especially the residual) occurred during winter, supporting the idea that stems and clover stolons are sink organs for C that is used when the production of fresh photosynthate is reduced (White, 1973; Poorter and Nagel, 2000; Meuriot et al., 2018).

The increase in ^{13}C recovered from roots (0–15 cm depth) in the irrigated treatment between autumn (T2) and winter (T3), corresponds with the reduction in ^{13}C recovered from the residual over the same period (Figure 4.4B–C). Hence, we suggest that during the autumn-winter period plants invest more photosynthate C in growing or maintaining root biomass, as a 'fitter' root system will give more advantages for acquiring resources during the spring growing season (Wedderburn et al., 2010). This investment of C in building root biomass will require nutrients, hence, effective nutrient management of the whole plant-soil system, including both above- and below-ground pasture biomass, is required to maintain SOC in grazed pastures (Richardson et al., 2019), as root derived-C contributes more to SOC storage than above-ground litter-derived C (Rasse et al., 2005; Kong and Six, 2010).

For our first hypothesis, we aimed to test whether lower root biomass in the summer irrigated treatment, would reduce the recovery of previously fixed photosynthate C in the soil during the autumn and winter periods compared to the dryland treatment. Our results do not support this hypothesis as they showed that in a perennial pasture system the production of photosynthate C is continuous, even under winter conditions. This production allows the transfer of C from plants to the soil resulting in a balance of inputs and outputs of photosynthate C partitioned in soil, hence, maintaining SOC content under the conditions of this study. Further research to assess the combined effects on SOC storage due to irrigation, fertilisation and grazing intensity will be required, as grazing intensity is regarded as one of the major factors affecting SOC storage in grazed pastures (Machmuller et al., 2015; Mohammad et al., 2019).

The decline in ^{13}C recovery from root biomass in the dryland and irrigated treatments from both soil depths (0–15 cm and 15–25 cm) by spring (T4, Figure 4.4C–D) can be attributed to a higher rate of pasture growth, hence higher dilution, and from the transfer of C from plant to soil to improve nutrient cycling (Jones et al., 2009; Kaiser et al., 2015; Liese et al., 2017; Vidal et al., 2018; Gorka et al., 2019). The fact that ^{13}C recovery in roots from the 15–25 cm depth was lower in the irrigated treatment indicates that loss and/or transfer of this photosynthate C from these deeper roots was higher than in the dryland treatment in a possible attempt by the plant to restore the deviation between above- and below-ground biomass allocation (Poorter and Nagel, 2000) caused by summer irrigation.

Our research provided only partial support for the second hypothesis that higher rates of above-ground dry matter production in the spring period would result in faster turnover of root C and higher accumulation of previously fixed (photosynthate) C in soil compared to the autumn and winter seasons. Although above-ground dry matter production was higher in the spring period and there was evidence of increased root turnover, there was no evidence that this lead to higher accumulation of the previously fixed C in the soil. Seasonal changes in the fate of photosynthate C in the 0–15 cm rhizosphere soil in this study had a negligible or statistically insignificant effect on the photosynthate C partitioned to non-rhizosphere soil or whole soil, where ^{13}C recovery was similar to the mass recovered at the end of the irrigation period (T1). These results not only indicate that inputs and outputs of photosynthate C from soil were balanced by the gain and losses of C in the rhizosphere soil, but also demonstrate that seasonal changes that affect rhizosphere soil were 'diluted' by the large volume of non-rhizosphere soil, supporting the idea that soil processes measured at larger scales are occurring in soil hotspots comprising small soil volumes (Kuzyakov and Razavi, 2019).

The decline in ^{13}C recovery from roots in the dryland treatment for both soil depths by T5 during summer (Figure 4.4C–D) occurred as a result of the conversion to an irrigated treatment, supporting our results obtained at the end of the previous summer irrigation (T1), when photosynthate C storage in the deeper roots was reduced likely due a faster turnover, as summer irrigation has been reported to increase root turnover in temperate pastures (Scott et al., 2012). The steady state of ^{13}C recovery in roots in the former irrigated treatment, and soil components for both treatments by T5 indicate that during summer the transfer of C from roots to soil was lower compared with spring, and that inputs and outputs of photosynthate C from soil were balanced and/or stabilised in the soil.

4.5.3 The short-term persistence of photosynthate C in soil particle size fractions

The significant increase in ^{13}C recovery for the > 250 µm (coarse POM) fraction from non-rhizosphere soil (0–15 cm depth, Figure 4.6A) under the dryland treatment between the end of the irrigation

phase (T1) and winter (T3), indicates that during the autumn and winter period, there was an accumulation of ^{13}C in the coarse POM fraction (Figure 4.6A). However, there was a subsequent decline in this ^{13}C recovered in the coarse POM fraction between winter (T3) and the next summer (T5), demonstrating that short-term losses of SOC associated with this fraction occurred. We did not determine the ^{13}C enrichment of soil particle size fractions in spring (T4), hence, it is not known if the decline in photosynthate C partitioned to coarse POM in the dryland treatment occurred during spring or summer.

In most terrestrial ecosystems, plant litter entering the decomposer food web acts as a primary source of energy for decomposer organisms, that in turn improve nutrient cycling for plant growth (Bardgett et al., 1998). Therefore, it is likely that the decomposition of the coarse POM fraction, regarded as less protected from soil decomposers (Gregorich and Beare, 2008), may have started during spring. However, it is not clear from this study whether the decomposition rates of POM were different between spring and summer. Furthermore, the ^{13}C recovered in the coarse POM fraction of non-rhizosphere soil (0–15 cm depth) in the irrigated treatment (Figure 4.6A) was lower than in the dryland treatment in winter (T3), indicating that there was a significant reduction in the storage of photosynthate C in this fraction during the autumn-winter period following irrigation.

The differences in ^{13}C recovered in the 53–250 µm (fine POM) fraction (non-rhizosphere soil, Figure 4.6B) also gives evidence that irrigation may have promoted coarse POM decomposition, as the decline in ^{13}C recovered in this fraction under the dryland treatment at T5 was paralleled by an increase in the ^{13}C recovered in the fine POM fraction. This transfer may have occurred faster in the irrigation treatment, which would explain the higher partitioning to the fine POM fraction at T1. A previous study involving a Lismore stony silt loam soil reported that irrigation of ryegrass-white clover pasture increased root turnover (Scott et al., 2012) and the abundance of herbivore soil mesofauna (Fraser et al., 2012), which provides a plausible mechanism for the higher partitioning of ^{13}C to fine POM in this study. Based on these findings there is evidence that irrigation of temperate pastures increases both the formation and decomposition of unprotected particulate organic matter, which aligns with the results of Apesteguía et al. (2015), where the incorporation of maize-derived organic C into small macroaggregates and microaggregates (250–2000 µm and 50–250 µm, respectively) was only observed under irrigation.

Interestingly, the mass of photosynthate C partitioned to the POM fractions (Figure 4.6A–B) in the irrigated treatment remained constant throughout the study, which indicates that the C partitioned to these fractions during irrigation is relatively stable over an annual cycle, despite changes in other plant components. According to the Soil Continuum Model (SCM) proposed by Lehmann and Kleber (2015), to explain the fate of organic litter entering SOM, it may be that the short-term storage of

SOC in POM fractions was related to an increase in soil aggregation. However, we did not measure aggregation or the distribution of ^{13}C in aggregates in this study.

Similar to the results for coarse POM fraction, we observed a significant increase in the ^{13}C recovered in the < 5 µm (Clay) fraction of non-rhizosphere soil (0–15 cm depth, Figure 4.6E) in winter (T3) in the dryland treatment. This increase in C associated with the clay fraction is well supported by the Microbial Efficiency-Matrix Stabilisation (MEMS) hypothesis proposed by Cotrufo et al. (2013), where higher quality plant litter inputs to soil can be used more efficiently by microorganisms stimulating SOM formation in the POM and mineral associated soil organic matter or MAOM. The fact that ^{13}C recovery in the clay fraction (non-rhizosphere soil) of the dryland treatment was similar to the recovery in the irrigated treatment at the end of the summer (T1), indicates that plant litter decomposition processes involved in the partitioning of photosynthate C to POM and MAOM (< 5 µm) fractions in the irrigated treatment occurred more rapidly.

The increase in ^{13}C recovered in the coarse POM and clay fractions of 15–25 cm whole soil (Figure 4.7A–E) displayed a similar pattern to that observed for the same fractions in the 0–15 cm soil. However, there were some differences compared with fractions from the 0–15 cm soil. In particular, the recovery of ^{13}C in the fine POM fraction tended to remain higher in the irrigated than the dryland treatment, from the cessation of irrigation over an entire annual cycle. This result indicates that C partitioned to fine POM in the 15–25 cm soil (Figure 4.7B) was enhanced by irrigation and remained relatively stable over the balance of the annual pasture growth cycle as a legacy effect of irrigation. Whereas the partitioning of C to this fraction may be explained by faster turnover of roots in the irrigated treatment (Scott et al., 2012), the stability of the C may be explained by the occlusion of fine POM in soil aggregates (Golchin et al., 1994; Lehmann and Kleber, 2015) as discussed before. It is not clear why there was an increase in the new C associated with 20–53 µm fraction of the irrigated treatment at T5 compared to the dryland treatment (Figure 4.7C). However, it is important to note that the C associated with the 20–53 µm fraction makes up a relatively small proportion of the whole soil C compared to other fractions. Interestingly, the decline in ^{13}C recovery of the 'stable' clay fraction in the dryland treatment by T5 may have been driven by desorption of C from this fine mineral fraction (Lehmann and Kleber, 2015), reaching similar values of saturation observed in the former irrigated pasture soil.

The aim of our third hypothesis was to test whether differences in the partitioning and turnover of C associated with POM fractions could be explain any difference in the C stored in irrigated versus dryland pasture soils. This study provides evidence that the C fixed during summer irrigation does not lead to greater or lesser storage of photosynthate C over an annual cycle compared to dryland pasture conditions. Although there was no difference in total C storage, there were temporal

differences between irrigated and dryland pastures in the partitioning of C to root biomass, rhizosphere and soil particle size fractions. Therefore, the previously reported losses of SOC from temperate irrigated grazed pastures (Mudge et al., 2017) may be driven by management that favours the turnover and decomposition of specific fractions, for example the effects of livestock treading in intensive grazing regimes on root turnover or the decomposition of POM. Further research on the combined effects of irrigation and other management practices on the persistence of SOM in the longer-term (> 1 year) is required to determine their impact on net storage of SOC under temperate grazed pastures.

4.6 Conclusions

This study found no differences in the persistence, over an annual pasture production cycle, of photosynthate C stored in the soil during summer irrigation compared to dryland conditions. However, irrigation did subsequently affect the spatial and temporal partitioning of C to root biomass, rhizosphere soil and soil size fractions, with greater partitioning of ^{13}C to the fine POM (53–250 µm) fraction of the previously irrigated pasture relative to dryland conditions. Although recovery of ^{13}C in the clay (< 5 µm) fraction of irrigated soil (0–15 cm) was initially higher than that of dryland surface soil, the increase in ^{13}C in the clay (< 5 µm) fraction of dryland soil during autumn-winter period resulted in no subsequent differences in the clay fraction ^{13}C recovery. We suggest that higher turnover of root derived-C and greater partitioning of photosynthate C to the POM fraction under irrigated pastures may be important to off-setting any effects of greater root biomass in dryland pastures on the longer-term storage of SOC. The previously reported losses of SOC from temperate irrigated grazed pastures may be driven by interactions with other management factors that favour the decomposition and turnover of C plant and soils fractions in the longer-term and requires further investigation.

4.7 Acknowledgements

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4.8 Supplementary data

Table 4.1. Standing biomass production of residual expressed as dry matter for the dryland and irrigated treatments. Values are means ± standard error of mean (*n* = 8 per treatment at T1, and *n* = 4 per treatment for T2–T5). The least significant difference (LSD) between means with significance level of 5% are given when P < 0.05 for sampling time (T), treatment (Tr) and interaction between sampling time and treatment (T x Tr).

Sampling time (T)	Treatment (Tr)		P value			LSD value
	Dryland	Irrigated	T	Tr	T x Tr	T
T1	2395 ± 135	2773 ± 234			0.123	420
T2	2608 ± 316	2518 ± 120			0.792	
T3	2944 ± 175	2707 ± 84.9			0.487	
T4	4022 ± 333	4526 ± 447			0.145	
T5	3874 ± 95.7	3742 ± 179	< 0.001	0.341	0.700	485

Table 4.2. Mean $\delta^{13}C$ values of plant-soil components at natural abundance (NA) and during the ^{13}C chase period at sampling times T1–T5 (1, 12, 125, 237 and 349 days after the last $^{13}CO_2$ labelling event) for the dryland and irrigated treatments. The least significant difference (LSD) between means with significance level of 5% are given when P < 0.05 for sampling time (T), treatment (Trt) and interaction between sampling time and treatment (T x Trt). Values in bold indicate significant differences between the dryland and irrigated treatments.

Isotopic composition of plant-soil components expressed in $\delta^{13}C$(‰)														
Component	**Depth (cm)**	**Treatment (Trt)**	**NA**	**Sampling time (T)**					**P value**			**LSD value**		
				T1	T2	T3	T4	T5	**T**	**Trt**	**T x Tr**	**T**	**Trt**	**T x Trt**
Herbage		Dryland		900.8	388.4	-16.7	-26.0	-28.9						
		Irrigated	-28.6	902.6	320.7	-20.4	-25.6	-29.4	< 0.001	0.064	0.005	20.8		29.5
Residual		Dryland		**517.7**	**337.2**	**162.0**	**85.6**	32.8						
		Irrigated	-29.1	**441.6**	**314.5**	**126.3**	**37.0**	25.6	< 0.001	< 0.001	0.162	34.2	19.8	
Roots	0–15	Dryland		**104.0**	104.7	**85.2**	32.9	7.8						
		Irrigated	-28.9	**134.8**	111.0	**139.6**	28.1	21.0	< 0.001	0.005	0.153	25.4	14.7	
	15–25	Dryland		**76.5**	**45.4**	**74.5**	**-1.3**	-20.2						
		Irrigated	-28.9	**54.4**	**61.3**	**57.4**	**-20.2**	-18.9	< 0.001	0.050	0.133	18.2	10.5	
Rhizosphere soil	0–15	Dryland		-5.34	-11.6	-11.5	-8.62	-11.7						
		Irrigated	-27.3	-4.16	-9.16	-10.9	-0.99	-9.47	0.003	0.070	0.555	4.76		
Non-rhizosphere soil	0–15	Dryland		-22.1	-20.6	-21.4	-21.3	-22.5						
		Irrigated	-27.5	-20.9	-21.1	-21.9	-21.4	-21.5	0.652	0.389	0.568			
Combined soil	0–15	Dryland		-20.7	-19.8	-20.3	-20.8	-21.9						
		Irrigated	-27.5	-19.9	-19.8	-20.6	-20.9	-20.9	0.250	0.390	0.850			
Whole soil	15–25	Dryland		-24.5	-23.9	-23.8	-24.1	-25.5						
		Irrigated	-27.4	-24.6	-24.9	-24.5	-24.8	-25.5	0.102	0.191	0.546			

Table 4.3. Mean $\delta^{13}C$ values of soil size particle fractions of non-rhizosphere soil (0–15 cm depth) at natural abundance (NA), and during the ^{13}C chase period at sampling times T1, T3 and T5 (1, 125 and 349 days after the last $^{13}CO_2$ labelling event) for the dryland and irrigated treatments. The least significant difference (LSD) between means with significance level of 5% are given when P < 0.05 for sampling time (T), treatment (Trt) and interaction between sampling time and treatment (T x Trt). Values in bold indicate significant differences between the dryland and irrigated treatments.

Isotopic composition of non-rhizosphere soil (0–15 cm depth) expressed as $\delta^{13}C$ (‰)											
Fraction (F)	**Treatment (Trt)**	**NA**	**Sample event (T)**			**P value**			**LSD value**		
			T1	T3	T5	T	Trt	T x Trt	T	Trt	T x Trt
	Dryland		8.29	17.4	**-0.06**						
>250 µm	Irrigated	-27.7	13.1	24.8	**11.9**	0.009	0.037	0.712	9.17	7.49	
	Dryland		**-23.0**	**-21.2**	**-16.4**						
53–250 µm	Irrigated	-28.5	**-16.1**	**-15.1**	**-13.2**	0.005	<0.001	0.312	2.64	2.16	
	Dryland		**-26.2**	-25.7	**-25.9**						
20–53 µm	Irrigated	-28.0	**-25.6**	-25.3	**-24.8**	0.107	0.004	0.262		0.43	
	Dryland		**-26.5**	**-26.7**	**-26.0**						
5–20 µm	Irrigated	-28.1	**-25.5**	**-26.2**	**-25.5**	0.003	<0.001	0.219	0.34	0.28	
	Dryland		**-24.5**	-23.6	-23.3						
<5 µm	Irrigated	-27.1	**-23.4**	-24.1	-23.4	0.054	0.005	0.011		0.68	0.68

Table 4.4. Mean δ^{13}C values of soil size particle fractions of whole soil (15–25 cm depth) at natural abundance (NA), and during the ^{13}C chase period at sampling times T1, T3 and T5 (1, 125 and 349 days after the last $^{13}CO_2$ labelling event) for the dryland and irrigated treatments. The least significant difference (LSD) between means with significance level of 5% are given when P < 0.05 for sampling time (T), treatment (Trt) and interaction between sampling time and treatment (T x Tr). Values in bold indicate significant differences between the dryland and irrigated treatments.

Isotopic composition of whole soil (15–25 cm depth) expressed as δ^{13}C (‰)											
Fraction (F)	**Treatment (Trt)**	**NA**	**Sample event (T)**			**P value**			**LSD value**		
			T1	T3	T5	T	Trt	T x Trt	T	Trt	T x Trt
>250 µm	Dryland		-6.60	3.43	-12.8						
	Irrigated	-27.6	-8.72	-4.83	-15.1	0.020	0.230	0.707	8.82		
53–250 µm	Dryland		**-25.9**	**-24.0**	**-23.9**						
	Irrigated	-28.6	**-23.4**	**-21.3**	**-20.9**	0.080	0.005	0.981		1.78	
20–53 µm	Dryland		-27.3	**-26.5**	**-27.1**						
	Irrigated	-28.3	-27.1	**-25.9**	**-25.3**	<0.001	<0.001	0.004	0.44	0.36	0.62
5–20 µm	Dryland		-27.2	-27.1	-26.9						
	Irrigated	-28.0	-26.9	-27.1	-26.7	0.059	0.133	0.702			
<5 µm	Dryland		-25.5	**-24.4**	-25.2						
	Irrigated	-27.1	-25.7	**-25.7**	-25.4	0.020	0.001	0.014	0.39	0.32	0.55

Chapter 5

Summer irrigation enhances the turnover of root and mineral-associated organic carbon under ryegrass-white clover pasture

5.1 Abstract

Summer irrigation of temperate managed pastures is used to increase above-ground pasture productivity. However, the effect of irrigation on soil organic C (SOC) storage under summer irrigated pastures is not well understood. The main objective of this study was to quantify the effect of summer irrigation on the short-term (< 1 year) persistence of previously assimilated C partitioned in the plant-soil system. A continuous $^{13}CO_2$ pulse labelling approach was used to ^{13}C label ryegrass (*Lolium perenne* L.) and white clover (*Trifolium repens* L.) pasture mesocosms during spring-like conditions, prior to imposing the irrigation and dryland treatments during summer.

These treatments were imposed on the ^{13}C-labelled mesocosms for 140 days after the cessation of ^{13}C labelling. The fate of the ^{13}C in the irrigated and dryland treatments was traced for a total of 343 days, over both the irrigation and post-irrigation periods, days 1 to 140 and days 141 to 343, respectively. This was determined on 5 destructive sampling dates (T1–T5), on days 1, 15, and 140, within the irrigation period, and on days 225 and 343 in the post-irrigation period. During the post irrigation period mesocosms were maintained under typical seasonal soil moisture regimes.

Over the irrigation period (140 days), irrigation increased the losses of the previously assimilated ^{13}C from above-ground pasture biomass compared to the dryland treatment, principally through the harvest and removal of ^{13}C in the herbage, which was approximately 3-fold in the irrigated treatment. Root biomass under irrigation was 2000 kg dry matter ha^{-1} lower. The ^{13}C recovered in root biomass (0–15 cm depth) in the irrigated treatment at the end of the irrigation (140 days) and post-irrigation (343 days) periods was lower than in the dryland treatment by approximately 70% and 60%, respectively.

The irrigation treatment reduced the persistence (increased the turnover) of ^{13}C previously assimilated in the above-ground (herbage) and below-ground (roots) plant biomass, but it did not affect the storage of ^{13}C in the soil after 343 days compared to dryland pasture conditions. However, the dryland pasture soil retained more of the previously assimilated ^{13}C in root biomass and the clay size fraction compared to the irrigated soil. This study provides evidence that summer irrigation can

increase the turnover of C in roots and the mineral associated SOC that may have important implications for the longer-term SOC storage under intensified pastoral production systems.

Keywords: Irrigation, pastures, photosynthate C and root turnover

5.2 Introduction

Grasslands cover approximately 70% of the world's agricultural area (Soussana and Lüscher, 2007) and contain about 20% of the global soil organic carbon (SOC) stocks (Stockmann et al., 2013). However, changes in agricultural practices and climate may shift the role of grassland soils from being a net sink to a net source of atmospheric carbon dioxide (CO_2) (Conant et al., 2017; Whitehead et al., 2018). Agricultural management may have a key role to play in determining the direction of this change. To achieve a net increase in SOC storage under managed grasslands requires either an increase in the rate of C inputs to the soil and/or an associated reduction in the rate of C loss (Conant et al., 2001; Kirschbaum et al., 2017; Whitehead et al., 2018).

Inputs of organic C (OC) to soil primarily occur via the transfer of photosynthate C, cycling of OC returns from plant biomass (above- and below-ground plant litter turnover) or recycling of C through the excreta of grazing animals in grazed pastures (Whitehead et al., 2018). Losses of SOC are driven by soil biological decomposition of soil organic matter (SOM) and associated CO_2 emissions (Paustian et al., 2000; Lal, 2004a; Schipper et al., 2007; Dungait et al., 2012). However, losses can also occur via leaching of dissolved organic C (DOC) (Ghani et al., 2007; Schipper et al., 2007; Sparling et al., 2016) and soil erosion (Boden et al., 2019).

Seasonal irrigation of temperate managed grasslands is used to increase above-ground pasture productivity during warmer and drier seasons, when soil water content limits plant growth (Scott et al., 2012; Condron et al., 2014; Moinet et al., 2017; Carmona et al., 2020). However, the specific effects of seasonal irrigation with respect to C inputs and outputs, and their net effect on SOC storage under perennial pastures are not well understood: contrasting responses to seasonal irrigation were previously reported, including gains (Kelliher et al., 2015), neutral effects (Condron et al., 2014; Moinet et al., 2017; Carmona et al., 2020) and reductions (Condron et al., 2014; Mudge et al., 2017) in SOC content, relative to dryland management. Thus, in terms of sustainability and understanding the potential for increasing SOC, there remains a need to clarify the effects of irrigation on the inputs and outputs of organic C that in turn determine the net SOC balance within temperate managed pastures.

Physical fractionation of SOM into particulate organic matter (POM) and mineral associated organic matter (MAOM) fractions, has assisted in developing the current understanding of the effects of agricultural practices on SOC formation, persistence and functioning (Cotrufo et al., 2019; Lavallee et al., 2020). The POM fractions (> 53 µm) consist of plant derived compounds at different stages of decomposition, with relatively short turnover and residence times in the soil < 10 years (Balesdent, 1996; Cotrufo et al., 2015; Poeplau et al., 2018; Lavallee et al., 2020). The MAOM fractions are associated with fine soil fractions (< 53 µm, silt and clay) consisting predominantly of low molecular weight compounds derived from both, plant and microbial activity (Kallenbach et al., 2016; Kögel-Knabner, 2017; Lavallee et al., 2020), with relative long residence times within the soil > 10 years (Feller and Beare, 1997; Baldock and Skjemstad, 2000; Lützow et al., 2006; Sokol et al., 2019).

Previous research has demonstrated that irrigation of a temperate ryegrass (*Lolium perenne* L.) and white clover (*Trifolium repens* L.) mixed pasture during summer, increased above-ground dry matter production (Scott et al., 2012; Condron et al., 2014; Moinet et al., 2017; Carmona et al., 2020) and the allocation of newly assimilated C to above-ground biomass, while irrigation reduced the allocation of C to root biomass (Carmona et al., 2020) resulting in smaller and shallower root systems when compared to an unirrigated dryland pasture (Stewart and Metherell, 1999; Scott et al., 2012; Carmona et al., 2020).

Irrigation has also been shown to affect the partitioning of C between soil particle and aggregate size fractions by enhancing the accumulation of OC in the fine POM (53–250 µm) size fraction (Carmona et al., 2020) and microaggregates (50–253 µm) (Gillabel et al., 2007; Apesteguía et al., 2015). However, these differences in pasture biomass allocation and C partitioning between SOC fractions were shown to have no effect on the net storage of recently assimilated photosynthate C in soil during the irrigation season (Carmona et al., 2020) or the stability of the newly formed SOC over the course of an annual pasture growth cycle (This thesis, Chapter 4).

Irrigation may also affect the net storage of C in soil by altering the turnover of C previously assimilated in plant and soil components when compared to dryland systems. Differences in the turnover of this pre-existing C, due to irrigation inputs, may occur as a direct function of soil water content effects on soil respiration (Sanaullah et al., 2012; Moinet et al., 2019) or as a product of the mineralisation flush that follows rewetting of dry soils (Harrison-Kirk et al., 2013; Canarini and Dijkstra, 2015; Zhang et al., 2020). Moreover, it has also been reported that summer irrigation applied to temperate grazed pastures increases root turnover (Scott et al., 2012), potentially by enhancing the abundance and activity of soil invertebrates (earthworms and herbivore mesofauna) when compared with a dryland pasture (Fraser et al., 2012).

The transfer of root derived-C to soil can occur directly though rhizodeposition (Jones et al., 2004; Cheng and Gershenson, 2007; Pausch and Kuzyakov, 2018) or indirectly by root turnover (Gill and Jackson, 2000; Eissenstat and Yanai, 2002). In the short-term, root derived-C contributes more to SOC than above-ground plant litter (Rasse et al., 2005; Kong and Six, 2010) and POM fractions are regarded as pivotal for the short-term storage of root derived-C in the soil (Kong and Six, 2010). However, this transfer of OC from roots to soil is regulated by processes within the leaves and roots in response to environmental conditions that affect plant growth (Jones et al., 2004). For example, under water stress conditions (e.g. drought) it has been reported that the production of photosynthate C and its transfer within the entire plant-soil system is reduced (Peñuelas et al., 2004; Chomel et al., 2019).

The transfer of C from roots to soil stimulates soil microbial decomposition of SOM improving plant nutrient availability (Jones et al., 2004; Vidal et al., 2018; Gorka et al., 2019), but it also contributes to losses of organic C from the soil via respired CO_2 and/or the leaching of DOC (Talbot et al., 2008; Paterson et al., 2016). However, adsorption of DOC to the MAOM fractions is possible via the microbial-DOC pathway that results from plant litter or POM decomposition (Cotrufo et al., 2015). Nevertheless, there are no published studies that have explicitly addressed the effects of irrigation on the persistence of previously assimilated C in plant and soil size fractions of managed pasture systems.

Therefore, the main objective of this study was to quantify the effect of summer irrigation on the short-term (< 1 year) persistence of previously assimilated C partitioned in the plant-soil system, including soil particle size fractions. The following hypotheses are proposed to explain the effects of summer irrigation on the short-term persistence of the previously assimilated photosynthate C partitioned in the plant-soil system relative to an unirrigated pasture:

1. Irrigation will reduce the persistence of previously assimilated photosynthate C within the below-ground plant and soil components as a result of an enhanced decomposition of this photosynthate C during the summer period.

2. Irrigation will also decrease the persistence of previously assimilated photosynthate C in the POM size fractions while increasing the accumulation of C to MAOM fractions.

5.3 Materials and Methods

Materials and methods that include plant-soil mesocosm establishment, soil properties, $^{13}CO_2$ continuous-pulse labelling, sampling procedures and calculations have been described in detail by

Carmona et al. (2020) (This thesis, Chapter 3). However, specific materials and methods relevant to this study are given below.

5.3.1 Experimental design

Mesocosms (15 cm wide x 25 cm high) were established (*n* = 66) and sown in May 2017 with a pasture mixture comprising of ryegrass (*Lolium perenne* L.) and white clover (*Trifolium repens* L.) at rates of 20 and 5 kg ha^{-1}, respectively. The soil was a Lismore stony silt loam (Pallic Firm Brown soil [New Zealand]; Udic Ustochrept [USDA]) (Hewitt, 2010).

Soil moisture and temperature probes (CS635-L 3-Rod 15cm TDR, Campbell Scientific, Inc. Logan, USA) were inserted into a subset of mesocosms (*n* = 8) to monitor soil moisture and temperature. During pasture establishment, the mesocosms' soil volumetric water content (VWC) was maintained at a target VWC of 20% by periodic watering using a manual volumetric dispenser.

Mesocosms were housed inside an unheated glasshouse for approximately 3 months (May 17–Aug 17) to allow the pasture to establish. Whenever the ryegrass reached the third leaf stage (Sean et al., 2016) the pasture was harvested by cutting to a residual height of 4 cm, a common grazing pasture height. To ensure the pasture nutrient requirements were not limiting, mesocosms were periodically fertilised with nitrogen (N) (15 g L^{-1} urea solution, equivalent to 50 kg N ha^{-1}) and P-K-S (19 kg P ha^{-1}, 159 kg K ha^{-1} and 16 kg S ha^{-1}) in solution (Monaghan et al., 2007; Morton et al., 2017).

5.3.2 The $^{13}CO_2$ continuous-pulse labelling

Prior to initiating the $^{13}CO_2$ labelling, a subset of established pasture mesocosms (*n* = 4) were destructively sampled to determine the natural abundance (NA) ^{13}C signatures of the plant and soil components. Simultaneously, another subset of mesocosms (*n* = 48) were placed into a sealed transparent (acrylic) plant growth chamber (2 m long × 1.2 m wide × 1 m high) in a randomised block design (*n* = 4 blocks) under the same soil VWC that ranged between 18–32%. Mesocosms were continuously pulse labelled through daily additions (equivalent to 0.5 g $Na_2{}^{13}CO_3$) of $^{13}CO_2$ for approximately 3 months over the late winter-spring period (Southern Hemisphere, 24 August 2017–04 Dec 2017), following the same $^{13}CO_2$ labelling procedure described in Carmona et al. (2020).

The concentration of CO_2 within the chamber was monitored continuously and controlled using a system consisting of a data logger (Campbell Scientific CR1000, USA) connected to an infrared gas analyser (LI-7000, LI-COR Biosciences, Lincoln, USA). The atmosphere within the plant growth chamber was maintained at an ambient CO_2 concentration (approximately 400 ppm) through additions of food grade $^{12}CO_2$ from a cylinder that was connected to the chamber. Following the

uptake of $^{13}CO_2$ at the end of each day, the chamber was opened and allowed to circulate fresh air to avoid high concentrations of CO_2 accumulating in the chamber overnight due to plant and soil respiration. Environmental variables (temperature, relative humidity and photosynthetically active radiation (PAR)) were continuously monitored within the chamber. The temperature and relative humidity inside the chamber were maintained under similar conditions to those in the glasshouse, using a fan-forced heat exchanger.

The soil VWC was managed to maintain a small soil water deficit to avoid drainage. The soil VWC of all mesocosms was monitored by regularly weighing each mesocosm (once per week), and when required, the plant growth chamber was open, and water was applied to the mesocosms manually using a volumetric dispenser to achieve the target soil VWC. The remaining mesocosms (*n* = 14) were maintained in a separate unheated glasshouse under similar environmental conditions to the plant growth chamber to act as controls for the pasture growing conditions and NA ^{13}C values outside the chamber.

5.3.3 Irrigation treatment

One day after the last $^{13}CO_2$ labelling event, designated sampling time T1 (05 Dec 2017), a subset of ^{13}C labelled mesocosms (*n* = 8) were destructively sampled to quantify the ^{13}C enrichment obtained within the plant-soil system at the start of the seasonal irrigation as described below. A subset of the control mesocosms (*n* = 4) were also destructively sampled.

The remaining ^{13}C labelled and control mesocosms (*n* = 40 and *n* = 10, respectively) were randomly allocated using a split-plot design to one of two soil VWC treatments: dryland vs irrigated. Treatments were applied over nearly 5 months (5 Dec 2017–23 April 2018). The soil VWC for the dryland treatment was maintained within a target range of 7–20%, while the soil VWC for the irrigated treatment was maintained between 24–33%. The dryland and irrigated treatments simulated an unirrigated pasture and a typical summer irrigation system, respectively.

The irrigated treatment was managed to maintain a soil water deficit in order to avoid drainage (soil VWC < field capacity = 39%). Soil mesocosm VWC was monitored by regular weighing each mesocosm (1–2 times per week). When required, water was applied manually using a volumetric dispenser. At the end of the irrigation period, the remaining mesocosms (after 2 more destructive samplings at T2 and T3 as described below) from both the dryland and summer irrigation treatments were placed under a similar soil VWC for approximately 7 months (23 April 2018–4 Nov 2018) to quantify the legacy effects of irrigation on the short-term fate of the previously assimilated ^{13}C.

5.3.4 Sampling and sample preparation

Mesocosms were destructively sampled on six dates distributed over three periods ($^{13}CO_2$ labelling, irrigation and post-irrigation) of the experiment as follows (Figure 5.1):

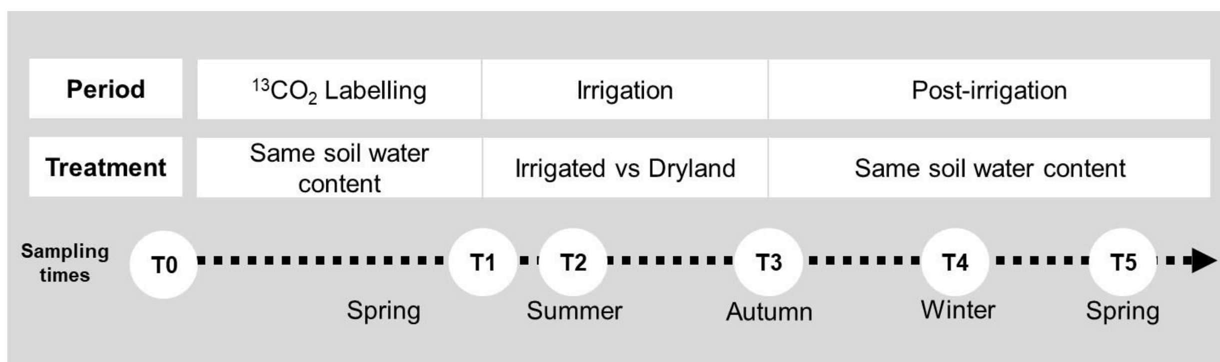

Figure 5.1. Timeline for the different experimental periods: labelling, irrigation and post-irrigation, with the correspondent destructive sampling times and associated seasons, where T1, T2, T3, T4 and T5 correspond to 1, 15, 140, 225 and 343 days, following the cessation of $^{13}CO_2$ labelling, respectively.

Labelling period

Sampling during the labelling period was as follows:

- T0 (25 Aug 2017): immediately before initiating $^{13}CO_2$ labelling (n = 4).
- T1 (05 Dec 2017): the first day following cessation of continuous $^{13}CO_2$ pulse labelling (3 months) and immediately prior to initiating the irrigated and dryland treatments (n = 8).

Irrigation period

Sampling during the irrigation period was as follows:

- T2 (19 Dec 2017): 15 days after cessation of ^{13}C labelling (irrigated vs dryland; n = 4 per treatment).
- T3 (23 Apr 2018): 140 days after cessation of ^{13}C labelling (irrigated vs dryland; n = 4 per treatment).

Post-irrigation period

Sampling during the post-irrigation period was as follows:

- T4 (17 Jul 2018): 225 days after cessation of ^{13}C labelling (irrigated vs dryland; n = 4 each) and 85 days after cessation of irrigation.
- T5 (12 Nov 2018): 343 days after cessation of ^{13}C labelling (irrigated vs dryland; n = 8 each) and 203 days after cessation of irrigation.

At each sampling time mesocosms were separated into above and below-ground C components following the same procedure described in detail in Carmona et al. (2020) (This thesis, Chapter 3). However, relevant aspects to this study are given below.

The above-ground plant C components were sampled as herbage biomass (cut to 4 cm grazing height), while ryegrass leaf meristems, stems and clover stolons were cut at the soil surface and combined as one plant component, hereafter referred to as the 'residual'. The soil column (15 cm wide x 25 cm high) was extracted from the mesocosm and divided into two soil depths: 0–15 and 15–25 cm.

Then, root biomass was manually collected from each depth and the soil was separated into the following soil C components: rhizosphere soil was separated from the wider non-rhizosphere soil, only for soil from the 0–15 cm depth, by shaking carefully the soil that was adhered to the roots previously sampled in this depth. In the 15–25 cm depth, rhizosphere soil was not separated from root biomass and the soil sampled in this depth is hereafter referred to as 'whole soil'.

Soil gravimetric water content (oven drying at 105°C for 24 h) was determined from non-rhizosphere soil (0–15 cm depth) and whole soil (15–25 cm depth) samples sieved using a 5.5 mm mesh. Samples of rhizosphere soil (0–15 cm), non-rhizosphere soil (0–15 cm) and the whole soil (15–25 cm) were sieved separately using a 2 mm-sieve and air-dried at 25°C. Prior to elemental and isotope analyses, the dried herbage, residual, roots, rhizosphere soil, non-rhizosphere soil and whole soil samples were finely ground (< 53 µm) using a bench top ring mill (BTRM, RockLabs, Auckland, NZ). To avoid cross contamination equipment was cleaned between samples using deionised water and ethanol.

5.3.5 Soil particle size fractionation

Subsamples (20–30 g) from the 2 mm sieved air-dried samples of rhizosphere soil (0–15 cm depth), non-rhizosphere soil (0–15 cm depth) and whole soil (15–25 cm depth) were fractionated into five soil size fractions: > 250 µm (coarse POM), 53–250 µm (fine POM), 20–53 µm (silt), 5–20 µm (fine silt), and < 5 µm (clay).

Soil particle size fractionation was carried out for the above soil components at sampling times T0, T1 and T3 (NA, *n* = 4 per soil component; labelled *n* = 8 per soil component; Dryland and Irrigated, *n* = 4 per soil component per treatment) using a method adapted from Qiu et al. (2010). Briefly, samples were dispersed in deionised water using an ultrasonic probe (BOSCH, UW 2200) for 60 s with sonication set at 60 J s^{-1}.

The dispersed soil suspension was passed through 250 µm and 53 µm sieves with the two POM fractions retained on the respective sieves. The < 53 µm material was separated into 20–53 µm, 5–20 µm and < 5 µm fractions by gravity, using their different rates of sedimentation and siphoning off the excess of water. A flocculent (calcium chloride dehydrate, $CaCl_2.2H_2O$) was added to the < 5 µm suspension to aid the sedimentation of the clay particles. The masses of the five fractions were obtained following oven drying at 60°C, before each fraction was homogenised using a mortar and pestle prior to analysis.

5.3.6 Isotopic composition after $^{13}CO_2$ labelling

Subsamples of the plant-soil C components and the soil size fractions were analysed for both C concentration, and the isotopic C composition using an elemental analyser (Sercon, model GSL, Crewe, UK) coupled to a continuous flow isotopic ratio mass spectrometer (Sercon, model 20-22, Crewe, UK). The ^{13}C isotope composition ($\delta^{13}C$) of the samples was expressed in units of per mil (‰) (Equation 5.1).

$$\delta^{13}C\ (‰) = \left[\left(\frac{R_{sample}}{R_{standard}}\right) - 1\right] \qquad [5.1]$$

Where *R* = the $^{13}C/^{12}C$ ratio for either the sample or the standard (0.01118) and the $\delta^{13}C$ values are hereafter expressed relative to the international standard Pee Dee Belemnite (PDB).

5.3.7 Partitioning of ^{13}C during irrigation and post-irrigation periods

The partitioning of ^{13}C between plant and soil components, including soil particle size fractions, was calculated as the mass of ^{13}C recovered within these components for each treatment (Equation 5.2–5.6). When applicable, values for the rhizosphere and non-rhizosphere soil from the 0–15 cm depth were used to calculate either a weighted average of $\delta^{13}C$ values or the mass of ^{13}C recovery expressed in units of mg $^{13}C\ m^{-2}$ for combined soil in the 0–15 cm soil depth.

The amount of C (mg C m^{-2}) in each plant-soil component or soil size fraction was calculated using Equation 5.2.

$$C = (TC/100) \times m \qquad [5.2]$$

Where *TC* is the total C (%) and *m* is the mass of each plant-soil component or soil size fraction per unit area (mg m^{-2}), using the surface area of the mesocosms (0.01767 m^2).

The fractional abundance (*F*) of the sample was calculated using Equation 5.3 modified from Stewart and Metherell (1999) as follows:

$$F = \frac{(\delta^{13}C + 1000)}{[(\delta^{13}C + 1000 + (1000/R_{standard}))]} \qquad [5.3]$$

Where $R_{standard}$ = the $^{13}C/^{12}C$ ratio for the standard. The $\delta^{13}C$ values are expressed relative to Pee Dee Belemnite (PDB).

The atom% ^{13}C excess was calculated as the difference between the fractional abundance of the heavier isotope after labelling (F_a) and the fractional abundance at NA level (F_{NA}) using Equation 5.4.

$$atom\%\ ^{13}C_{excess} = (F_a - F_{NA}) \times 100 \qquad [5.4]$$

The mass of ^{13}C recovered expressed in mg m^{-2} ($^{13}C_{mass}$) within each plant and soil component was calculated using Equation 5.5.

$$^{13}C_{amount} = \frac{atom\%\ ^{13}C_{excess}}{100} \times C \qquad [5.5]$$

5.3.8 Statistical analysis

Factorial analysis of variance (ANOVA) based on a split plot design using GenStat (18th edition, Lawes Trust, Harpenden, UK) was performed to compare the effects of seasonal irrigation on the persistence and losses of photosynthate C from the plant-soil system between the dryland and irrigated treatments. Plant and soil components, including soil particle size fractions data were analysed with a model defined by treatment and sampling time as fixed factors. However, sampling time was excluded as a fixed factor when data did not meet ANOVA assumptions of normality and the equal variance. Residual plots and the Shapiro-Wilk test were used to check the normality and the equal variance assumptions of ANOVA. When ANOVA assumptions were met without Log-transformed data, the Fisher's Protected Least Significant Difference test (LSD) with a significance level of 5% was used to determine the difference between the labelled, dryland and irrigated treatments means. Where Log transformation of data was required, the Fisher's Protected Least

Significant Ratio (LSR), with a significance level of 5% was used to assess differences of means between treatments as follow in Equation 5.6.

if a/b ≥ LSR ∴ a ≠ b [5.6]

Where *a* and *b* are the larger and smaller means, respectively, and independent of the treatment, LSR is the Least Significant Ratio between treatment means.

As herbage dry matter production and its Log-transformed data did not meet the ANOVA assumptions, a two samples t-test was run to determine differences between treatment means using GenStat (18th edition, Lawes Trust, Harpenden, UK).

5.4 Results

5.4.1 Environmental conditions

Average soil VWC during the $^{13}CO_2$ labelling period for labelled mesocosms was similar to the long-term 18-year mean in situ soil VWC (NIWA, 2018), except in August 2017 where mesocosms were set up and their soil VWC was conditioned to approximately 32% during September 2017 (Figure 5.2A). During the irrigation period soil VWC for the dryland treatment was on average 14.5 ± 2.7 % (± standard deviation), which was in the range of the 18-year mean in situ soil VWC for the Lincoln area during the same period (17.2 ± 4.8%, NIWA 2018). The soil VWC in the dryland treatment varied from a minimum of 7.5% to a maximum of 19.4%. The soil VWC for the irrigated treatment during the irrigation period averaged 24.5 ± 2.4 % (± standard deviation) and varied from 20.1% to 30.9%. During the post-irrigation period soil VWC was similar between the dryland and irrigated treatments and within the range of the 18-year mean soil VWC in field conditions (Figure 5.2A).

Average soil temperature (0–15 cm depth) during the labelling period was higher than the 12-year mean soil temperature (0–30 cm depth) under field conditions (17.7 ± 0.4°C and 10.2 ± 0.72°C, respectively) (Figure 5.2B). During the irrigation period mean soil temperatures (0–15 cm depth) in the dryland and irrigated pastures were similar, approximately 20°C and were 4°C higher than the 12-year mean in situ soil temperature (0–30 cm depth) of 16.2 ± 1.12°C. During the post-irrigation period soil temperatures were similar between the dryland and irrigated pastures and were approximately 5°C higher than under field conditions during the same period (Figure 5.2B). Air temperatures in the plant growth-labelling chamber ranged from a minimum of 5.4°C in August 2017 to 34.4°C in December 2017 at the end of the labelling (Figure 5.2C). Mean air temperature in the labelling chamber during labelling was 17.7 ± 4.3°C, which was approximately 6°C higher than the 30-year mean outdoor air temperature for Lincoln over this period (11.4 ± 2.9°C). During the

irrigation and post-irrigation periods average air temperatures in the glasshouse, where both treatments were maintained after labelling, were in the range of the 30-year mean outdoor air temperatures.

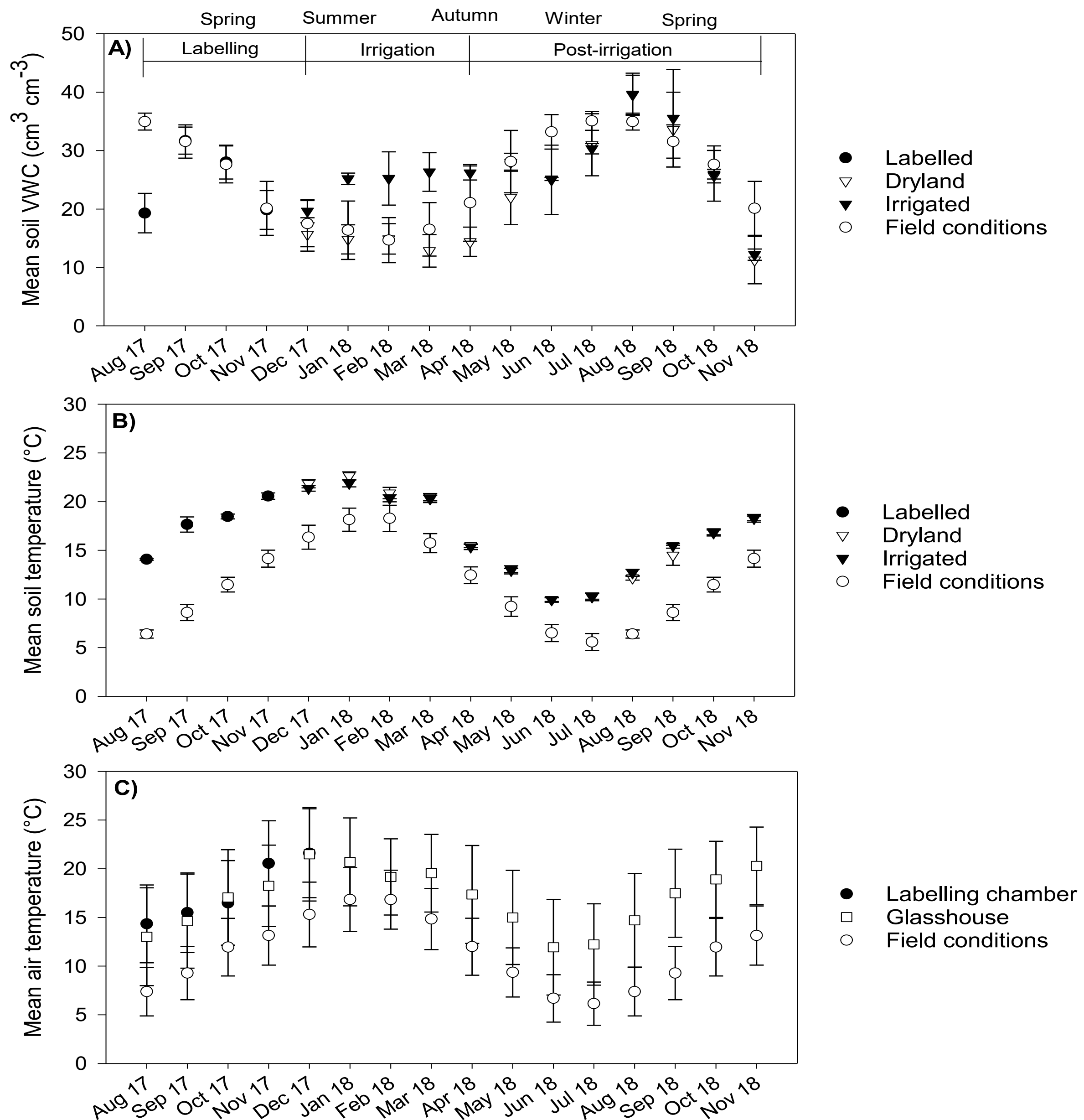

Figure 5.2. Monthly time series of mean values during the $^{13}CO_2$ labelling, irrigation and post-irrigation period for: A) Soil volumetric water content (WVC) for the ^{13}C-labelled, dryland and irrigated treatments, and the 18-year mean soil VWC in field conditions, B) Soil temperature for the ^{13}C-labelled, dryland and irrigated treatments (0–15 cm depth), and the 12-year mean soil temperature in field conditions (0–30 cm depth), and C) Air temperature in the labelling chamber, glasshouse and the 30-year mean outdoor mean air temperature. Error bars represent ± 1 standard deviation of the mean.

5.4.2 Pasture biomass

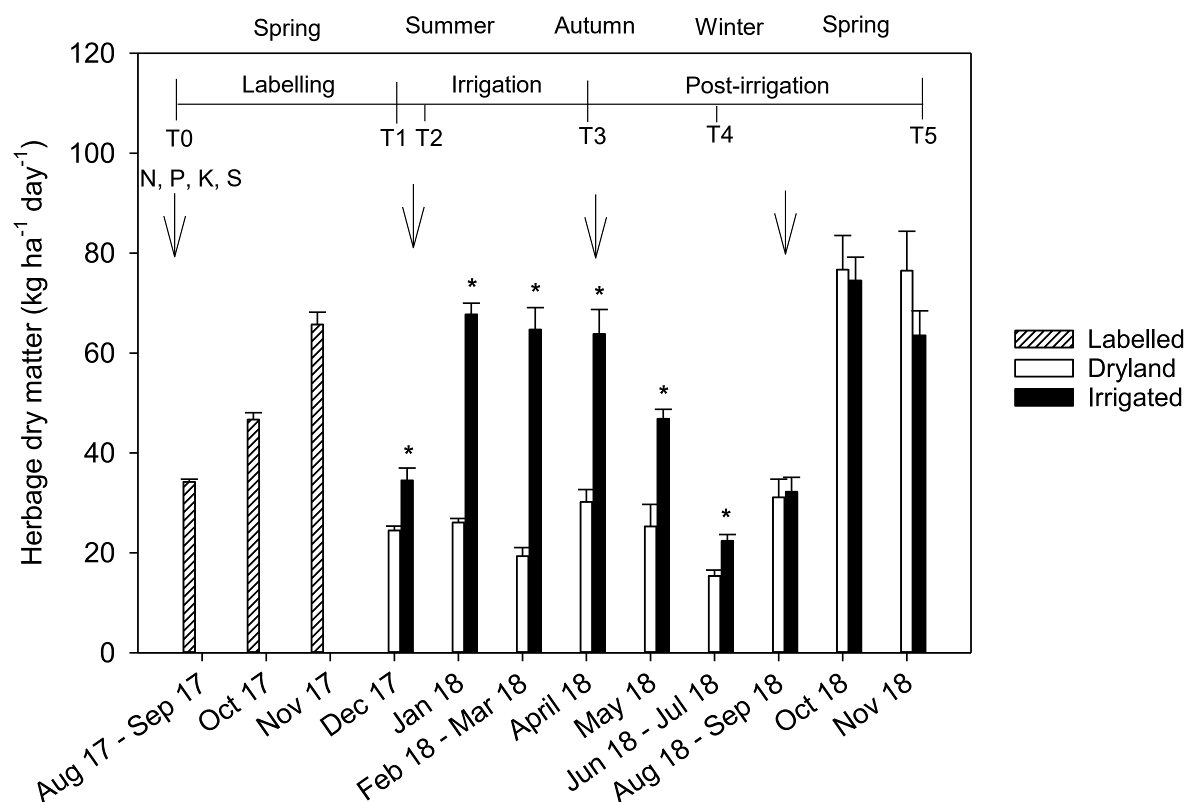

Figure 5.3. Mean values of the daily herbage dry matter production (kg ha^{-1} day^{-1}) for the Labelled mesocosms during the $^{13}CO_2$ labelling between sampling times T0 and T1, for the irrigated and dryland treatments during the seasonal irrigation between T1–T3, and post-irrigation period between T3 and T5. Error bars represent 1 standard error of the mean and asterisk symbol (*) represent significant differences between the dryland and irrigated treatments at P < 0.05. Arrows indicate a fertilisation event with the addition of nitrogen (N), phosphorus (P), potassium (K) and sulphur (S).

During the $^{13}CO_2$ labelling period (25 Aug 2017–04 Dec 2017) a steady increase in daily herbage dry matter production was observed. The highest rate of production was in Nov 2017 with 66 ± 2.5 kg dry matter ha^{-1} day^{-1} (P < 0.001, Figure 5.3). At the beginning of the irrigation period (05 Dec 2017) a decrease in herbage production rates occurred relative to Nov 2017, however, herbage growth remained higher in the irrigated treatment (35 ± 2.5 kg dry matter ha^{-1} day^{-1}) compared with the dryland treatment (24 ± 0.8 kg dry matter ha^{-1} day^{-1}) (P = 0.004, Figure 5.3).

At the end of December, after the addition of fertilisers (N, P, K and S), daily herbage dry matter production increased significantly in the irrigated treatment during the January 2018–April 2018 period, with growth rates similar to those observed in November 2017. During this period, the irrigated treatment produced approximately 3-fold more herbage dry matter than the dryland

treatment ($P < 0.001$, Figure 5.3). A significant irrigation effect on herbage dry matter production rates was still observed in May 2018 and the June–July 2018 period, with the previously irrigated treatment producing more herbage than the dryland treatment ($P < 0.001$, Figure 5.3). After this time, between August 2018 and November 2018, there was no irrigation effect on the herbage production rate ($P = 0.811$) but production rates increased in October 2018.

The standing dry matter of the residual plant material (meristems and clover stolons) was similar between the dryland and irrigated treatments during the seasonal irrigation at sampling times T2 ($P = 0.654$) and T3 ($P = 0.339$), equal to approximately 1500 kg dry matter ha^{-1} (Figure 5.4A). During the post-irrigation period, under winter and spring like conditions, a significant effect of the previous summer irrigation was observed at T4 ($P = 0.006$) and T5 ($P = 0.012$), with higher residual standing mass in the previously irrigated treatment.

In the 0–15 cm depth, the standing root mass at T2 (15 days since irrigation started) was similar between the dryland and irrigated treatments ($P = 0.589$, Figure 5.4B). However, by T3 (after 140 days of irrigation), root mass was approximately 2000 kg dry matter ha^{-1} lower in the irrigated when compared with the dryland treatment ($P = 0.003$ and LSD2–4 = 1402). This was due to an increase in root biomass in the dryland treatment. At T4, during the post-irrigation period, no effects of irrigation were observed on the standing root mass. By T5, during spring growth conditions, root mass was again lower in the irrigated treatment ($P = 0.005$, LSD5 = 991.6, Figure 5.4B). Root mass in the 0–15 cm depth did not vary under the irrigated treatment during the irrigation and post-irrigation periods when compared with the dryland treatment.

In the 15–25 cm depth, the standing root mass was not affected by the seasonal irrigation at T2 and T3, being similar between the dryland and irrigated treatments ($P = 0.833$ and $P = 0.175$, respectively, Figure 5.4C). Despite no differences in root mass between the treatments by T3, there was a significant increase in root biomass under the dryland treatment relative to T2 ($P < 0.001$ and LSDtime > 695.1), but not in the irrigated treatment (LSDtime < 695.1). At T4, root mass (15–25 cm depth) was again similar between the dryland and irrigated treatments ($P = 0.541$, Figure 5.4C). A decrease in root biomass under the dryland treatment was observed relative to T3 ($P < 0.001$ and LSDtime > 695.1), while no significant changes were observed in root biomass under the irrigated treatment relative to T3 (LSDtime < 695.1). At T5, under spring conditions, despite root mass in the 15–25 cm depth increasing for both treatments relative to T4 ($P < 0.001$ and LSDtime > 695.1), the increase was lower in the irrigated treatment compared with the dryland treatment ($P = 0.006$ and LSD5 = 695).

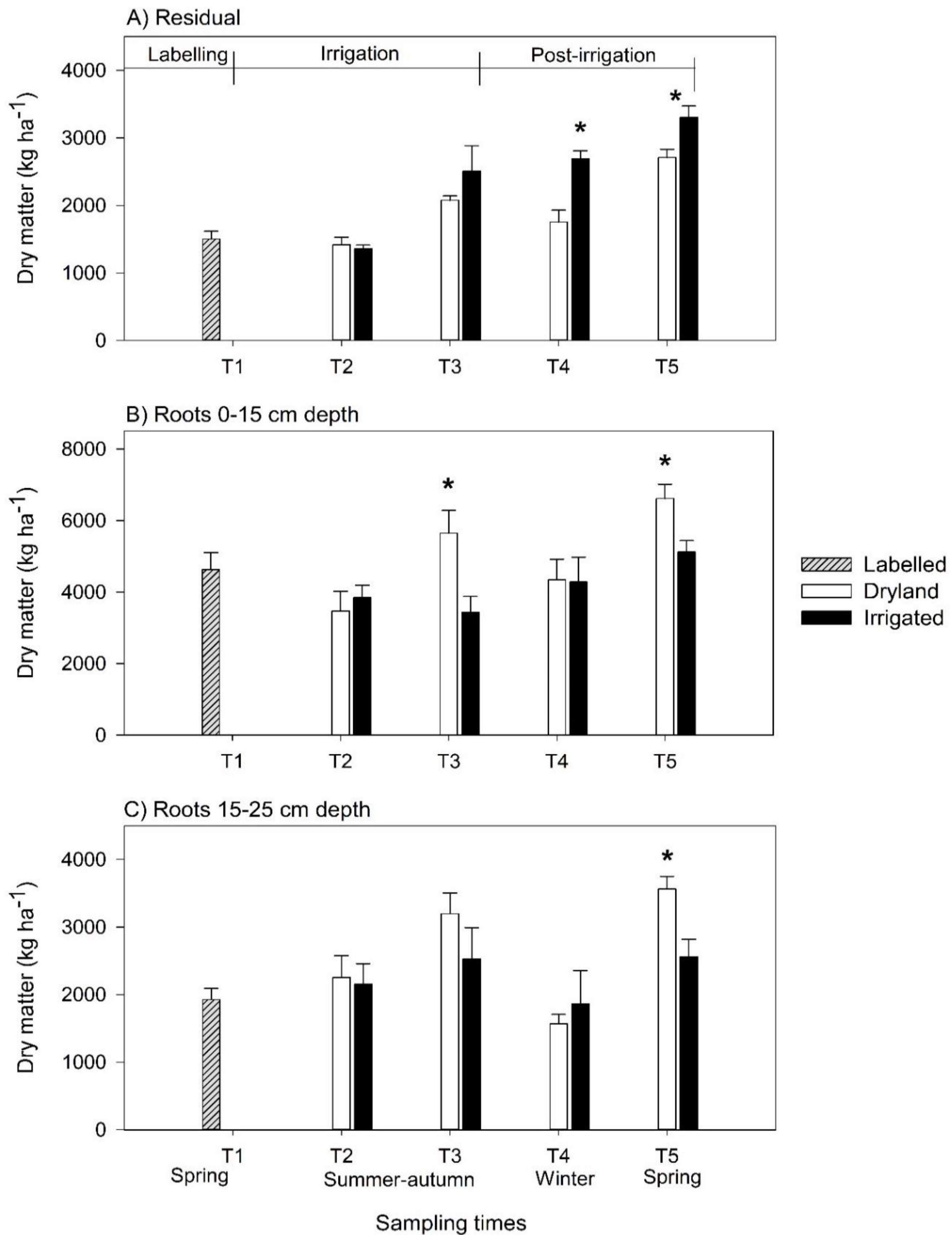

Figure 5.4. Mean values of the residual and root biomass in kg of dry matter ha^{-1} of: A) Residual (meristems and clover stolons), B) Roots in the 0–15 cm depth, and C) Roots in the 15–25 cm depth, for labelled mesocosms at the end of the $^{13}CO_2$ labelling period at sampling time T1. For the dryland and irrigated treatments during the seasonal irrigation period between sampling times T1 and T3, and during the post-irrigation period between sampling times T3, T4 and T5. Error bars represent 1 standard error of the mean and asterisk symbol (*) represents a significant difference between the dryland and irrigated treatments at P < 0.05.

5.4.3 Quantity of ^{13}C recovered within the Plant-soil system

The ^{13}C isotopic composition (expressed as $\delta^{13}C$ values) and the mass of total C within the above- and below-ground plant and soil components, including soil particle size fractions, that were used to calculate the ^{13}C recovery after the $^{13}CO_2$ labelling period at sampling time T1, during the seasonal irrigation period at T2 and T3, and over the post-irrigation period at T4 and T5, are shown in Supplementary data, Tables 5.1–5.4. The ^{13}C recoveries in the above- and below-ground plant and soil components are expressed in units of mg ^{13}C m^{-2}.

The ^{13}C recovery in above- and below-ground plant components

At sampling time T1 (1 day after the last labelling event), the quantity of ^{13}C recovered in herbage at the start of irrigation was 116 mg ^{13}C m^{-2} (Figure 5.5A). By T2, after 15 days of irrigation, ^{13}C recovery in herbage had decreased in both the dryland and irrigated treatments relative to T1 ($P < 0.001$ and LSDtime > 9.68), with a greater decrease in the irrigated compared with the dryland treatment ($P = 0.041$ and LSD > 5.94).

After 140 days of irrigation by T3, ^{13}C recovery in herbage had decreased further relative to T1 ($P < 0.001$ and LSD time > 4.03), with no differences between the dryland and irrigated treatments ($P = 0.326$, Figure 5.5A). By T4, during the post-irrigation period, ^{13}C recovery was lower under the irrigated compared with the dryland treatment ($P= 0.003$ and LSD > 0.051) and by T5, no ^{13}C was recovered in herbage from either the dryland or the irrigated treatments.

The ^{13}C recovery in the residual plant material at T1 was 177 mg ^{13}C m^{-2} (Figure 5.5B). By T2, ^{13}C recovery in the residual had decreased in the irrigated pasture (t-test $P = 0.023$) relative T1, and as a result, it was lower than the dryland treatment ($P = 0.022$ and LSD < 22.8), where ^{13}C recovery in the residual did not change significantly relative to T1 (t-test $P = 0.401$). By T3, ^{13}C recovery in the residual had decreased further for both the dryland and irrigated treatments relative to T2 ($P = 0.007$ and LSDtime > 16.13). However, ^{13}C recovery in the irrigated treatment was again lower compared with the dryland treatment ($P = 0.002$, and LSD > 22.8, Figure 5.5B). At T4 and T5, during the post-irrigation period, ^{13}C recovery in the residual continued to decrease relative to T3 ($P < 0.001$ and LSDtime > 22.2), with no difference between the treatments ($P = 0.158$ for T4 and $P = 0.105$ for T5).

Recovery of ^{13}C in roots from the 0–15 cm depth at T1 was 460 mg ^{13}C m^{-2} (Figure 5.5C). By T2, ^{13}C recovery in roots had decreased for both the dryland and irrigated treatments relative to T1 ($P < 0.001$ and LSDtime > 63.1) with no difference between the treatments ($P = 0.409$). At T3 the ^{13}C

recovered in roots (0–15 cm depth) decreased significantly in the irrigated treatment by approximately 70% when compared with the dryland treatment ($P < 0.001$ and $LSD2–4 > 89.3$, Figure 5.5C), where the ^{13}C recovery in roots (0–15 cm depth) did not change significantly relative to T2 ($P < 0.001$ and $LSDtime < 63.1$).

During the post-irrigation period (T4), the ^{13}C recovery in roots (0–15 cm depth) under the irrigated treatment was similar to the amount recovered at T3 ($P < 0.001$ and $LSDtime < 63.1$), but this was again lower compared with the dryland treatment ($P = 0.036$ and $LSD2–4 = 89.3$, Figure 5.5C). However, a decrease from root (0–15 cm depth) ^{13}C recovery in the dryland treatment occurred at T4 relative to T3 ($P < 0.001$ and $LSD > 63.1$). By T5, ^{13}C recovery in roots (0–15 cm depth) did not vary significantly for either the dryland or irrigated treatments relative to T4 ($P <0.001$ and $LSD < 63.1$). However, ^{13}C recovery for these roots at T5 was still lower in the irrigated treatment (48 mg ^{13}C m^{-2}) by approximately 60% when compared with the dryland treatment (131 mg ^{13}C m^{-2}) ($P = 0.012$ and $LSD5 > 63.1$).

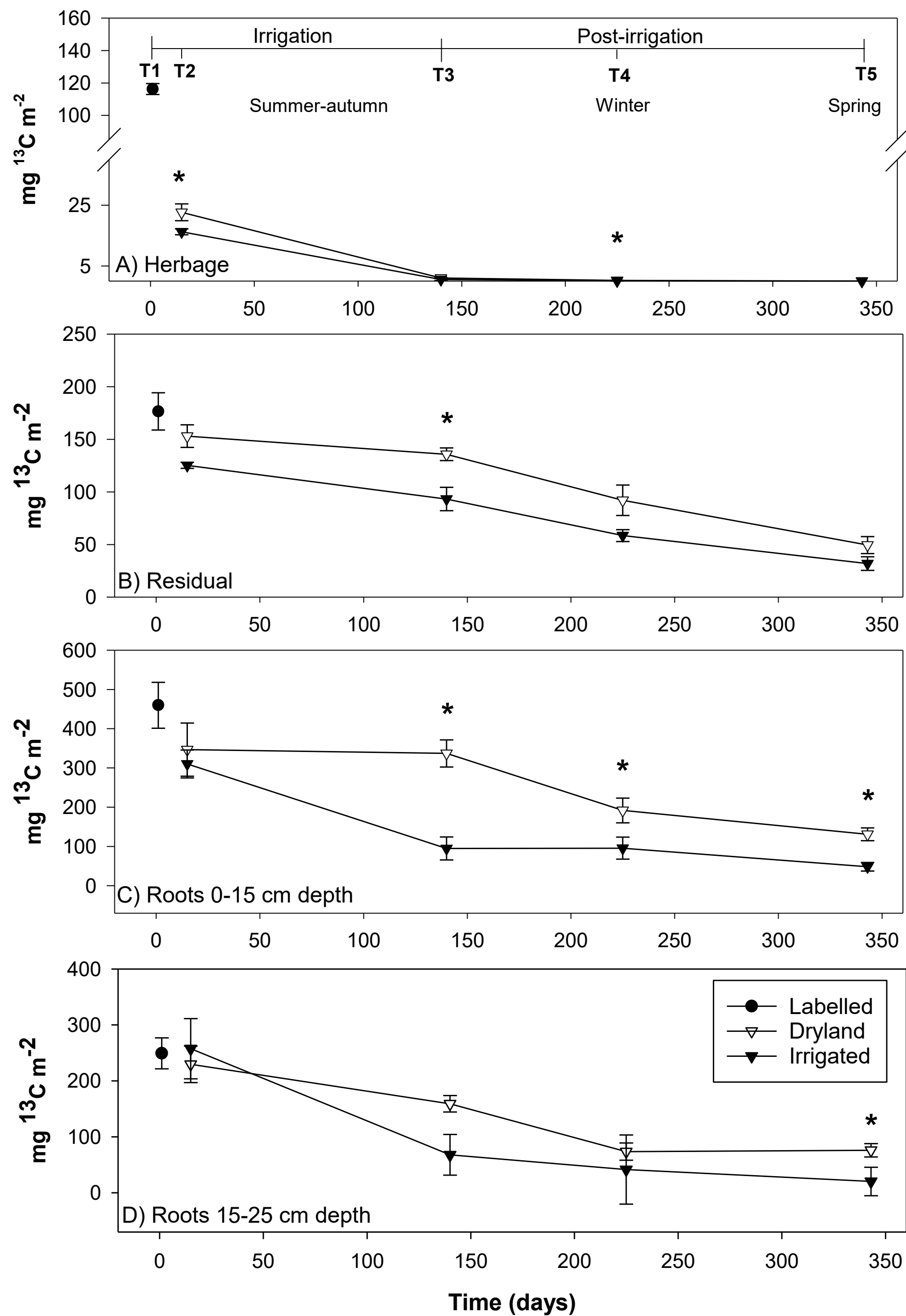

Figure 5.5. The ^{13}C recovery (mg ^{13}C m^{-2}) one day after the last $^{13}CO_2$ labelling event and at the beginning of the seasonal irrigation (sampling time T1), during the irrigation period at 15 and 140 days (sampling times T2 and T3), and the post-irrigation period at 225 and 343 days since the last ^{13}C labelling event (T4 and T5) for: A) Herbage, B) Residual (meristems and clover stolons), C) Roots in the 0–15 cm depth, and D) Roots in the 15–25 cm depth. Error bars represent 1 standard error of the mean and asterisk symbol (*) represents a significant difference between the dryland and irrigated treatments at $P < 0.05$. Note the break in the y-axis for ^{13}C recovery in herbage.

Recovery of ^{13}C in roots from the 15–25 cm depth at T1 was 249 mg $^{13}C\ m^{-2}$ (Figure 5.5D). By T2, ^{13}C recovery in these roots was similar to ^{13}C recovery at T1 (P < 0.001 and LSRtime < 1.91), with no difference between the treatments (P = 0.877 and LSR2–4 < 2.49). By T3, ^{13}C recovery in roots (15–25 cm depth) decreased in the irrigated treatment (P < 0.001 and LSRtime > 1.91), while in the dryland pasture ^{13}C recovery remained similar relative to T2 (P < 0.001 and LSRtime < 1.91). However, there were no significant treatment differences in ^{13}C recovery in roots (15–25 cm) at this time (P = 0.070, Figure 5.5D).

The ^{13}C recovery in roots (15–25 cm depth) under the dryland treatment at T4 had decreased relative to T3 (P < 0.001 and LSRtime > 1.91), but there was no treatment effect on the ^{13}C recovery from these deeper roots at T4 (P = 0.270, Figure 5.5D). The ^{13}C recovery in roots (15–25 cm) at T5 was lower in the irrigated compared with the dryland treatment (P < 0.001 and LSR5 > 1.91, Figure 5.5D), as a result of the decrease in ^{13}C recovery in these roots under the irrigated treatment (P < 0.001 and LSRtime > 1.91).

The ^{13}C recovery in soil components

At the start of irrigation (T1), ^{13}C recovered in rhizosphere soil from the 0–15 cm depth was 35.5 mg $^{13}C\ m^{-2}$ (Figure 5.6A). The ^{13}C recovered in the rhizosphere soil (0–15 cm) was similar between the dryland and irrigated treatments over the irrigation period between T2 (P = 0.891) and T3 (P = 0.355). There was some evidence (P = 0.070) that the ^{13}C recovered in rhizosphere soil (0–15 cm depth) of the dryland treatment increased in the post-irrigation period between T3 and T4. At T5, the ^{13}C recovery was similar between treatments T5 (P = 0.647).

The ^{13}C recovery in non-rhizosphere soil from the 0–15 cm depth at T1 was 122 mg $^{13}C\ m^{-2}$ (Figure 5.6B). By T2, after 15 days of irrigation, ^{13}C recovery in non-rhizosphere soil from the 0–15 cm depth did not vary relative to T1 (P < 0.001 and LSDtime < 33.2), and it was similar between the dryland and irrigated treatments (P = 0.619). At the end of the irrigation period (T3), ^{13}C recovery in non-rhizosphere soil (0–15 cm depth) had increased in the dryland treatment (P < 0.001 and LSD > 33.2) and it was also higher when compared with the irrigated treatment (P = 0.049 and LSD2–4 = 47).

In the post-irrigation period between T3 and T4, ^{13}C recovery in non-rhizosphere soil (0–15 cm depth) decreased in the dryland treatment (P < 0.001 and LSDtime > 33.2, Figure 5.6B), while in the irrigated pasture ^{13}C recovery remained similar to T1. However, the difference between the treatments was not statistically significant at T4 (P = 0.071). By T5, ^{13}C recovery in non-rhizosphere soil (0–15 cm depth) had decreased in the irrigated treatment (P < 0.001 and LSDtime > 33.2) and it

was similar to ^{13}C recovery in the dryland treatment (P = 0.612), which remained unchanged from T4.

When ^{13}C recoveries in rhizosphere and non-rhizosphere soils from the 0–15 cm depth were totalled (Figure 5.6C), no significant differences between the treatments were observed in this ^{13}C recovery during the irrigation and post-irrigation periods (P > 0.05). Moreover, the only significant change over time in this combined ^{13}C recovery from the 0–15 cm soil depth was observed in the dryland treatment between T3 and T4 (P = 0.006 and LSDtime > 43.9), where a decreased in ^{13}C recovery occurred. The ^{13}C recovery in whole soil (rhizosphere soil not separated from non-rhizosphere soil) from the 15–25 cm depth at T1 was 34.1 mg $^{13}C\ m^{-2}$ (Figure 5.6D). In contrast to the combined soil from the 0–15 cm depth, ^{13}C recovery in whole soil (15–25 cm depth) did not vary over the irrigation or post-irrigation periods (P = 0.326) with similar recoveries between the dryland and irrigated treatments (P > 0.05).

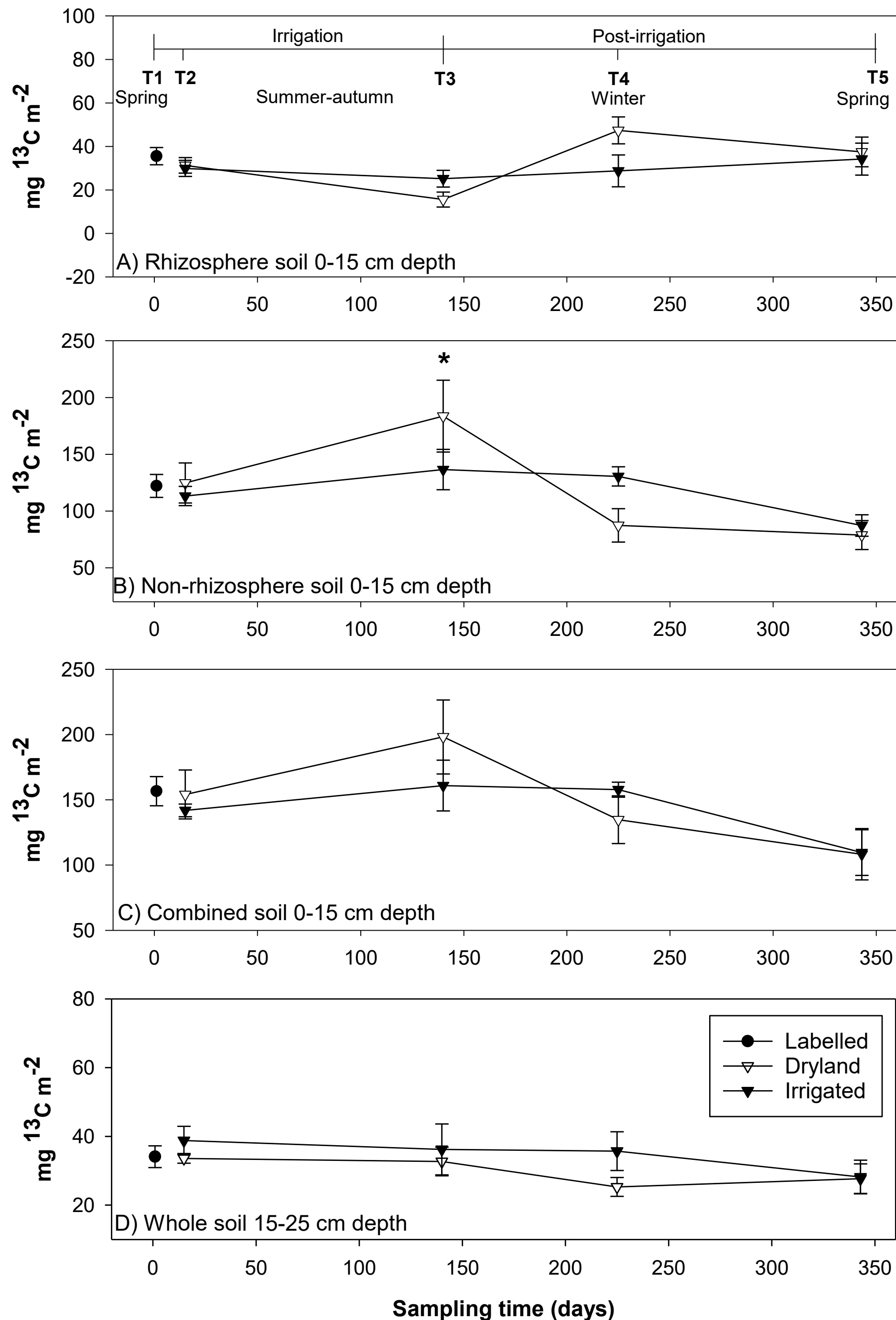

Figure 5.6. Quantity of ^{13}C recovered (mg ^{13}C m^{-2}) at sampling time T1 (1 days after the last $^{13}CO_2$ labelling event), during the irrigation period at T2 and T3 (15 and 140 days since the last labelling event), and the post-irrigation period at T4 and T5 (225 and 343 days since the last labelling event) for: A) Rhizosphere soil (0–15 cm depth), B) Non-rhizosphere soil (0–15 cm depth), C) Combined soil (0–15 cm depth), and D) Whole soil (15–25 cm depth). Error bars represent 1 standard error of the mean and asterisk symbol (*) represents significant difference between the dryland and irrigated pastures at $P < 0.05$.

The ^{13}C recovery in soil particle size fractions

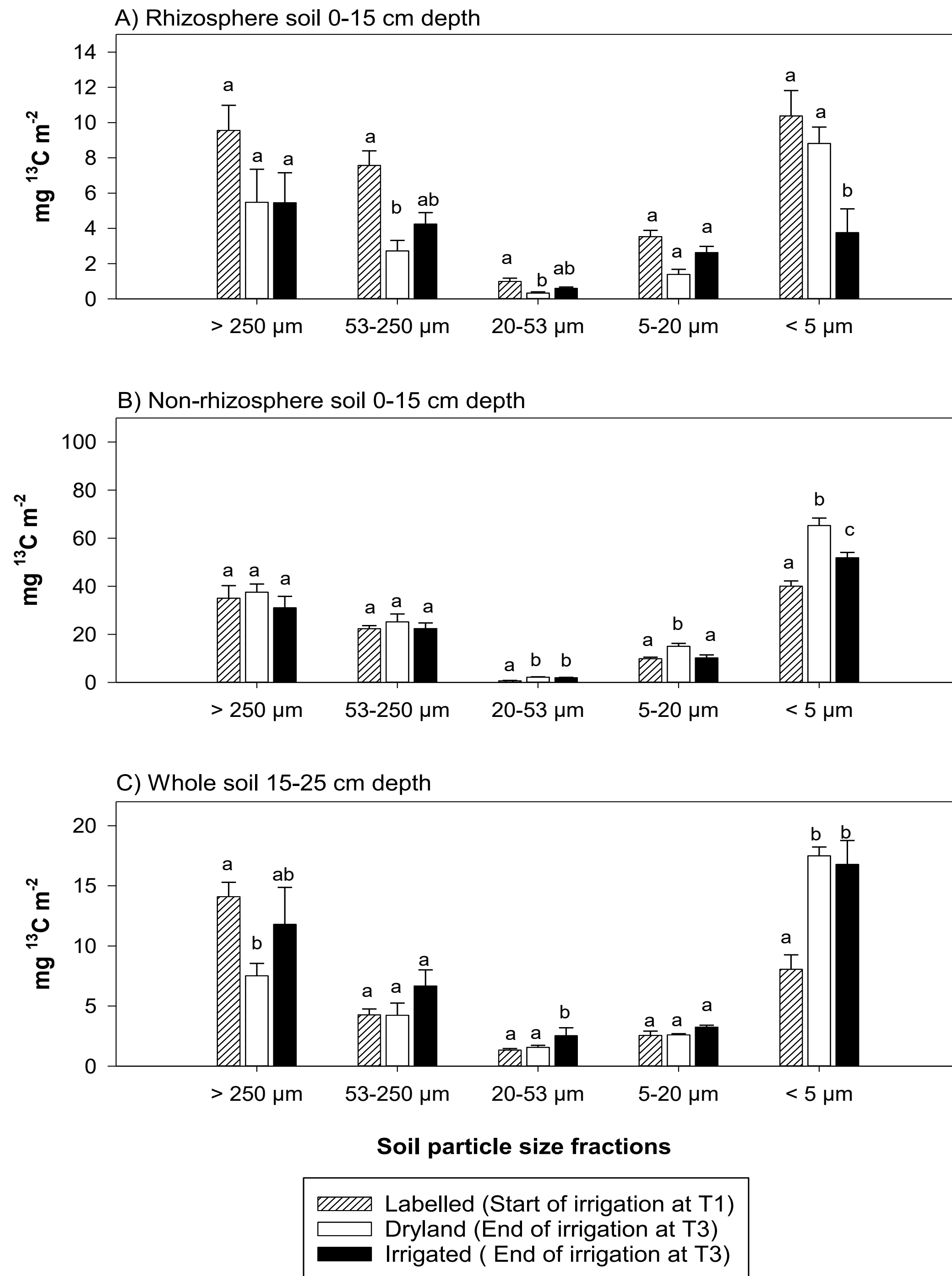

Figure 5.7. Quantity of ^{13}C recovered (mg ^{13}C m^{-2}) in soil particle size fractions for labelled mesocosms at 1 day after the last $^{13}CO_2$ labelling event and at the start of the irrigation period at sampling T1, and for the dryland and irrigated treatments at the end of irrigation after 140 days since the last ^{13}C labelling event at sampling time T3 from: A) Rhizosphere soil (0–15 cm depth), B) Non-rhizosphere soil (0–15 cm depth), and D) Whole soil (15–25 cm depth). Error bars represent 1 standard error of the mean. Letters on the top of the bars indicate significant or not significant differences between the labelled, dryland and irrigated treatments.

At the end of irrigation at T3 (140 days after the last labelling event), significant differences between the dryland and irrigated treatments in ^{13}C recovery among soil size fractions from rhizosphere soil (0–15 cm depth, Figure 5.7A) were only observed in the < 5 µm fraction (P = 0.029 and LSD > 3.51), with lower ^{13}C recovery in the irrigated treatment, while in the dryland treatment it remained similar to the quantity recovered at T1 (1 day after the last ^{13}C labelling event). Despite no significant differences in ^{13}C recoveries between the dryland and the irrigated treatments for the 53–250 µm and 20–53 µm fractions (fine POM and coarse silt, respectively), the ^{13}C recovery in the dryland treatment in these fractions at T3 had decreased when compared with the same fractions at T1 (P = 0.039 and LSD > 4.29 for 53–250 µm, and P = 0.008 and LSR > 2.09 for 20–53 µm, Figure 5.7A). The ^{13}C recovery in the > 250 µm and 5–20 µm fractions (coarse POM and fine silt) from rhizosphere soil (0–15 cm depth) did not show significant variations between the labelled mesocosms at T1 and the dryland and irrigated treatment at the end of the irrigation at T3 (P = 0.039 and LSD < 4.29 for > 250 µm, and P = 0.029 and LSD < 3.51 for the 5–20 µm, Figure 5.7A).

The ^{13}C recovery in soil fractions from the non-rhizosphere soil (0–15 cm depth, Figure 5.7B), differed between the dryland and irrigated treatments at T3 in the 5–20 µm and < 5 µm (clay) fractions (P <0.001 and LSD > 4.28 for both fractions), where both fractions had higher recoveries in the dryland treatment. However, the ^{13}C recovery in the < 5 µm fraction also increased in the irrigated treatment relative to T1 (Figure 5.7B). The ^{13}C recovery in the 20–53 µm fraction from non-rhizosphere soil (0–15 cm depth) also increased for the dryland and irrigated treatment relative to T1 (P < 0.001 and LSD > 0.53), but there were no difference between treatments at the end of the irrigation period (Figure 5.7B). In the > 250 µm and 53–250 µm fractions ^{13}C recovery from non-rhizosphere soil (0–15 cm depth) at T3 did not vary due to treatment from the ^{13}C recovery at T1(P = 0.681, Figure 5.7B).

Recovery of ^{13}C in soil fractions from whole soil (15–25 cm depth, Figure 5.7C), only showed a significant difference between the dryland and irrigated treatments at T3, in the 20–53 µm fraction (P = 0.031 and LSD > 0.98), as the^{13}C recovery in the 20–53 µm fraction was higher in the irrigated treatment, while in the dryland treatment it remained similar to T1. In the < 5 µm fraction ^{13}C recovery from whole soil (15–25 cm depth) was similar between the dryland and irrigated treatments at the end of irrigation at T3, but ^{13}C recovery increased relative to T1 (P = 0.002 and LSD > 5.13, Figure 5.7C). For the whole soil the ^{13}C recovery in the > 250 µm fraction decreased in the dryland treatment at T3 (P = 0.015 and LSD > 4.98), while in the irrigated treatment ^{13}C recovery was similar to T1. However, this difference between the dryland and the irrigated treatments was not statistically significant (P = 0.098, Figure 5.7C). At T3, the ^{13}C recovery in the 53–250 µm and 5–20

μm fractions from the whole soil (15–25 cm depth) did not vary relative to T1 (P = 0.375 for the 53–250 μm and P = 0.331 for the 5–20 μm, Figure 5.7C).

5.5 Discussion

5.5.1 Irrigation effects of pasture biomass allocation

Irrigation increased herbage dry matter production and lowered root biomass during summer-autumn irrigation period in the irrigated when compared with the dryland treatment (Figure 5.3 and Figure 5.4). These changes in pasture biomass allocation due to irrigation are consistent with the 'functional equilibrium' theory, which states that plants will shift their biomass allocation towards above- or below-ground organs as a strategy to improve the uptake of the limiting resources (e.g. light, nutrients and water) (Lambers, 1983; Poorter and Nagel, 2000; Körner, 2015).

Therefore, with water and nutrient supplied through irrigation and fertilisation, during the long summer days, plants would be expected to shift their biomass allocation towards above-ground components in order to enhance canopy structure and subsequently light interception (Wardlaw, 1990; Minchin et al., 1994). In contrast, when plant growth is limited by available soil water content, as in the dryland treatment, biomass allocation is focused on building a larger and/or deeper root system to improve water uptake (Poorter and Nagel, 2000; Sanaullah et al., 2012; Körner, 2015), and photosynthetic activity decreases due to stomata closure that consequently, reduces both evapotranspiration and CO_2 assimilation (Peñuelas et al., 2004; Zhou et al., 2019).

The observed differences in pasture biomass allocation, to above- and below-ground components as a result of irrigation are consistent with other studies in temperate pastures on a similar soil (i.e. Lismore stony silt loam), where irrigation significantly increased herbage production (Scott et al., 2012; Condron et al., 2014; Moinet et al., 2017; Carmona et al., 2020) while lowering root biomass (Stewart and Metherell, 1999; Scott et al., 2012; Carmona et al., 2020).

The results of the current study go further by demonstrating that the irrigation-induced shift in pasture biomass allocation (i.e. enhanced herbage dry matter production and lower root biomass), also persisted over the post-irrigation period: higher rates of above-ground plant biomass production persisted until the middle of the following winter (T4, Figure 5.3), with associated higher residual biomass and lower root biomass over the following spring period (T5, Figure 5.4). This appears to be the first study to report that summer irrigation can have persistent effects over an annual pasture growth cycle, post-irrigation.

A possible mechanism to explain this legacy effect of irrigation favouring above-ground biomass allocation could be associated with greater reserves of photosynthates (White, 1973) allowing higher herbage growth during winter, where low temperatures and irradiance limits the production of photosynthates (Saggar and Hedley, 2001; Dodd and Mackay, 2011). Alternatively, water limitations in the dryland treatment that reduced herbage production may have also limited the production and subsequent storage of photosynthates thus limiting the potential for pasture growth (Poorter and Nagel, 2000) during the post-irrigation period.

5.5.2 Irrigation effects on the persistence of photosynthate C within the plant-soil system

Above-ground plant components

Losses of C from a specific plant organ can occur via catabolic reactions (i.e. cell respiration), by the transfer of C to other plant organs (Jones et al., 2004; Meuriot et al., 2018) and/or by biomass removal (e.g. grazed pasture systems) (Skinner, 2008; Whitehead et al., 2018). The rapid loss of ^{13}C from herbage biomass in both the irrigated and dryland treatments over the irrigation period, and then its complete removal from the system over the post-irrigation period (Figure 5.5A), was undoubtedly due to biomass removal during the simulated grazing events. However, leaf respiration could have also contributed to the lower ^{13}C recoveries in herbage, especially during the winter season, where leaf respiration has been reported to dominate C losses in temperate pastures (Saggar and Hedley, 2001).

The lower mass of ^{13}C recovered in herbage in the irrigated (approximately 6% less), when compared with the dryland treatment at T2 (Figure 5.5A), can be explained by the higher growth rates of herbage in the irrigated treatment, that not only allowed the removal of herbage biomass more frequently, but also the dilution of the ^{13}C enrichment due to the inputs of 'fresh' photosynthate C that resulted in lower $\delta^{13}C$ values in the irrigated treatment relative to the dryland treatment over this period (Supplementary data Table 5.1). Higher growth rates of herbage biomass in the irrigated treatment until the middle of winter, in addition to respiration losses, could also explain the lower ^{13}C recovery for herbage in the irrigated treatment at T4, when compared with the dryland treatment (Figure 5.5A).

The lower ^{13}C recovery in residual biomass under the irrigated treatment (approximately 24% less) when compared with the dryland treatment at the end of the irrigation period, at T3 (Figure 5.5B), may have been the result of a greater mass of ^{13}C being reallocated to other plant organs, as leaf meristems, stems and clover stolons, which comprised the residual and which are regarded as sink

organs for carbohydrates reserves. These reserves are maintained for the purpose of restoring plant growth, especially leaf growth (Meuriot et al., 2018) after environmental perturbations that reduce photosynthesis activity (e.g. leaf defoliation or low soil water content) (Poorter and Nagel, 2000).

Hence, it is likely that in the irrigated treatment the reallocation of ^{13}C from the residual, to restore leaf growth after more frequent grazing events, may have played an important role in lowering the persistence of ^{13}C in the irrigated residual during the irrigation period. In contrast, plants in the dryland treatment, where low water supply affected the production of photosynthates, stored a greater mass of ^{13}C in the residual biomass until the alleviation of water deficit (Poorter and Nagel, 2000). This is supported by the observation that when this water deficit was alleviated in the autumn-winter period a reduction in the residual ^{13}C occurred in the dryland treatment over the following autumn-winter period (Figure 5.5B).

However, the fact that residual biomass was higher in the irrigated compared with the dryland treatment over the post-irrigation period (Figure 5.4A), indicates that a higher quantity of non-labelled or "fresh" photosynthate C was partitioned and stored in this plant component, diluting the ^{13}C previously allocated in the residual. Lower residual $\delta^{13}C$ values in the irrigated treatment over both, the irrigated and post-irrigation periods (Supplementary Table 5.1) not only supports this idea of a higher dilution of the residual ^{13}C in the irrigated relative to the dryland treatment, but also provide evidence that C partitioning to above-ground biomass was favoured as a legacy effect of the previous summer irrigation.

Below-ground plant and soil components

Root biomass is considered one of the main sources of soil C (Rasse et al., 2005; Kong and Six, 2010) that enhances nutrient cycling through the microbial decomposition of organic compounds deposited below-ground (Jones et al., 2004; Vidal et al., 2018; Gorka et al., 2019). This transfer of root derived-C to soil can occur through rhizodeposition from living roots (Jones et al., 2004; Pausch and Kuzyakov, 2018) and via root turnover, which is considered a pivotal process for enabling nutrient cycling and SOC storage in most terrestrial ecosystems (Gill and Jackson, 2000; Eissenstat and Yanai, 2002).

Summer irrigation applied to temperate managed pastures has been reported to increase root turnover when compared with an unirrigated pasture system (Scott et al., 2012), hence, the lower ^{13}C recovery in root biomass under the irrigated treatment at the end of irrigation, especially in the

0–15 cm depth, (Figure 5.5C) may have occurred due to higher losses of root derived-^{13}C as a consequence of faster root turnover.

Faster root turnover results in faster translocation of nutrients within the entire plant-soil system (Gill and Jackson, 2000) supporting the higher above-ground pasture productivity maintained over the summer period promoted through irrigation. It has been reported that in pastures the translocation of nutrients occurs primarily after the senescence of fine roots, which are considered an important nutrient source for pasture plants (Gordon and Jackson, 2000).

Therefore, further research on the combined effects of summer irrigation and nutrient management is needed to understand the causal mechanisms driving the faster root turnover under irrigated pasture systems. For example, Richardson et al. (2019) proposed a paradigm shift for nutrient management in agricultural systems to enable soil C storage suggesting that the fertilisation of the entire plant-soil system be considered rather than the crop only.

In addition to root ^{13}C losses being a consequence of faster root turnover, root respiration may have also favoured losses of root biomass ^{13}C in the irrigated treatment over the irrigation period, and this would have also occurred in the dryland treatment during the post-irrigation period (Figure 5.5C–D). Although root respiration was not measured in this study, Sanaullah et al. (2012) reported that under a soil water deficit (i.e. drought) root respiration was significantly reduced relative to optimum soil water conditions, hence, any dilution of root biomass ^{13}C as a consequence of root respiration may have occurred faster in the irrigated treatment when compared with the dryland treatment.

Moreover, Moinet et al. (2017) reported that under field conditions with a similar soil type to that used in this study (i.e. Lismore stony silt loam soil) and with a ryegrass-white clover dominant mixed pasture, irrigation resulted in higher total soil respiration (roots and soil) rates through spring and summer compared to an unirrigated pasture. In contrast, the reduction in root respiration in the dryland treatment may be explained by limitations to photosynthetic activity due to the associated water deficit that, in turn, resulted in lower production and partitioning of photosynthate C within the above- and below-ground plant components (Poorter and Nagel, 2000).

A lower photosynthate C production rate in the dryland treatment over the summer period would also decrease the transfer of root derived-C to the entire soil food web, as it has been reported that under drought conditions the flow of photosynthate C from plant to the soil food web is relative to optimum soil water conditions (Chomel et al., 2019). Such an effect would explain the higher

retention of ^{13}C in root biomass and non-rhizosphere soil in the 0–15 cm depth, in the dryland treatment at the end of the irrigation period (Figure 5.5C–D).

These findings infer that in an irrigated temperate pasture the losses of photosynthate C from roots and non-rhizosphere soil, in the upper soil depth (0–15 cm), occur during the summer-autumn period due to irrigation maintaining soil moisture, while in a dryland system these losses will occur later when the soil water deficit is alleviated by rainfall in the autumn-winter period. Hence, the current soil data suggest that the inputs and outputs of root derived-C to the soil are balanced resulting in a neutral effect of irrigation on short-term SOC storage between the irrigated and dryland pastures, at least in the first annual cycle of the conversion from dryland to irrigated management.

A neutral effect of irrigation on SOC stocks over an annual cycle under field conditions was reported by Moinet et al. (2017), where irrigation and nitrogen addition did not lead to a net increase nor decrease in SOC accumulation, despite increasing above-ground pasture productivity relative to a dryland pasture. Results of Moinet et al. (2017) support the current findings and those presented in Chapter 4, where over an annual cycle irrigation did not increase or decrease the content of newly formed SOC when compared with a dryland pasture system.

One year after ^{13}C was assimilated by the pasture there was no evidence that irrigation had altered the quantity of new C (^{13}C) recovered in the bulk soil, relative to the dryland system (Figure 5.6). However, more of the new C assimilated over the spring period was retained in root biomass of the dryland than the irrigated treatment one year after ^{13}C labelled ceased. In this regard, the root biomass results support our first hypothesis, which stated that irrigation would reduce the persistence of previously assimilated ^{13}C in the below-ground plant and soil components. However, it is not known to what extent the lower storage of new C in roots of irrigated pastures may contribute to lower storage of C in soils over multiple annual cycles of irrigation compared to dryland pastures.

Therefore, further research in to the observed reduction of the pasture root system (observed over an annual period in the current study) and associated root turnover, is still required to understand the long-term persistence of photosynthate C within the pasture soil, as it could result in lower inputs of root-derived C to the soil, which may explain the reduction of SOC stocks under long-term irrigated grazed pastures reported by Mudge et al. (2017) and Condron et al. (2014) in temperate climate conditions.

5.5.3 Irrigation effects on the short-term persistence of photosynthate C within soil particle size fractions

Summer irrigation reduced the recovery of ^{13}C in the < 5 µm (clay) size fraction of rhizosphere soil (0–15 cm depth) relative to the dryland treatment (Figure 5.7A), indicating that losses of the previously assimilated photosynthate C from this fraction were higher. Such losses of clay associated ^{13}C in the rhizosphere soil (0–15 cm depth) may have occurred through microbial respiration, as irrigation provides optimal soil water content for microbial activity over the drier and warmer summer period.

Vidal et al. (2018) observed *in situ* the transfer of photosynthate C from immature root cells (apoplastic pathway) to the microbial community, mainly bacteria that surrounded the peripheral root cells. Moinet et al. (2019) also observed that elevating soil water content, through irrigation, in a temperate grazed pasture increased soil respiration by enhancing root and rhizosphere soil respiration. This supports the possibility that enhanced respiration of root derived-^{13}C in the rhizosphere soil (0–15 cm depth) resulted in the higher losses observed from the clay ^{13}C soil fraction.

However, the transfer of ^{13}C from the rhizosphere clay fraction (0–15 cm) to the non-rhizosphere soil in the same or deeper soil depths (e.g. whole soil in the 15–25 cm depth) via DOC leaching, may have also contributed to the reduction of ^{13}C in the rhizosphere soil clay fraction, as C associated with the fine mineral soil size fractions (< 53 µm, MAOM) are primarily low molecular weight compounds (Grandy and Neff, 2008) derived directly from root exudation (Farrar et al., 2003; Jones et al., 2004) or from microbial activity (Kallenbach et al., 2016) that can be readily transferred in the soil as DOC (Ghani et al., 2007; Cotrufo et al., 2015).

The vertical transfer of ^{13}C as DOC from rhizosphere soil (0–15 cm depth) to the whole soil (15–25 cm depth) could, for example, explain the increase in the ^{13}C recoveries within the clay fraction of the 15–25 cm soil depth (Figure 5.7C). However, the increase and magnitude of ^{13}C recovery in the clay fraction was similar between the dryland and irrigated treatments (Figure 5.7C), and it does not explain the lower ^{13}C recovery in the clay fraction from the rhizosphere soil (0–15 cm depth) in the irrigated treatment, giving support to the idea that ^{13}C from the clay fraction was loss via microbial respiration (Figure 5.7A–B).

Moreover, ^{13}C recoveries from the non-rhizosphere soil (0–15 cm) and the whole soil (15–25 cm) at the end of the irrigation period did not increase in the irrigated treatment. In contrast, ^{13}C recovery in non-rhizosphere soil (0–15 cm depth) increased in the dryland treatment during this time (Figure

5.7B–D). Higher ^{13}C recovery in the fine silt (5–20 µm) and clay (< 5 µm) fractions of non-rhizosphere dryland soil compared with the irrigated treatment, is consistent with the increase in ^{13}C recovery from the non-rhizosphere soil in the dryland treatment at the cessation of irrigation (Figure 5.7B).

The absence of change or slight increase in the ^{13}C recovery within the rhizosphere MAOM fractions (fine silt and clay) in the dryland treatment at the end of the irrigation period (Figure 5.7A), and the fact that the total C content of these MAOM fractions was approximately 2-fold lower when compared with the irrigated treatment (Supplementary Table 5.4) indicates that the inputs of 'fresh' photosynthate C were higher in the irrigated treatment that subsequently enabled the production and transfer of photosynthate C to the soil food webs. This is consistent with previous reports of reduced photosynthate C inputs under dryland conditions (Chomel et al., 2019).

Such a reduction in the transfer of C to soil microorganisms in the dryland treatment may have allowed the storage of ^{13}C in the MAOM size fractions from the rhizosphere and non-rhizosphere soil in the 0–15 cm depth in this treatment. These findings are consistent with the previously observed higher recovery of recent photosynthate C in rhizosphere soil of dryland pasture compared to irrigated pasture in Chapter 3 and reported as Carmona et al. (2020).

Interestingly, in the irrigated treatment the low recovery of ^{13}C in the rhizosphere clay fraction observed over a relatively short time (140 days, Figure 5.7A, suggests that soil MAOM despite being regarded as a more 'stable' pool of SOM with longer residence times in the soil (Feller and Beare, 1997; Baldock and Skjemstad, 2000; Lützow et al., 2006; Sokol et al., 2019), is in fact a very dynamic SOM pool within the rhizosphere soil. This aligns well though with the nature of rhizosphere soil, considered one of the most important and highly dynamic soil microbial hotspots regulating SOC cycling, and hence, C flows in terrestrial ecosystems (Kuzyakov and Domanski, 2000; Pausch and Kuzyakov, 2018; Kuzyakov and Razavi, 2019).

This highlights the importance of the scale at which soil processes are measured and modelled (e.g. rhizosphere soil vs non-rhizosphere, bulk soil vs soil fractions) when assessing the impacts of agricultural and soil management practices on SOC-climate interactions, and the importance of managing C flows rather than C stocks (Lehmann and Kleber, 2015).

In contrast to the clay fraction in the rhizosphere soil (0–15 cm), irrigation maintained the short-term (140 days) storage of the ^{13}C previously partitioned in the 53–250 µm (fine POM) and 20–53 µm (coarse silt) fractions relative to the dryland treatment (Figure 5.7A). Irrigation has been reported to increase the OC stored in microaggregates (50–200 µm) and/or POM size fractions (53–250 µm)

relative to unirrigated crops (Gillabel et al., 2007; Apesteguía et al., 2015) or dryland pastures systems (Carmona et al., 2020).

In this study the ^{13}C dynamics within aggregates was not measured, but the maintenance of ^{13}C recovered in the POM fractions may have occurred by limiting the accessibility of decomposer to this ^{13}C through the occlusion into microaggregates (Six et al., 2002; Six et al., 2004; Dungait et al., 2012). Moreover, in the rhizosphere soil, Vidal et al. (2018) also observed complex interactions between roots, bacteria and fungi regulating the transfer and potential protection of photosynthate C in the soil, by binding photosynthate C with iron oxides with roots, bigger mineral particles (e.g. quartz) and surrounding bacteria in microaggregates, that were highly associated with fungal hyphae.

Thus, irrigation may have favoured biological, chemical and physical interactions that resulted in the maintenance of ^{13}C associated with the fine POM fraction in the rhizosphere soil, which also explains the greater persistence of ^{13}C in the coarse POM for the 'whole soil' in the 15–25 cm depth, where rhizosphere soil was not separated (Figure 5.7A).

However, these results do not support the second hypothesis, which stated that irrigation would enhance the decomposition of ^{13}C associated with the POM fractions while increasing the partitioning of this previously assimilated ^{13}C to the MAOM fractions, potentially via the microbial-DOC pathway of SOM formation from plant litter decomposition as proposed by Cotrufo et al. (2015).

Plant litter and POM decomposition represent an important source of OC for soil decomposer communities, that in turn also facilitate nutrient cycling within the entire plant-soil system (Bardgett et al., 1998). However, the quality of plant litter and POM, especially the N content, is considered one of the main factors controlling their decomposition (Bardgett et al., 1998; Moorhead and Sinsabaugh, 2006; Cotrufo et al., 2013; Lavallee et al., 2020), due to the stoichiometric requirements for microbial growth and SOM formation (Hessen et al., 2004; Chen et al., 2014).

As discussed previously, summer irrigation of temperate ryegrass-white clover pastures on stony silt loam soils, similar to this study, has been reported to increase root turnover (Scott et al., 2012), soil respiration (Moinet et al., 2017), and the abundance of earthworms and herbivore soil mesofauna (Fraser et al., 2012). These findings indicate that summer irrigation may have favoured the formation and short-term (< 1 year) storage of the POM fractions by increasing root turnover in order to meet the stoichiometric C and N ratio for soil microbial growth, especially when considering a high nutrient demand system such as a summer irrigated pasture. Summer irrigation increases above-

ground productivity and the uptake of nutrients as N (Moinet et al., 2017) and phosphorus (P), which cycle closely within SOM under temperate pastures (Rumpel et al., 2015).

This study provides evidence that summer irrigation can increase the turnover of root derived-C as well as the formation and turnover of C associated with POM and MAOM fractions compared to dryland management. These findings demonstrate the value of combining ^{13}C labelling with SOM fractionation to understand the effects of agricultural management practices on SOC storage (Poeplau et al., 2018; Cotrufo et al., 2019; Lavallee et al., 2020).

5.6 Conclusions

Although the fact that irrigation reduced the short-term persistence (increased the turnover) of ^{13}C previously assimilated in the above-ground (herbage) and below-ground (roots) plant biomass, it did not affect the storage of ^{13}C in the soil after 334 days when compared to the dryland pasture. However, the dryland pasture soil retained more of the previously assimilated ^{13}C in root biomass and MAOM fraction compared to the irrigated soil. This study provides evidence that summer irrigation can increase the turnover of C in roots and MAOM and this may have important implications for the longer-term SOC storage under intensified pastoral production systems.

5.7 Acknowledgements

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5.8 Supplementary data

5.8.1 Isotopic composition of plant and soil components after the $^{13}CO_2$ labelling period

Table 5.1. The mean of $\delta^{13}C$ (‰) values for plant and soil components at natural abundance (NA), at 1 day after the last $^{13}CO_2$ labelling event at sampling time T1; for the dryland and irrigated treatments over the irrigation (T2–T3) and post-irrigation (T4–T5) periods. The least significant difference (LSD) of means with significance level of 5% compares means between treatments at each sampling time is provided when P < 0.05. Values in bold represent significant differences between the dryland and irrigated treatments at each sampling time.

Isotopic composition $\delta^{13}C$(‰)																
Component	**Depth (cm)**	**NA**	**Treatment**	**Sampling times (days)**					**P-value**				**LSD-value**			
				1	15	140	225	343								
				T1	T2	T3	T4	T5	T2	T3	T4	T5	T2	T3	T4	T5
			Dryland		**112.1**	**-25.0**	-29.7	-30.1								
Herbage		-30.6	Irrigated	381.5	**40.7**	**-28.6**	-30.1	-30.1	<0.001	0.035	0.952	0.932	25.3	3.11		
			Dryland		**223.2**	**120.2**	**91.2**	**16.5**								
Residual		-30.8	Irrigated	260.6	**203.2**	**62.3**	**24.7**	**-6.7**	<0.001	<0.001	0.006	0.040	17.7	10.9	30.3	21.9
			Dryland		195.3	**117.4**	**77.6**	**15.1**								
	0–15	-30.5	Irrigated	201.2	175.4	**29.9**	**20.5**	**-8.0**	0.249	<0.001	0.011	<0.001		23.6	32.3	11.2
			Dryland		217.9	**114.4**	103.5	**22.9**								
Roots	15–25	-30.0	Irrigated	264.1	263.9	**46.9**	46.3	**-5.2**	0.256	0.016	0.079	<0.001		43.2		13.1
			Dryland		-8.97	-11.9	-13.6	-18.6								
Rhizosphere soil	0–15	-27.4	Irrigated	-9.48	-12.4	-15.7	-13.7	-20.2	0.248	0.064	0.885	0.474				
Non-rhizosphere			Dryland		-24.3	-22.7	-25.0	-25.4								
soil	0–15	-27.4	Irrigated	-24.1	-24.3	-23.7	-24.0	-25.4	0.923	0.346	0.721	0.138				
			Dryland		-23.7	-22.5	-23.8	-24.8								
Combined soil	0–15	-27.4	Irrigated	-23.4	-23.7	-23.2	-23.5	-24.8	0.743	0.433	0.549	0.945				
			Dryland		-25.9	-25.9	**-26.3**	-26.4								
Whole soil	15–25	-27.4	Irrigated	-25.9	-25.6	-25.9	**-25.8**	-26.4	0.588	0.419	0.039	0.952			0.51	

5.8.2 Mass of C of plant and soil components

Table 5.2. The mean values of total C mass (g of C) for plant and soil components at 1 day after the last $^{13}CO_2$ labelling event at sampling time T1, and for the dryland and irrigated treatments over the irrigation (T2–T3) and post-irrigation irrigation (T4–T5) periods. The least significant difference (LSD) of means with significance level of 5% compares means between treatments at each sampling time is provided when P < 0.05. Values in bold represent significant differences between the dryland and irrigated treatments at each sampling time.

Mass of C (g)															
Component	**Depth (cm)**	**Treatment**	**Sampling times (days)**												
			1	12	140	225	343	**P-value**				**LSD-value**			
			T1	T2	T3	T4	T5	T2	T3	T4	T5	T2	T3	T4	T5
		Dryland		**0.26**	0.30	**0.45**	0.46								
Herbage		Irrigated	0.46	**0.37**	0.37	**0.38**	0.46	< 0.001	0.469	0.045	0.593	0.04		0.06	
		Dryland		0.98	1.46	**1.20**	**1.78**								
Residual		Irrigated	0.98	0.87	1.66	**1.70**	**2.21**	0.507	0.499	0.007	0.001			0.35	0.24
		Dryland		2.46	**3.71**	2.92	**4.76**								
	0–15	Irrigated	3.20	2.44	**2.46**	2.90	**3.74**	0.145	0.009	0.965	0.003		0.92		0.65
		Dryland		1.52	1.82	0.88	**2.34**								
Roots	15–25	Irrigated	1.36	1.38	1.68	1.16	**1.74**	0.674	0.684	0.398	0.014				0.47
		Dryland		2.84	**1.68**	**5.51**	6.72								
Rhizosphere soil	0–15	Irrigated	3.25	3.26	**3.45**	**3.30**	7.62	0.737	0.009	0.002	0.474		1.27	1.27	
		Dryland		62.6	63.3	58.1	55.4								
Non-rhizosphere soil	0–15	Irrigated	59.8	57.9	58.5	61.1	53.6	0.061	0.058	0.226	0.305				
		Dryland		65.4	64.9	63.6	62.1								
Combined soil	0–15	Irrigated	63.0	61.2	61.9	64.4	61.2	0.067	0.196	0.705	0.599				
		Dryland		34.9	34.2	36.6	34.4								
Whole soil	15–25	Irrigated	35.4	36.2	38.1	34.2	34.4	0.677	0.325	0.209	0.990				

5.8.3 Mass of C in soil size particle size fractions

Table 5.3. The mean of $\delta^{13}C$ (‰) values for soil particle size fractions for rhizosphere soil (0–15 cm depth), non-rhizosphere soil (0–15 cm) and the whole soil (15–25 cm) at natural abundance (NA), at 1 day after the last $^{13}CO_2$ labelling event at sampling time T1 (labelled) and, for the dryland and irrigated treatments at the end of the irrigation period at T3. The least significant difference (LSD) of means with significance level of 5% compares means between the labelled, dryland and irrigated treatments is provided when P < 0.05.

Isotopic composition $\delta^{13}C$(‰)							
Soil component	**Fraction (µm)**	**Sampling time**				**P-value**	**LSD-value**
		T0	T1	T3			
		NA	Labelled	Dryland	Irrigated		
Rhizosphere soil (0–15 cm depth)	>250	-29.0	41.9	37.9	20.0	0.042	16.6
	53–250	-29.2	8.64	2.61	-4.29	0.319	
	20–53	-29.6	-17.7	-21.6	-21.6	0.360	
	5–20	-28.1	-19.9	-21.2	-21.5	0.137	
	< 5	-26.3	-16.7	-18.5	-18.4	0.160	
Non-rhizosphere soil (0–15 cm depth)	>250	-27.9	-9.42	5.58	-7.61	0.222	
	53–250	-28.6	-22.3	-21.0	-19.8	0.050	2.34
	20–53	-27.2	-26.9	-26.2	-26.2	<0.001	0.38
	5–20	-28.1	-26.8	-26.4	-26.7	0.002	0.19
	< 5	-27.1	-25.1	-23.9	-24.5	<0.001	0.33
Whole soil (15–25 cm depth)	>250	-28.5	-15.9	-20.5	-16.8	0.300	
	53–250	-28.6	-26.5	-26.4	-26.6	0.023	1.51
	20–53	-28.8	-27.6	-27.5	-26.3	0.001	0.64
	5–20	-27.9	-27.4	-27.4	-27.3	0.270	
	< 5	-27.1	-26.4	-25.5	-25.8	<0.001	0.41

5.8.4 Soil size fractions

Table 5.4. The mean values of total C mass (g of C) for soil particle size fractions for rhizosphere soil (0–15 cm depth), non-rhizosphere soil (0–15 cm) and the whole soil (15–25 cm) at 1 day after the last $^{13}CO_2$ labelling event at sampling time T1 (labelled) and, for the dryland and irrigated treatments at the end of the irrigation period at T3. The least significant difference (LSD) of means with significance level of 5% compares means between the labelled, dryland and irrigated treatments is provided when P < 0.05.

Soil component	Fraction (µm)	Sampling time			P-value	LSD-value
		T1	T3			
		Labelled	Dryland	Irrigated		
Rhizosphere soil (0–15 cm depth)	>250	0.22	0.13	0.18	0.265	
	53–250	0.33	0.15	0.28	0.014	0.12
	20–53	0.19	0.07	0.13	0.024	0.09
	5–20	0.73	0.35	0.67	0.025	0.30
	< 5	1.76	0.80	1.81	0.024	0.80
Non-rhizosphere soil (0–15 cm depth)	>250	2.99	3.22	2.49	0.575	
	53–250	5.73	5.36	4.08	< 0.001	0.40
	20–53	3.13	3.56	3.11	0.127	
	5–20	13.5	14.0	11.5	< 0.001	0.79
	< 5	32.9	32.7	32.0	0.072	
Whole soil (15–25 cm depth)	>250	1.90	1.56	1.67	0.378	
	53–250	3.37	3.05	2.68	0.103	
	20–53	1.92	1.99	1.61	0.116	
	5–20	8.07	7.66	7.74	0.612	
	< 5	18.7	18.2	20.9	0.087	

Chapter 6

Summary, conclusions and recommendations for future research

6.1 General overview

There is a general consensus that identifying agricultural management practices that can balance the trade-off between the demand to increase food production with the need to maintain, or ideally increase, SOC storage is essential. Increasing SOC stocks is not only vital for mitigating global climate change but also to guarantee food security and other important soil ecological functions (Lal, 2004a; Lal, 2016). The use of seasonal irrigation to enhance above-ground pasture productivity in temperate regions such as New Zealand is increasing. However, the specific impact of irrigation and the causal mechanisms altering SOC storage under these managed pastures are not well understood (Whitehead et al., 2018). There have been contradictory responses of SOC stocks to irrigation that have been reported: these include increases (Kelliher et al., 2015), no differences (Condron et al., 2014; Hunt et al., 2016; Moinet et al., 2017), and reductions (Condron et al., 2014; Mudge et al., 2017) in SOC when compared with dryland pasture management.

This PhD study aimed to measure the effects of summer irrigation on the net assimilation, partitioning and short-term persistence of photosynthate C within the entire pasture plant-soil system, in order to gain a better understanding of the mechanisms driving the changes in SOC stocks under irrigated pastures.

The specific objectives of this research study were:

1. To quantify the effects of seasonal summer irrigation versus dryland management on the net assimilation and partitioning of photosynthate C in the above- and below-ground components of the plant-soil system, including soil particle size fractions (Chapter 3).

2. To quantify the short-term persistence (over an annual pasture growth cycle) and re-distribution of photosynthate C between above- and below-ground C components after a summer irrigation cycle where pasture had been ^{13}C labelled under irrigation or non-irrigated conditions prior to the start of the annual cycle (Chapter 4).

3. To quantify the effects of irrigation on the short-term persistence of photosynthate C previously assimilated and partitioned within the plant-soil system, when pasture had been

^{13}C labelled under similar soil moisture conditions, prior to the start of a summer irrigation cycle (Chapter 5).

To address these specific objectives two continuous $^{13}CO_2$ pulse labelling experiments were performed using ^{13}C-labelled mesocosms established with a ryegrass (*Lolium perenne* L.) and white clover (*Trifolium repens* L.) mixed pasture. Mesocosms were packed with a shallow Lismore stony silt loam soil. Ryegrass-white clover mixed pastures are the predominant species composition of the grazed pastures in New Zealand (Dodd and Mackay, 2011). Lismore stony soils are present in areas of New Zealand (e.g. Canterbury) were summer irrigation of pastures has been steadily increasing for the last two decades (Ministry for the Environment & Stats NZ, 2019a). These soils tend to require summer irrigation to be managed as highly productive pasture systems.

Section 6.2 summarises the key results of this thesis, while Section 6.3 provides the main conclusions of this study, where after Section 6.4 reflects on how the results obtained from this PhD research have contributed to an increased understanding of the effects of summer irrigation on SOC storage under temperate managed pastures. Finally, Section 6.5 presents recommendations for future research.

6.2 Summary of key results

6.2.1 Chapter 3: Seasonal irrigation affects the partitioning of new photosynthate carbon in soil

Chapter 3 addressed the first specific objective of this PhD research, which was to quantify the effects of irrigation versus dryland management on the net assimilation and partitioning of new photosynthetically derived-C in the above- and below-ground components of the plant-soil system, including soil particle size fractions. This chapter focused specifically on results from the first destructive sampling (T1) of ^{13}C-labelled mesocosms in the first $^{13}CO_2$ labelling experiment, after ryegrass-white clover pasture mesocosms had been $^{13}CO_2$ pulse labelled daily for three months over the summer-early autumn period, under either simulated irrigation or dryland soil water conditions. The key results included in this chapter were:

At the end of the ^{13}C labelling period under the irrigated and dryland treatments it was observed that, compared with dryland conditions:

1. Irrigation increased the partitioning of new photosynthate C (^{13}C) to above-ground leaf biomass but reduced the partitioning of C to root biomass in the deeper soil layer (15–25 cm depth).

2. Irrigation reduced the accumulation of new photosynthate C (^{13}C) in rhizosphere soil.

3. Irrigation did not increase or decrease the net allocation of new photosynthate C (^{13}C) to the soil, despite an increase in herbage dry matter production and a reduced root biomass.

4. Irrigation increased the mass of new photosynthate C (^{13}C) in the 53–250 µm (fine POM) and < 5 µm (clay) soil particle size fractions.

6.2.2 Chapter 4: Fate of carbon fixed and partitioned to plant and soil fractions during summer irrigation over an annual pasture growth cycle

Chapter 4 addressed the second specific objective of this PhD thesis that was to quantify the short-term persistence and re-distribution of photosynthate derived-^{13}C between above- and below-ground C components, following a summer irrigation cycle over an annual pasture growth cycle. Chapter 4 focussed on studying the fate of photosynthate derived-^{13}C that had been previously assimilated and partitioned to plant and soil components during the summer irrigation period. The plant and soil ^{13}C was traced over 349 days using data from all five destructive sampling times (T1–T5) on days 1, 12, 125, 234 and 349 post labelling, with all mesocosms after sampling time (T1) maintained under uniform seasonal soil moisture contents. The key results reported in this chapter were:

1. After 349 days (T5), approximately 50% of the initial ^{13}C mass recovered in root biomass and rhizosphere soil in the 0–15 cm soil depth remained, with no differences between the irrigated and dryland treatments.

2. After 349 days (T5), approximately 100% of the initial ^{13}C mass recovered at 1 day (T1) in non-rhizosphere soil (0–15 cm depth) and whole soil (15–25 cm depth) remained, and again there were no differences between the irrigated and dryland treatments.

3. There were differences in the temporal re-distribution of ^{13}C due to summer irrigation within root biomass and rhizosphere soil (0–15 cm depth), the POM (> 53 µm) and clay (< 5 µm) size fractions of non-rhizosphere soil (0–15 cm depth) and whole soil (15–25 cm depth) over the following autumn-winter period.

4. After 349 days (T5), contributions of ^{13}C to the 53–250 µm fine POM fraction continued to be higher in the whole soil in the 15–25 cm depth under irrigation when compared with the dryland treatment.

6.2.3 Chapter 5: Summer irrigation enhances the turnover of root and mineral-associated organic carbon under ryegrass-white clover pasture

Chapter 5 addressed the third specific objective of this PhD thesis that was to quantify the effects of summer irrigation or dryland management on the short-term (343 days) persistence and partitioning within the plant-soil system of photosynthate derived-^{13}C, previously assimilated prior to summer irrigation, over the irrigation and post-irrigation periods. Chapter 5 presented changes in ^{13}C recoveries within the plant-soil system from the second continuous $^{13}CO_2$ pulse labelling experiment where ryegrass-white clover pasture mesocosms were ^{13}C-labelled under the same soil water content during spring-like conditions. Then, summer irrigation was imposed one day after ^{13}C labelling ceased. The fate of ^{13}C was traced over 343 days using data from five destructive sampling times (T1–T5) on days 1, 15, 140, 225 and 343, respectively, as follows: labelling period (spring), irrigation period (summer-earlier autumn) and post-irrigation period (late autumn, winter and spring). Therefore, Chapter 5 examined the effect of summer irrigation on the turnover of ^{13}C as irrigation was imposed after the $^{13}CO_2$ labelling. Whereas, Chapters 3 and 4 examined the effect of irrigation on the net assimilation and partitioning of ^{13}C as a result of the irrigation cycle applied during the $^{13}CO_2$ labelling.

The key results reported in this chapter were:

During the irrigation and post-irrigation periods it was observed that, compared with dryland conditions:

1. Summer irrigation changed the allocation of pasture biomass towards above-ground plant components resulting in lower allocation to root biomass at the end of the irrigation period. This effect of summer irrigation on reducing the pasture root system persisted until the following spring.

2. Irrigation increased the turnover of ^{13}C previously assimilated in root biomass over both, the irrigation and post-irrigation periods, especially in the 0–15 cm soil depth, with root biomass ^{13}C recoveries lower by 70 and 60% in the irrigated treatment at the end of the irrigation (140 days) and post-irrigation (343 days) periods, respectively.

3. Irrigation did not affect the short-term (343 days) storage of ^{13}C in the soil, but did change the temporal partitioning of ^{13}C within soil particle size fractions of rhizosphere and non-rhizosphere soil in the 0–15 cm depth and within the whole soil in the 15–25 cm depth.

4. These differences in the temporal partitioning within soil size fractions were driven mainly by irrigation favouring the contribution of ^{13}C to the > 250 µm (coarse POM) and 53–250 µm (fine POM) soil particle size fractions, while increasing the turnover of ^{13}C partitioned previously in the clay (< 5 µm) fraction.

6.3 Conclusions

This research demonstrated that summer irrigation applied to ryegrass-white clover pastures despite increasing above-ground pasture productivity, had no effect on the net mass of photosynthate C in the soil at the end of the irrigation cycle, or in the short-term (< 1 year), when compared with a dryland pasture system.

However, irrigation did affect the partitioning of photosynthate derived-^{13}C in soil relative to the dryland pasture system. Summer irrigation favoured the accumulation of ^{13}C into the fine POM (53–250 µm) fraction and enhanced the inputs and outputs of ^{13}C from the clay (MAOM < 5 µm) fraction. The ^{13}C of the POM fraction is most likely to have been derived from root turnover and the ^{13}C of the < 5 µm fraction could be derived from the adsorption of root exudates and microbial derived-^{13}C.

The re-distribution of ^{13}C within POM and MAOM fractions occurred earlier in the irrigated treatment over the summer and autumn seasons, while in the dryland treatment the re-distribution of ^{13}C occurred over the following autumn, winter and spring seasons. These results provide evidence for faster turnover and decomposition of root and mineral-associated OC under irrigated pastures.

Further evidence supporting the finding of faster root C turnover due to summer irrigation was the result showing the irrigated pasture root system reduced in mass and depth when compared with the dryland pasture, as the irrigated pasture allocated more resources to above-ground biomass. Results from this study also showed that this effect of irrigation favouring above-ground pasture productivity can persist over an annual pasture growth cycle, especially during the autumn and spring seasons following the cessation of summer irrigation.

These findings challenge the predictions made for some SOC simulation models that assume that increasing above-ground productivity through irrigation will increase SOC storage. These results also point towards testing and validate the modelling assumptions regarding MAOM as a 'stable' SOC cycling pool, with longer residence times in the soil relative to POM. Therefore, the data provided in this study may be used to inform SOC models in order to improve the predictions of SOC under temperate managed pastures.

6.4 Reflection on the current work

The literature review in Chapter 2 identified that a better understanding of the impacts of irrigation on SOC storage under temperate managed pastures is needed, and that there is limited data available on how irrigation affects the mechanisms controlling the balance between inputs and outputs of OC from the soil. Accordingly, this PhD research program was focused on measuring the effects of irrigation on the net assimilation, partitioning and short-term persistence of photosynthate C within the entire plant-soil system.

Results from Chapter 3 showed that at the end of a summer irrigation period there were differences in the partitioning of photosynthate derived-^{13}C within the above- and below-ground plant components, and within the different soil bulk components (e.g. rhizosphere and non-rhizosphere soil) and particle soil size fractions, as a result of higher soil water content (irrigated treatment) when compared with a lower soil water content (dryland treatment). However, the partitioning of photosynthate derived-^{13}C in each plant and soil C component was calculated in terms of the total mass of ^{13}C recovered within the entire plant-soil system, without considering losses of ^{13}C through plant (autotrophic) and soil (heterotrophic) respiration. This implied that the mass of ^{13}C recovered within each component of the plant-soil system under both, the irrigated and dryland treatments was overestimated.

This approach based on the net assimilation of photosynthate C instead of the gross assimilation does not consider that photosynthesis and respiration rates may have been different between the irrigated and dryland treatments, as these two processes involved in the transfer of C through the atmosphere-plant-soil system are strongly influenced by soil water content (Peñuelas et al., 2004; Brown et al., 2009; Joseph et al., 2014; Zhou et al., 2016).

Joseph et al. (2014) reported that the photosynthetic capacity of browntop grass (*Agrostis capillaris* L.) declined significantly when soil volumetric water content fell below 20%. Thus, given that ryegrass and white clover are C3-photosynthesis pathway plants as is the browntop grass, it would be expected that the photosynthetic capacity of the ryegrass-white clover pasture may have behaved in a similar way under the dryland treatments (Chapters 3 and 5), as soil volumetric water contents consistently fell below 20%.

However, soil water deficit also decreases plant and soil respiration. Root respiration has been reported to be more sensitive to water stress than soil microbial respiration (Hunt et al., 2004; Curtin et al., 2012; Sanaullah et al., 2012). Moinet et al. (2019) reported that soil respiration was positively correlated with soil water content and it was dominated primarily by the autotrophic

component (root respiration). It remains unknown to what extent the soil water contents in the dryland treatments may have limited plant and soil respiration relative to the irrigated treatments.

Plant and soil respiration were not measured in this study due to logistical constraints. These respiration measurements would have provided data to quantify a mass balance between the effects of summer irrigation on the inputs (photosynthesis) and outputs (respiration) of photosynthate C from the soil. However, results from this study inform about the net effect of summer irrigation in terms of the accumulation of newly formed SOC over the irrigation period (Chapter 3) and its short-term (< 1 year) persistence and turnover following the cessation of irrigation (Chapter 4), and when summer irrigation was applied (Chapter 5). These results may be important to determining the net storage of SOC under irrigated pastures in the longer-term.

Another important assumption considered in this study was that losses of photosynthate C via DOC did not occur. The irrigated treatments applied in Chapter 3 and Chapter 5, and soil water contents over the following autumn and spring periods were managed to avoid drainage. However, considering the low water storage capacity and high permeability of the shallow stony soil used, losses of OC via DOC would have occurred if the soil water content was equal or higher than soil water content at soil field capacity, similar to losses of N via leaching, especially over the winter period that are well documented from these stony soils (Carrick et al., 2013; Cichota et al., 2016). The range of soil water content managed for the irrigated treatments in this study provides evidence that the inputs of water through irrigation can be optimised to increase above-ground pasture productivity while avoiding losses of nutrients from the soil and maintaining SOC storage under managed pastures.

The nutrient management in terms of N fertilisation applied to both the dryland and irrigated treatments was 200 kg N ha^{-1} per year (split in 4 single applications of 50 kg N ha^{-1}), which is a common rate use for dairy pastures in New Zealand (Dairy NZ, 2012). However, this range of N input is higher relative to the annual average range for New Zealand's pastures of 15–110 kg N ha^{-1} reported by Monaghan et al. (2007). The C:N ratio is known to affect SOC storage (Cotrufo et al., 2019) as the formation and accumulation of SOM from the microbial decomposition of OC requires that the microbial nutrient demands are met (Hessen et al., 2004; Chen et al., 2014), especially the stoichiometric ratios between C, N and P in pastures systems (Rumpel et al., 2015). In this context, the relatively high inputs of N applied in this study allowed to measure the effects of irrigation on the accumulation and short-term persistence of the newly formed SOC under both treatments avoiding N being a limiting factor. Therefore, when studying the effects of irrigation on SOC it is

crucial to understand the synergistic effects of irrigation and nutrient status on C transfer within the entire plant-soil system.

This research has identified two key mechanisms that may be driving the different responses of SOC under temperate managed pastures to summer irrigation:

1. Irrigation promotes faster turnover of root derived-C that results in a reduced root system relative to dryland pastures supporting the findings reported by (Scott et al., 2012). Therefore, summer irrigation affects the dynamics of the root C pool under pastures that is considered the main OC input to the soil (Rasse et al., 2005; Kong and Six, 2010) and has direct effects on SOC functioning and storage.
2. Irrigation favours the formation and storage of the fine POM (53–250 μm) fraction while increasing the turnover of the MAOM size fraction relative to dryland systems. Therefore, soils under irrigated pastures are biologically more active. This could explain greater storage of SOC under dryland compared with irrigated pasture systems in the longer-term. POM fractions are pivotal for the short-term SOC storage (Kong and Six, 2010; Cotrufo et al., 2019) and are very sensitive to microbial decomposition when soil physical disturbances occur (Cotrufo et al., 2019; Lavallee et al., 2020).

6.5 Recommendations for future research

The work in this PhD program raises further questions that need to be addressed in future research to improve our understanding on the mechanisms driving the different responses of SOC to irrigation, for example:

1. What are the causal mechanisms driving the faster root turnover under irrigated pastures?

2. Does the faster root turnover and lower root biomass result in lower root derived-C inputs to the soil over the long-term (several irrigation cycles)? If yes, this could explain the reduced SOC stocks observed under irrigated pastures relative to dryland (rainfed) pastures reported by Condron et al. (2014) and Mudge et al. (2017).

3. In the present work irrigation reduced root biomass (higher root turnover) of ryegrass and white clover species. Do similar effects occur under more diverse pastures?

4. The effects of irrigation were observed using ryegrass and white clover species but only one soil type was used (Lismore stony silt loam soil). Understanding if the effects would be consistent across soils with different textures and mineralogy would also assist our

understanding of C cycling, losses and gains, and SOC storage in soils. Are the observed effects consistent across soil types?

5. How significant is nutrient management of the irrigated and dryland pasture treatments in terms of short-term storage of newly formed SOC?

6. Does irrigation enhance cumulative respiration flux? What fractions of the soil are sensitive to changes in soil moisture with respect to respiration?

7. What are the trade-offs between irrigation and nutrient management to increase pasture productivity and the need for maintaining or increasing SOC stocks under grazed pastures?

Recommendations for future research that can potentially address the above questions are presented in the following sections:

6.5.1 Improving the understanding of the mechanisms driving faster root C turnover under irrigated pastures

Results from Chapters 3 and 5 of this thesis have shown that summer irrigation reduced root biomass of a ryegrass-white clover pasture established at mesocosm scale in a stony Lismore silt loam soil, when compared with dryland conditions. Chapter 5 demonstrated that at mesocosm scale, irrigation also increased the turnover of photosynthate C partitioned previously to root biomass - results that are supported by measurements under field conditions in a similar pasture-soil system by Scott et al. (2012). Results from Chapters 3, 4 and 5 showed that irrigation favoured the formation and maintenance of the POM soil size fractions and Fraser et al. (2012) observed that there was a higher abundance of earthworms and herbivore soil mesofauna under a long-term summer irrigated ryegrass-white clover pasture on a Lismore stony silt loam soil. Based on all these findings, it was then suggested that greater activity of root-feeding soil invertebrates under the irrigated pasture may have favoured the transfer of root fragments to POM, especially to the fine POM (53–250 µm) fraction, resulting in a smaller and shallower root system relative to dryland conditions.

Therefore, future studies examining root turnover and POM formation rates along with measurements tracing the transfer of photosynthate C into soil food webs (e.g. mycorrhizal fungi, bacteria and soil herbivore mesofauna) will provide a better understanding of the mechanisms and vectors regulating the transfer and accumulation of root derived-C in the soil under irrigated pastures. The use of stable C isotopes ($^{13}C/^{12}C$) can be a useful method to measure the effects of irrigation on the transfer of root derived-C in to the soil food webs, similar to the study by Chomel et

al. (2019) where the effect of drought on the incorporation of recent photosynthate C into soil food webs was measured. Results from such studies could help to determine whether summer irrigation is increasing not just above-ground but also below-ground pasture productivity resulting in faster above- and below-ground C cycling.

Future research studying the effects of summer irrigation on the dynamics of C transfer within the entire plant-soil system are highly recommended to measure C losses via plant and soil respiration, as the percentage of C recovered within the plant-soil system after isotopic labelling does not reflect the effects of soil water content on fluxes of C passing through the different plant and soil components. As an example, the significance of CO_2 fluxes that occurs after wetting dry soils (Birch effect) that is observed under field conditions (Birch, 1958; Borken and Matzner, 2009), may differ with regular irrigation preventing the soil drying out. Multiple short dry/wet events under irrigation may accelerate C fluxes through the microbial biomass (Navarro-García et al., 2012; Slessarev and Schimel, 2020).

Irrigation favoured the partitioning of root derived-C to the POM soil fractions (higher root turnover) while increased the turnover of the MAOM (clay fraction) under the ryegrass-white clover pasture relative to dryland conditions. It remains unknown whether this effect was enhanced due to the presence of the N fixing-white clover plants whose high plant litter quality associated with low C and N ratios would increase its microbial decomposition (Hessen et al., 2004; Cotrufo et al., 2013; Chen et al., 2014) and subsequent accumulation in the soil without water deficit conditions. In this regard, measuring the effects of summer irrigation on the inputs and accumulation of root derived-C in the soil with and without the presence of legumes, along with measurements of root traits (e.g. root mass, rooting depth and root diameter) and respiration fluxes will also improve our understanding about how legumes can modify the effects of irrigation on the inputs and storage of SOC resulting from root turnover.

Furthermore, this PhD research also suggested that a key causal factor driving the faster root turnover under summer irrigated ryegrass-white clover pastures compared with dryland pastures could be related to greater nutrient requirements of the entire pasture-soil system under irrigation, as root turnover is one the main sources of OC to soil decomposers, whose activity regulates C and nutrient cycling in terrestrial ecosystems (Bardgett et al., 2005). Future research measuring the synergistic effects of summer irrigation and different nutrient management options (e.g. frequency and rates of N application) on the inputs and outputs of OC in the soil from root derived-C will assist with the development of more 'applied' research or larger farm scale experiments to determine whether summer irrigation is a sustainable management practice in terms of C and N cycling.

For example, dual ^{13}C and ^{15}N labelling experiments could be useful to study the C and N dynamics within the plant-soil system under irrigated pastures. Alternatively, stoichiometric measurements of C, N and P ratios of the entire plant-soil system, including microbial biomass measurements (Hessen et al., 2004; Rumpel et al., 2015) may provide a robust approach to studying the effects of irrigation on SOC and nutrient cycling under temperate pastures.

6.5.2 Improving our understanding on the POM and MAOM dynamics under managed pastures

The current research demonstrated that summer irrigation enhances the formation and short-term (< 1 year) storage of POM. However, further research on how continued irrigation in conjunction with other variables will affect the storage of SOC in the form of POM is required. This is because of the accumulation of SOC, in this case POM due to irrigation, does not necessarily equate to higher persistence in the soil (Cotrufo et al., 2015). Variables include management practices like grazing intensity or tillage; land use change (intermittent and short cultivation periods on pasture soils); and climate conditions (e.g. higher soil temperatures). As an example, soil compaction from livestock trampling in a high intensity grazing regime could enhance POM decomposition, as uncomplexed SOM is regarded as very sensitive to soil physical disturbances (Cotrufo et al., 2019; Lavallee et al., 2020). However, irrigation management during a pasture phase of a mixed cropping system could potentially build OC and nutrients in the soil through the accumulation of POM that would be available over the cropping phase.

Therefore, a better understanding of the functional characteristics of POM and MAOM could provide information about the effects of agricultural management practices on SOC storage (Cotrufo et al., 2019; Lavallee et al., 2020). Studies of POM dynamics under irrigated pastures could be achieved by establishing plot trials with different stocking rates, then measure the C content of the POM size fractions. Alternatively, soil samples archived from long-term irrigated and dryland pastures experimental trials could be physically fractionated into POM and MAOM fractions to build a chronosequence of effects of irrigation on these soil C size fractions relative to dryland pastures.

Furthermore, chromatographic analytical methods such as GC-MS or ^{13}C-NMR (nuclear magnetic resonance) could be used to identify if there are differences between irrigated and dryland pastures in terms of C sources (i.e. plant or microbial) and the biochemical composition of SOC. For example, tannins and polyphenols are abundant constituents of plant litter and hence, observed in POM > 53 µm while some amino-sugar are specific microbial compounds observed in SOM fractions < 38 µm (Kögel-Knabner, 2002; Grandy and Neff, 2008). A key constraint to such studies is that physical fractionation of soil into size fractions is a very time-consuming method.

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