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**Water use efficiency of grazed pastures
under contrasting diversity**

A thesis
submitted in fulfilment
of the requirements for the degree
of
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by
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Abstract

Agriculture must increase food production to support a growing global population; however, this required increase in production is potentially restricted by freshwater supply for irrigation. Consequently, relying on irrigation to enhance agricultural production is only a partial solution and increasing water use efficiency (WUE) is an important priority. Increasing pastoral diversity has been shown to increase pasture production, especially during warm and dry growing conditions. The positive effect of increasing diversity is typically linked to complementarity in plant traits such as rooting depth and facilitation among species or the inclusion of plants with divergent life histories that use water more efficiently. However, studies of differences in evaporation (E) and WUE between pastures of contrasting diversity at the ecosystem scale are scarce. The objective of this thesis was to contrast seasonal WUE and production strategies of a traditional ryegrass-clover and a more diverse pasture at the ecosystem scale. This study was conducted on an intensively managed commercial dairy farm in the Waikato region of New Zealand.

The first objective of this thesis (Chapter 3) was to quantify spatial and temporal variation in E from traditional ryegrass-clover pastures. These baseline measurements of E from intensively managed pastures are important for informing water resource decision making and validation of hydrologic models and remote sensing methods. Evaporation was measured simultaneously over existing ryegrass-clover pasture at 3 sites on the same farm using the eddy covariance (EC) technique. At an annual timescale spatial (770 – 783 mm) and temporal (759 – 776 mm) variations in E were less than 3%. This low variation largely occurred because E was strongly controlled by net radiation ($r^2 = 0.81$, $p < 0.01$, daytime, half-hourly), which did not vary much between sites and years. However, seasonally E was strongly limited when volumetric moisture content (VMC) declined below permanent wilting point. Grazing events, that removed about 55% of leaf material, had no effect on E during autumn and winter but reduced E by up to 5% during summer and spring and it was likely soil water E was compensating for reductions in transpiration. Agreement between E measured by eddy covariance (E_{EC}) and FAO-56 reference crop modelled E (E_o)

was good when soil moisture limitation was not occurring. However, during periods of soil moisture limitation, E_o exceeded E_{EC} and a correction factor was needed. The water stress coefficient (K_s) and a simple three bin VMC correction factor (K_{VMC}) was trialled and both approaches improved agreement between modelled and measured E but each method had limitations. Further study is needed to determine a simple and robust routine to model E from temperate pastures under soil moisture limitation.

The objective of Chapter 4 was to compare, evaporation, gross primary production (GPP), and ecosystem WUE (EWUE) between a traditional ryegrass-clover pasture and a more diverse pasture which included multiple grasses, legumes, and herbs. It was hypothesised that the more diverse pasture, which included deeper rooting species, would be more productive during dry and warm periods because of increased access to soil water and increased WUE associated with the inclusion of more legumes. Carbon exchange and E was measured between September 2012 and June 2015 using a paired EC experimental design with a pre-treatment period (September 2012 to April 2013) to identify any pre-existing site differences. A new ryegrass-clover (New Rye) and a new diverse (New Mix) pasture were established in April 2013 following herbicide application by the direct drill method. Between June 2013 and June 2015 above ground harvestable dry matter (DM) production was also measured. Post-treatment, GPP was higher at New Mix during both dry (4.0%) and wet (8.8%) summer conditions and these increases were supported by DM production measurements. Evaporation rates were not significantly different and consequently both EWUE (GPP/E) and harvest WUE (HWUE, DM/E) were higher at New Mix during summer conditions. No differences in production (GPP and DM) were found during shoulder season conditions while E was significantly lower at New Mix (5.8%) resulting in higher shoulder season EWUE. Both GPP and DM production were lower at New Mix during cool winter conditions while E was not different resulting in lower cool season EWUE and HWUE at New Mix. At an annual scale both production and EWUE were similar between treatments because summer increases at New Mix were compensated for by winter increases at New Rye. Consequently the strategic integration of both ryegrass-clover and more

diverse swards on different parts of a farm would likely maintain more even year-round productivity.

Increasing plant diversity was shown to increase production during warm dry and warm wet conditions and WUE during warm wet and shoulder season conditions (Chapter 4). However, differences between treatments were small (~ 5%) and this likely occurred because ryegrass was a dominant species at both sites. The optimal mix of species is expected to vary spatially dependent on climate, soil type, and plant water requirements. Consequently, a rapid and cost effective method to screen for productive pasture plant species and mixes with high WUE *in situ* at farm scale is needed. The objective of Chapter 5 was to test the correlation between WUE calculated from bulk leaf $\Delta^{13}\text{C}$ (a measure of intrinsic WUE, WUE_i) and EWUE by comparing the seasonal progression of bulk leaf $\Delta^{13}\text{C}$ and EWUE measured at the paddock scale using EC. Mixed species bulk leaf biomass samples were harvested pre-grazing, dried, sub-sampled, ground, and the ratio of ^{13}C to ^{12}C was measured. After accounting for the seasonal changes in the atmospheric vapour pressure deficit (VPD) on WUE_i , following Farquhar et al. (1982), strong positive correlations were found between WUE calculated from $\Delta^{13}\text{C}$ ($\text{WUE}_{\Delta^{13}\text{C}}$) and EWUE ($r^2 > 0.79$, $p < 0.01$) at both New Rye and New Mix. Additional seasonal measurements of production and $\Delta^{13}\text{C}$ on individual plant species grown together at New Mix found important differences in $\text{WUE}_{\Delta^{13}\text{C}}$ and production among co-existing pasture species. These results indicated $\Delta^{13}\text{C}$ was a suitable tool for comparing WUE between different pasture swards and, importantly, differences in $\text{WUE}_{\Delta^{13}\text{C}}$ between co-existing pasture species indicated pasture mixtures could be manipulated to optimise WUE.

Through this PhD some of the first replicated field-scale measurements of E from intensively grazed pastures were published and spatial and temporal variation in E was low because of the dominant control by net radiation (Chapter 3). Chapter 4 demonstrated increasing pasture diversity had a small (~5%) but important positive effect on warm season EWUE and production and shoulder season EWUE. Ryegrass was a dominant species in both treatments and it is possible larger improvements in EWUE could have been achieved by further optimising pasture mixtures in the sward. Chapter 5 examined the correlations between

EWUE and $WUE_{\Delta^{13}C}$ with the goal of developing a rapid and cost effective method to compare WUE between pasture swards, and thereby optimise species mixtures. Strong correlations were shown in addition to important differences in $WUE_{\Delta^{13}C}$ between co-existing pasture species. Combined the findings of Chapters 4 and 5 strongly indicated that mixtures of pasture species within a sward could be manipulated to increase EWUE. However, it is expected that optimal species mixtures are site specific depending on soil, climate and plant water requirements. Following further ecosystem and plant level study to confirm results found at this site, I envisage $\Delta^{13}C$ measurements could be used by farm advisors, alongside production monitoring, to optimise species selection within a continuously varying spatial context to maximise WUE and farm production.

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Chapter One

1.1 Introduction

Freshwater resources are under increasing pressure on a global scale largely as a result of population growth and changes in precipitation patterns resulting in more droughts in some areas (Kundzewicz and Gerten, 2015). Simultaneously, agriculture needs to provide food for an expanding global population. Given the restrictions in freshwater supply, increasing reliance on irrigation is not always sustainable. Therefore, identifying approaches to improve water use efficiency (WUE) and drought resilience of our food production systems is critical. In the pastoral sector, plant species are needed that either use water more efficiently while maintaining agronomic production or are able to access greater soil moisture reserves (White and Snow, 2012).

New Zealand's efficient agricultural production is tightly linked to year-round pasture growth and this efficiency is critical for New Zealand farmers to compete in the global market (Pembleton et al., 2015). Year-round intensively grazed pastures cover about 33% of New Zealand's land area (Ministry for the Environment, 2009) and pasture growth in most of these areas is reliant on rainfall and soil water storage for late summer and autumn growth (not irrigated). Traditionally, these intensively managed pastures have been dominated by a simple binary mix of perennial ryegrass (*Lolium perenne*) and white clover (*Trifolium repens*) because of the ease of establishment and management, and the high production of quality herbage that is grown throughout most of the year (Kemp et al., 1999). While drought is not a new problem, areas of New Zealand have experienced more frequent and longer periods of soil moisture limitation in recent years which maybe a consequence of climate change (Clark et al., 2011). At high temperatures and under dry conditions ryegrass and clover pastures are low yielding and herbage quality declines (Charlton and Stewart, 1999). Consequently, interest in more diverse pastures, including grasses, legumes, and herbs, that produce more biomass during warm and dry conditions is increasing (Pembleton et al., 2015).

While ryegrass-clover pastures are well suited to New Zealand's typically cool, temperate, and moist climate, increasing species diversity is likely advantageous under less favourable conditions (nutrient and water limitations). Despite the often debated importance of diversity for ecosystem function, from a production perspective the advantages of additional species are typically related to the diversity of plant traits as opposed to the number of species (Sanderson et al., 2006; Tilman et al., 2002). Diversity of traits allows for complementarity in resource use and facilitation among species (Pembleton et al., 2015). For example, Pang et al. (2013) showed that in a pasture system hydraulic lift through deeper rooting lucerne increased soil water content in surface soils that could be utilised by shallow rooted companion species while Mueller et al. (2013) and Hoekstra et al. (2014) have shown that the depth of water extraction increases when species were grown together compared to when grown in monoculture. Desirable traits to cope with water limitations include deeper roots, high root to shoot ratios, and increased photosynthetic performance resulting in higher WUE. Other physiological attributes that regulate WUE include the leaf mass/area ratio, leaf N and P concentration (nutrient availability), dark respiration, and leaf lifespan (Wright et al., 2004).

More diverse pasture mixtures have been shown to provide increased dry matter (DM) production during summer periods while maintaining similar annual cumulative DM production to traditional perennial ryegrass and white clover pasture systems (Edwards, 2013). In New Zealand, commonly used alternative pasture species include grasses such as tall fescue (*Festuca arundinacea*) cocksfoot (*Dactylis glomerata*), timothy (*Phleum pratense*), and prairie grass (*Bromus willdenowii*), legumes such as lucerne (*Medicago sativa*) and red clover (*Trifolium pratense*), and herbs such as plantain (*Plantago major*) and chicory (*Chicorium intybus*). Edwards (2013) found milk solid (MS) production was similar when cows were offered the same allowance of simple and more diverse pasture while Woodward (2013) showed cows grazed on more diverse pastures produced more milk solids per kg DM consumed than cows grazed on traditional ryegrass-clover pastures. The inclusion of these species in pastoral systems has been limited because of problems with persistence and low winter growth causing early spring feed shortages. However, with careful management to maximise

spring production the strategic use of these forages may provide higher quality forage and animal production in summer-dry environments (Brown et al., 2005).

In summary, dry summers threaten farm profitability and this threat may increase in future as climate continues to change (Clark et al., 2011). Review of the literature suggests increasing the diversity of plant traits may help mitigate against dry summer periods but we are uncertain whether production gains are attributable to increases in WUE (e.g. Nobilly, 2015) or deeper root systems accessing more water (e.g. Brown, 2004). Prior to this thesis, little work had been done in New Zealand, and globally, comparing ecosystem scale evaporation and WUE between simple and more diverse pastures. Key research gaps identified during review of literature (Chapter 2) were; (1) the need for evaporation measurements from traditional ryegrass-clover pastures as a baseline measurement; (2) the need for ecosystem scale comparison of evaporation and WUE between ryegrass-clover and more diverse pastures; (3) and the need for a simple and robust tool to screen pasture mixes and individual species for high WUE (given optimum pasture mixes are likely site specific dependent on climate and soils). These gaps contribute to farmer uncertainty regarding the benefits and trade-offs of increasing pasture diversity and consequently reduce farmer uptake.

1.2 Objectives

The objective of this thesis was to examine the potential benefits of increasing pastoral diversity on WUE of intensively grazed pastures. The objective was achieved by comparing evaporation, gross primary production, and harvestable production between a simple binary ryegrass-clover and a more diverse pasture at the paddock scale using a novel paired eddy covariance approach. Current knowledge was extended by measuring paddock scale water and carbon exchange and controlling variables at high time resolution and linking these measurements to harvestable production. Such data provides insight into how we may manipulate agro-ecosystems by using increased species diversity to improve WUE and consequently production and profitability. However, during review of literature it became apparent that baseline paddock scale evaporation measurements from traditional ryegrass-clover pastures were scarce and this gap

needed filling prior to moving onto the effect of species diversity. Consequently, specific questions were:

Question 1

What is the annual evaporative flux of water from traditional ryegrass-clover pastures and how does the flux vary spatially and temporally as a result grazing and the seasonal progression of controls?

To answer this question, evaporation was measured at three sites and compared from daily to annual time scales. At one site, measurements continued over three consecutive years to examine inter-annual variability. Controlling factors were also examined including the effect of grazing induced changes in leaf area and finally measurements were compared to modelled evaporation.

Question 2

Is increased summer production from more diverse pastures related to increased access to soil water or increased WUE and what are the differences in seasonal plant growth and production characteristics?

To answer this question, water vapour and carbon fluxes measured by eddy covariance, and pasture dry matter (DM) production, was compared over a two-year period at seasonal and annual timescales between a newly sown ryegrass-clover and a newly sown more diverse pasture.

Question 3

Can natural carbon isotope discrimination ($\Delta^{13}\text{C}$) during photosynthesis be used to compare WUE between pasture swards at the paddock scale and consequently allow quick and cost effective monitoring of WUE at paddock and farm scale?

To answer this question, bulk biomass pasture samples were harvested within the footprint of two eddy covariance towers prior to grazing throughout one complete year (2015). The harvested biomass integrated leaf level $\Delta^{13}\text{C}$ over the period since the previous grazing event. WUE, as calculated from $\Delta^{13}\text{C}$ using the

Farquhar et al. (1982) method, was then compared to ecosystem WUE (EWUE) as measured by eddy covariance.

1.3 Thesis structure

This thesis begins with a review of literature (Chapter 2) that initially has a broad focus to provide background and context for the project. The review is then divided into three sections to provide context for the subsequent chapters.

Chapters 3 to 5 present research results that address the questions outlined above (Section 1.2) and are written in manuscript form for ease of submission for publication. Chapter 3 was published in July 2016 in *Agriculture, Ecosystems, and Environment* and focused on evaporation and controls from intensively grazed ryegrass-clover pastures. Chapter 4 compared EWUE and harvest WUE (HWUE) between a traditional ryegrass-clover and a more diverse pasture over a three-year period. Chapter 5 compared EWUE, as measured by eddy covariance, to WUE calculated from carbon isotope discrimination during photosynthesis. Carbon isotope discrimination has not been widely used to identify efficient pasture species and given the method is simple and cost effective it has great potential for selecting species based on site specific climate and soil characteristics. Finally, Chapter 6 presents a summary of the research findings across the breadth of the thesis and suggests potential opportunities for future research.

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Chapter Two

2 Literature review

2.1 Introduction

Water supply strongly restricts plant production on a global scale exceeding losses due to all other biotic and environmental factors (Law et al., 2002). Additionally, climate change models predict that the frequency and severity of drought is going to increase in some locations (Jongen et al., 2011) and hence future agricultural production losses will likely increase because of water scarcity. Therefore, identifying approaches to improve water use efficiency (WUE) and drought resilience of agricultural systems is critical to maintain or increase agronomic production to feed a growing global population. In the pastoral sector plant species with the ability to access greater soil moisture reserves or increase WUE while maintaining production are needed (White and Snow, 2012).

Grazed pastures cover ~26% of the global ice free land area (Steinfeld et al., 2006) while in New Zealand high producing exotic grasslands cover ~ 33% of the land surface (8.9 million ha) (Ministry for the Environment, 2009). Traditionally New Zealand pasture systems have been dominated by perennial ryegrass (*Lolium perenne*) and white clover (*Trifolium repens*) with our warm temperate maritime climate generally being well suited to ryegrass. However, ryegrass is not a drought tolerant species (Brown et al., 2005; Musgrave and Daly, 2004) and following a number of recent severe droughts in New Zealand interest in diversifying pastoral systems by including more drought tolerant or water efficient species is increasing.

Our current understanding of WUE in managed temperate grazing systems is limited because of the scarcity of work done at the ecosystem scale. The paucity of research in this area can largely be explained by the difficulty and expense in making measurements of water and carbon fluxes at an appropriate scale (Fisher et al., 2005).

2.1.1 Purpose and structure

The following review begins by introducing diverse pastures and their potential advantages in water limited environments. The eddy covariance technique used to measure water and carbon fluxes for this research is then introduced. Other measurement techniques are identified in Section 2.3 but detailed description was outside the scope of this review. The review is then broken into three parts with the objective being to identify current knowledge and consequently direction for future research to address gaps.

- ❖ Section 2.4 focuses on evaporation measurements from managed grasslands, controls, and modelling.
- ❖ Section 2.5 focuses on WUE measurements from managed grasslands including seasonal and inter-annual variation and controls.
- ❖ Section 2.6 discusses the potential to use natural carbon isotope discrimination during photosynthesis to measure paddock scale WUE.

2.2 Increasing pastoral diversity

In New Zealand, a simple ryegrass and white clover mix is commonly used under intensive dairy grazing because it is high yielding and tolerant of a wide range of environmental conditions and management (Brown et al., 2005). However, both species have poor drought tolerance that can limit production during dry summer and autumn periods. During these dry periods herbage quality declines with metabolisable energy (ME) typically reducing from about 11 MJ kg⁻¹ dry matter (DM) in spring to 7.7 – 9 MJ kg⁻¹ DM in summer and crude protein declines from about 21.5% to 14% over the same period (Brown et al., 2005).

Plant production during drought conditions is strongly influenced by their strategic use of water. Important plant traits that relate to water use strategies include water acquisition efficiency and WUE (Moreno-Gutierrez et al., 2012). Deep roots are desirable to increase access to soil water and high root to shoot ratios can increase water uptake relative to transpiration. WUE is strongly related to leaf stomatal regulation strategies and the photosynthetic assimilation rate. The photosynthetic assimilation rate is dependent on nutrient availability, particularly

N which strongly influences the carboxylation rate (Hussain et al., 2011). Other physiological attributes that can affect WUE include the leaf mass/area, dark respiration, and leaf lifespan (Wright et al., 2004).

Alternative pasture species of interest both globally and within New Zealand include grasses such as tall fescue (*Festuca arundinacea*) cocksfoot (*Dactylis glomerata*), timothy (*Phleum pratense*), and prairie grass (*Bromus willdenowii*), legumes such as lucerne (*Medicago sativa*) and red clover (*Trifolium pratense*), and herbs such as plantain (*Plantago major*) and chicory (*Chicorium intybus*). Plantain, chicory, lucerne, and red clover all have deep central tap roots and have been shown to produce greater herbage production relative to ryegrass and white clover in summer-dry environments (Section 2.2.1). The inclusion of these species in pastoral systems has been limited because of problems with persistence and low winter growth causing early spring feed shortages. However, with careful management to maximise spring production the strategic use of these forages may increase farm production (Brown et al., 2005).

2.2.1 Dry matter production

Increased species diversity has been shown to lead to increased pasture production from grazed pastures both in New Zealand (Edwards, 2013; Nobilly et al., 2013; Ruz-Jerez et al., 1991) and internationally (Kirwan et al., 2007; Sanderson et al., 2006; Skinner et al., 2004). Increased production from more diverse pastures has been attributed to deeper rooting species accessing more soil water (Skinner et al., 2006), higher WUE (Skinner, 2008) and increases in optimum temperatures for photosynthesis leading to higher productivity during warm wet summers (Goh and Bruce, 2005; Skinner and Dell, 2016). Skinner and Dell (2016) compared production over a nine-year period between a grass clover mix and a more diverse mix which included additional grasses along with chicory and lucerne in Pennsylvania. In eight out of nine years the more diverse mix produced more biomass with a mean annual increase of 31%. Seasonal increases were 34% in spring, 30% in summer, and 26% in autumn, with summer increases being larger during wet summers (Skinner and Dell 2016).

In New Zealand, Nobilly (2015) compared pasture production between binary ryegrass-clover mixes and a range of more diverse mixes under both irrigation and partial irrigation. Under partial irrigation (higher water stress) production from all treatments declined but declines were highest for the binary mixes and lowest for the most diverse mixes. Those mixes that included the deep tap rooted legumes (lucerne and red clover) had the lowest relative reduction in productivity. Under full irrigation production was highest from the more diverse treatments and on average the more diverse pastures produced an additional 1.6 t DM ha⁻¹ yr⁻¹ over the two-year trial. Most of the additional DM grew over the summer months. The diverse pastures were found to have lower ME kg⁻¹ DM⁻¹ but given the higher productivity still produced higher ME ha⁻¹. Both Edwards (2013) and Woodward (2013) found milk solid (MS) production was similar when dairy cows were offered the same allowance of simple (ryegrass-clover) and more diverse pasture.

2.2.2 Root depth and distribution

Deep rooted plant species are commonly found in water limited environments because of their ability to tolerate drought (Canadell et al., 1996) and consequently deeper roots are a desirable trait in seasonally water stressed pastures. Few studies have compared maximum rooting depth of pasture species in field conditions, largely because of the labour intensive nature of root biomass measurements. Researchers typically focus on root distribution within the upper 1 m of soil and these studies show pasture plants concentrate their roots in the near surface zone. For example, Gentile et al. (2003) measured root biomass of three forage species (tall fescue, lucerne, and chicory) to 1 metre depth in southwestern Uruguay on a silty clay loam and found that half of the root biomass for all three species was concentrated in the top 20 cm of soil. In the Netherlands, on a sandy soil, Deru et al. (2012) measured root density of three grass species (ryegrass, tall fescue, and cocksfoot) to 32 cm depth and found that most of the root biomass was concentrated in the upper 8 cm (ryegrass 83%, tall fescue 65%, and cocksfoot 76%). On a fine sandy loam in southern Canada, Bolinder et al. (2002) measured root biomass in seven perennial grass species and two perennial legume species and reported that after the first year 54% of roots were concentrated in the upper 15 cm. In New Zealand, and one of the few studies to have compared root biomass between a ryegrass-clover pasture and a more diverse pasture (to 300 mm

depth), McNally et al. (2015) found root biomass was higher for the moderately diverse pasture in all seasons in addition to more diversity in root traits.

Despite a large proportion of grassland species concentrating their roots near the surface (~ top 20 cm), Canadell et al. (1996) have shown the mean maximum rooting depth in temperate grasslands globally is 2.6 m. However, rooting depth is likely highly dependent on plant species and soil type. Moot et al., (2008) showed that perennial ryegrass grown on the deep Waikanui silt loam (high water storage capacity) extracted water to a maximum depth of 1.5 m and was extracting a considerable proportion of its water from below 1 m depth. In contrast, ryegrass grown on the Lismore stony loam (low soil water storage) extracted most of its water close to the surface. A similar trend was shown for lucerne. In the deep Wakanui soil, lucerne extracted 328 mm of water down to depths of at least 2.3 m, a large proportion of water was extracted from between 1 and 2.3 m (~ 50%). In contrast, on the Lismore stony soil lucerne only extracted 131 mm of water to 2.3 m depth and about 70% came from above 1 m depth. These findings demonstrate that depth of water extraction is dependent on both plant traits and soil type.

Ultimately, a mix of rooting depths is likely desirable to reduce competition between plant species (White and Snow, 2012). Commonly used pasture species (e.g. ryegrass, white clover) concentrate their roots in the near surface zone and root mass density generally declines exponentially with depth while water extraction declines linearly with depth (Woodward et al., 2001). This indicates a few roots at greater depth are important for water uptake, especially during dry periods.

2.2.3 Water movement through the soil and plant

Plants access water stored in the soil root zone and plant available water (held at tensions above -1500 kPa) is highest in well-structured loam and silt loam soils and relatively low in coarse sandy soils (low total water storage) and fine clay soils where a large proportion of soil water is held at tensions in excess of wilting point (McLaren and Cameron, 1996). The movement of water from the soil, through the plant and into the atmosphere is driven entirely by the physical process of evaporation (see Section 2.4) at the leaf surface. A continuous film of

liquid water moves from the soil into the plant root as a result of negative pressure created in the plants xylem vessels as water is lost from leaves via transpiration. As the soil water content declines plant roots produce the hormone ABA that is transported to the leaves and triggers a reduction in stomatal conductance and consequently transpiration. Plant species differ in their stomatal response to soil drying. Stomatal conductance is generally higher in plants adapted to fertile and moist conditions when the environment is favourable but such species (isohydric) typically reduce conductance at higher soil moisture contents relative to those adapted to dryer conditions (anisohydric) (McDowell et al., 2008; Schulze et al., 1994).

During dry periods when surface soil moisture content is low, hydraulic redistribution of soil water can occur at night through plant roots (e.g Pang et al 2013). During the day plant-water potential is lower than the soil and consequently soil water moves into plant roots and flows toward the leaves as a result of negative potential generated by transpiration. However, at night when stomata close and evaporative demand is low plant water potential can equilibrate with the soil water potential of deeper soil layers that are in contact with plant roots. Plant roots can then become passive hydraulic conduits that transfer soil water from areas of high potential at depth to areas of low potential nearer the surface (Sardans and Penuelas 2014). For example, Pang et al. (2013) showed that in a pasture system hydraulic lift through deeper rooting lucerne increased soil water content in surface soils that could be utilised by shallow rooted companion species. Additionally, Skinner (2008) found evidence for night time transfer of soil moisture from below 50 cm to the surface in both a simple ryegrass-clover and a ryegrass-clover-chicory mix largely because both mixtures had roots below 50 cm depth. The findings of Pang et al., (2013) and Skinner (2008) indicate deep rooted pasture species have the potential to increase water availability to shallow rooted companion species.

2.3 Water and carbon flux measurement by eddy covariance

Eddy covariance (EC) flux measurements are made by correlating changes in vertical wind velocity, measured using a sonic anemometer, with fluctuations in CO₂ and H₂O molar concentrations measured with fast response infrared gas

analysers. High frequency data are generally collected at 10-20 Hz (sampling frequency needs to be high enough to measure the smallest fastest eddies) and summed for each half hour period (summing shorter intervals can miss fluxes contributed by very large eddies) (Burba, 2005). For accurate measurement of flux densities between a vegetated surface and the atmosphere a number of site conditions are required including flat terrain with uniform upwind vegetation, steady atmospheric conditions, and sensors capable of recording the fastest and smallest eddies (Baldocchi, 2008; Burba, 2005). Despite these complexities the approach has important advantages when studying exchange processes at the paddock or ecosystem scale (Baldocchi, 2008). These include the ability to measure fluxes directly across useful spatial scales (few hectares up to hundreds of hectares depending upon height of instrumentation) at time resolutions from half hourly up to multiple years with simultaneous measurement of multiple potential forcing soil, meteorological, and biological drivers.

At the ecosystem scale, eddy covariance techniques do not measure plant CO₂ assimilation and transpiration directly but instead measures total evaporation (E) and net ecosystem production (NEP). To calculate ecosystem WUE, (see Section 2.5.1 for definitions) NEP must be partitioned into gross primary production (GPP) and ecosystem respiration (ER). Daytime ER is typically modelled based the temperature response of night time NEP because at night $NEP \approx ER$ and the modelled temperature response is used to estimate daytime ER (Reichstein et al., 2005). Daytime ER is then added to daytime NEP to derive GPP. More recently, this approach is being questioned because ER is likely lower at equivalent temperatures during the daytime when photosynthesis is occurring (Oikawa et al., 2017) and consequently GPP is likely overestimated. However, a preferred routine approach to partition GPP and ER from NEP has not yet been established.

2.3.1 Uncertainties

There is inherent uncertainty in EC derived measurements as a result of measurement, data processing, partitioning, and gap filling procedures.

The largest uncertainty in flux measurement is likely associated with the apparent lack of energy balance closure. Typically, the sum of the latent and sensible heat

flux is only about 80% of available energy (Wilson et al., 2002) and hence the law of conservation of energy is violated (Leuning et al., 2012). The proportion of unexplained imbalance is often used as an indication of data quality for all fluxes because it is assumed if the energy balance is not closed other fluxes (e.g. CO₂) could also be imperfect. Energy imbalance is thought to be associated with multiple factors including, but not limited to, measurement and data processing error and the potential mismatch between the latent heat (*LE*) flux footprint and point measurements of R_n and energy storage terms (Leuning et al., 2012; Wilson et al., 2002). Leuning et al. (2012) concluded closure could be improved substantially with careful attention to all sources of measurement and data processing error and suggested phase lags and incorrect estimates of energy storage terms were likely responsible for a large proportion of the remaining energy imbalance. While forcing closure has been recommended in the past (e.g. Twine et al., 2000) by increasing *LE* and sensible heat (*H*) (while maintaining the Bowen ratio) the mechanisms underlying the lack of energy budget closure are still under debate (Stoy et al., 2013) and forced closure may result in flux estimates that exceed realistic limits (e.g. Allen et al., 2011).

The largest source of known uncertainty associated with data processing is the potential bias introduced by the developed turbulence threshold chosen (friction velocity, u^*) (Campbell et al., 2015). Fully developed turbulence is central to the eddy covariance method and consequently periods of low turbulence are removed based on site specific turbulence thresholds that are critical for accurate measurement. Uncertainty associated with the choice of threshold for developed turbulence is often determined by comparing annual flux sums over a range of plausible turbulence thresholds (e.g. Campbell et al., 2015). Uncertainty associated with the gap filling method chosen is dealt with in multiple ways. Common approaches include statistical analysis of measurement-model residuals, statistical analysis of multiple gap filling runs with random artificial creation of gaps (Campbell et al., 2015), and comparison of variance between a range of gap filling methods (e.g. Eichelmann et al., 2016).

2.4 Evaporation

Evaporation (E) is defined as the process where liquid water is transformed to the gaseous phase and is synonymous with the term ‘evapotranspiration’ (Srinivasan et al., 2016). Evaporation is typically a large term in the catchment water balance and a major component of the Earth’s surface energy budget that in turn determines atmospheric circulation patterns and weather phenomena (Kelliher et al., 1993). Accurate quantification of E is important in agricultural systems for managing soil moisture deficits and irrigation scheduling to optimise dry matter production (Green et al., 1984) and to avoid water wastage through drainage and the ensuing environmental damage associated with nutrient leaching. The common pathway for exchange of water vapour and CO_2 through plant stomata also alludes to the importance of evaporation as a key ecosystem process (Dolman et al., 2014; Kelliher et al., 1993). This section begins with a broad review of the factors that control E and its partitioning before focusing on the effect of variation in leaf area through grazing. This is followed by discussion of evaporation modelling approaches before finally reviewing evaporation measurements from managed grasslands with a focus on temperate grazed pastures.

2.4.1 Controls of evaporation rates

The rate of evaporation is dependent on energy supply, available water, and plant physiological control (Dolman et al., 2014). The conversion from liquid water to vapour requires vast inputs of energy (2.45 MJ kg^{-1} at 20°C) which is supplied by direct solar radiation or indirectly from the atmosphere through turbulent exchange (Monteith, 1965) and consequently the evaporation rate is weather dependent. Water availability is coupled to climate through precipitation, Zhang et al. (2001) showed there was a strong relationship between annual precipitation and evaporation globally. The importance of plant physiology in controlling evaporation varies spatially depending on plant growth strategies, water supply, and the scale of observation (Jarvis and McNaughton, 1986).

The components that contribute to ecosystem evaporation include water transpired through plants and into the atmosphere via stomata (E_T), evaporation of soil water (E_S), and free water evaporated from plant leaves and stems via interception (E_I). In general, all three components of evaporation are strongly dependent on solar

radiation (Dolman et al., 2014) but other factors such as water supply and plant physiology become increasingly important in certain circumstances. For example, during the onset of drought plant stomatal control can constrain transpiration and ultimately drought can inhibit both transpiration and soil water evaporation (Lambers et al., 2008). Similarly, the contribution of interception is dependent on rainfall frequency and the aerodynamic properties of the vegetation in addition to energy input (Dolman et al., 2014). Ultimately, many interacting factors control energy and water availability and physiological constraints and these factors and their interactions are shown in Figure 2.1.

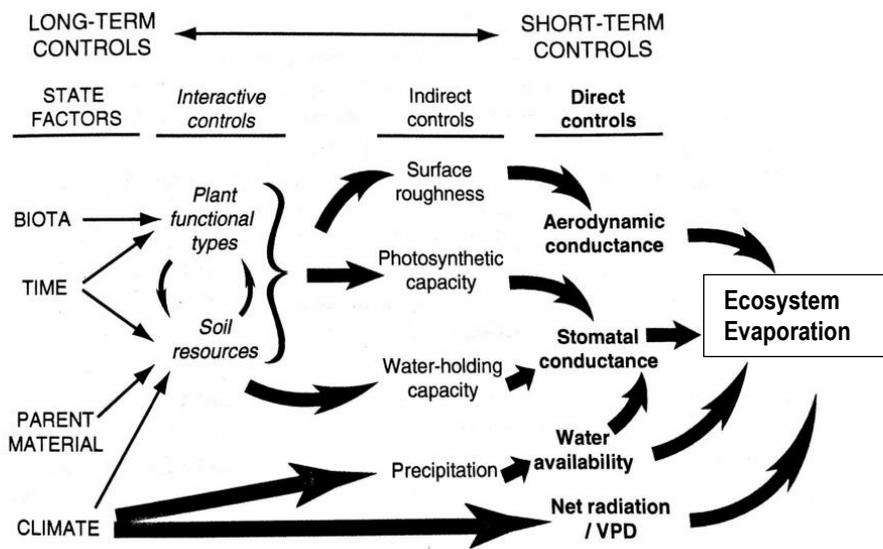


Figure 2.1 Factors controlling spatial and temporal variation in evaporation at an ecosystem scale. Thicker arrows depict the most important factors; however, this importance varies between ecosystems (Figure from Lambers et al. (2008)).

Historically, there have been opposing opinions on the relative importance of plant physiology versus supply and demand of water and energy in controlling evaporation (Jarvis and McNaughton, 1986). Small scale experiments by plant physiologists in cuvettes and plant chambers found plant stomata played a dominant role in mediating transpiration. In contrast, meteorologists argued that providing soil moisture was not limiting, weather conditions and in particular available energy was the dominant control (largely because of the large latent heat requirements of evaporation). These conflicting opinions were largely related to the scale of observation and the degree of decoupling (Ω , defined at canopy scale

by Equation 1 where Ω takes a value between 0 and 1) between the leaf and the overlying air stream. At the scale of individual stomata plants have perfect control of transpiration by controlling individual pore conductance. However, even at the leaf scale stomatal control over transpiration can range from weak to strong depending largely on leaf morphology. For small leaves, where ambient air mixes strongly with individual leaves strong coupling can occur between the leaf surface and the vapour pressure deficit (VPD) of the ambient air and consequently stomatal conductance largely controls transpiration rates. In contrast, for large smooth leaves the mixing of ambient air can be limited by leaf boundary layer resistance and in such conditions the leaf is poorly coupled to the ambient VPD and changes in stomatal conductance may have little effect on transpiration rates (Jarvis and McNaughton, 1986).

$$\Omega = \frac{\varepsilon+1}{\varepsilon+1+\frac{g_a}{g_s}} \quad \text{Eqn. 1}$$

where ε is s/γ , s is the slope of the relationship between saturation vapour pressure and temperature (kPa K^{-1}), γ is the psychrometric constant (0.067 kPa K^{-1}), g_a (m s^{-1}) is bulk aerodynamic conductance, and g_s is surface conductance (m s^{-1}).

At the canopy scale the decoupling coefficient is often used to determine the relative control of plant stomata and the VPD versus available energy in controlling evaporation (Hirano et al., 2015). In forest ecosystems, high surface roughness typically drives deep mixing of ambient air into the canopy resulting in weak decoupling (Ω approaches 0) and in such circumstances surface conductance and VPD strongly control transpiration. In contrasts, for aerodynamically smooth canopies (typical of grasslands) where poor mixing results in strong decoupling (Ω approaches 1), the effect of surface conductance on transpiration is relatively small compared to radiation and temperature (Jarvis and McNaughton, 1986). In general, across all ecosystem types when soil moisture is not limiting, as the scale of interest increases and the reference point for the saturation deficit moves higher above the canopy changes in surface conductance can easily be overlooked because of the increasing importance of available energy on ecosystem E (Jarvis

and McNaughton, 1986). Through the decoupling coefficient Jarvis and McNaughton (1986) were able to reconcile long standing differences in opinion regarding the importance of plant stomata verses supply and demand of water and energy in regulating transpiration and simultaneously advanced understanding of scaling from plant leaf to ecosystem and region.

2.4.2 Evaporation of soil water

Evaporation of soil water can have an important influence on ecosystem evaporation especially when leaf area is low, for example after an intensive grazing event or following cultivation and seeding. Soil water evaporation follows a three-stage sequence. During stage 1, when surface soil water is non-limiting, soil evaporation is only limited by weather conditions and most importantly available energy. Stage 2 begins when soil moisture content in the upper soil layer declines below a critical threshold and soil evaporation is limited by soil hydraulic properties that control the movement of liquid water and vapour to the soil surface. At stage 3 there is little liquid water movement in the soil and the rate of evaporation from the soil surface becomes negligible (Ventura et al., 2006). Wilson et al. (1997) showed that the critical threshold at which stage 1 ended was typically about -3.0 MPa, well below permanent wilting point (PWP, -1.5 MPa). Earlier experimental work from bare soil columns suggested stage 1 could persist for about 4 days with a rapid decline in evaporation between days 4 - 11 (Wilson et al., 1991). Kerr and McPherson (1978) reported that evaporation from bare soil was initially 3.4 mm day⁻¹ following vegetation removal but declined over a period of days to 1.3 mm day⁻¹. In comparison, evaporation over adjacent pasture (paspalum) was about 4.7 mm day⁻¹ with LAI greater than 3.0. Differences between the vegetated and non-vegetated site were largest during midday conditions when R_n was high suggesting evaporation was becoming limited by soil hydraulic properties (stage 2).

2.4.3 Effect of leaf area and grazing on evaporation

Intensive grazing events cause sudden large reductions in leaf area (from in excess of 4 m² m⁻² to less than 1 m² m⁻²) thereby modifying the surface energy budget through changes in surface albedo and energy partitioning (Frank, 2003). In an intensively grazed New Zealand pasture system grazing events occur up to 12

times annually and these events may have important implications for WUE and ecosystem modelling.

The relative partitioning of transpiration, interception, and soil water evaporation is dependent on leaf area (Allen et al., 1998b; Kelliher et al., 1995) and rainfall event frequency and size (Ataroff and Naranjo, 2009). When leaf area is high transpiration is the dominant source of ecosystem evaporation except immediately following rainfall when interception loss occurs. In grasslands, the relative contribution of interception is dependent on rainfall event size. For example, following small rainfall events (< 10 mm) the proportion of interception loss can be large (> 25%) while for large rainfall events (> 50 mm) the proportion of interception loss is small (< 10%) (Ataroff and Naranjo, 2009). Leaf area and rainfall frequency also control the relative contribution of soil water evaporation to total ecosystem evaporation. As rainfall frequency increases, resulting in a wet soil surface, and leaf area declines the relative contribution of soil water evaporation will increase because more radiation will reach the moist soil surfaces (Bremer et al., 2001; Frank, 2003). Therefore, reduction in leaf area through grazing likely reduces the transpiration component of ecosystem evaporation; however, depending on soil moisture content, increased evaporation of soil water may compensate (Falge et al., 2005). The interacting factors which control this partitioning are shown in Figure 2.2.

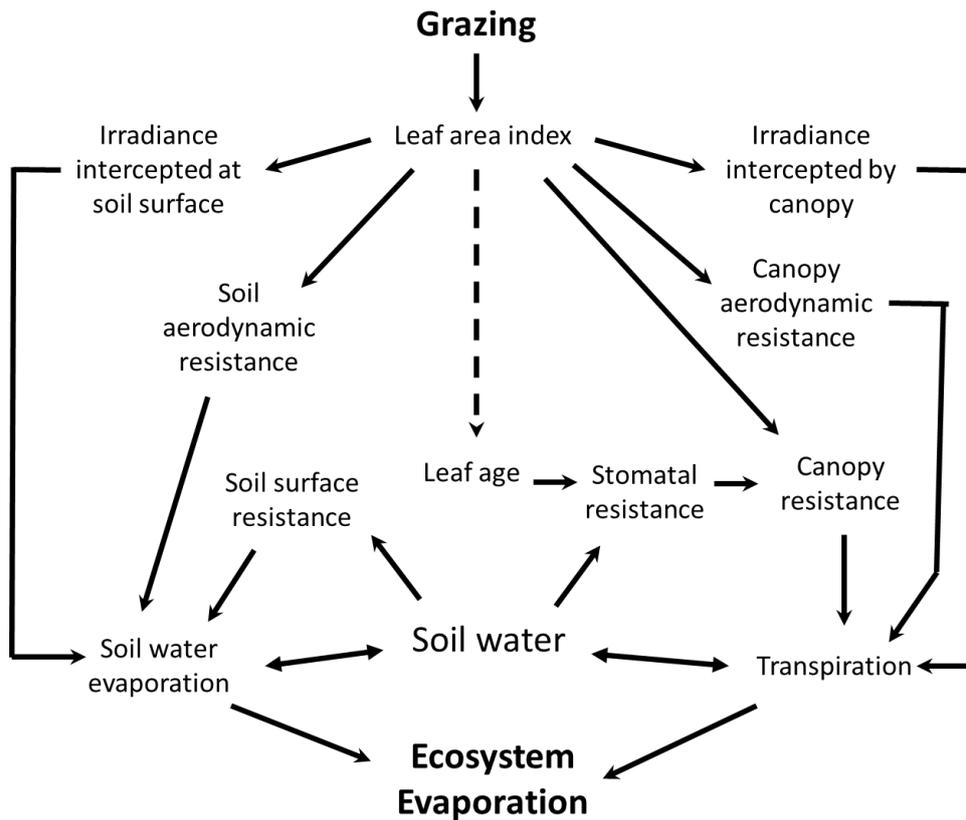


Figure 2.2. Effect of grazing events on evaporation through reduction in leaf area (Figure redrawn from Bremer et al. (2001)).

Reduction in leaf area through grazing has in some circumstances been shown to reduce evaporation while in other circumstances appears to have had no effect. Wang et al. (2012) found that grazing events had very little effect on evaporation in a semi-arid steppe ecosystem (Inner Mongolia, China) largely because increased soil evaporation compensated for reduced transpiration. Shuttleworth et al. (1989) found no correlation between LAI and evaporation rates over prairie grasslands and suggested the interplay between soil water and plant contributions likely moderated the effect of leaf area. Early work by the International Satellite Land Surface Climatology Project found evaporation was similar between grazed and un-grazed sites despite variation in LAI (Stewart and Verma, 1992). However, Frank (2003) found evaporation was 7% lower from a cattle grazed prairie in the Northern Great Plains compared to a non-grazed prairie (Frank, 2003). Bremer et al. (2001) also reported a 6.1% reduction in evaporation from a grazed tall grass prairie compared to non-grazed prairie. They found that daily evaporation could be reduced by as much as 40% when differences in green leaf

area were large and the soil surface was dry (Bremer et al., (2001) provide a brief summary of the mechanisms which affect evaporation through grazing, see Figure 2.1). Day and Detling (1994) found evaporation was lower from heavily grazed prairie grass grazed by prairie dogs in South Dakota compared to lightly grazed prairie.

Since the inception of this thesis a number of further studies have been published (including work from this thesis – see Chapter 3) which continue to show variation in the response of evaporation to grazing. Graham et al. (2016) found evaporation was relatively insensitive to sharp reduction in leaf following intensive grazing. They modelled leaf area based on known grazing times and found weak correlations ($r^2 = 0.06$) between leaf area and evaporation at the grazed site but stronger correlations ($r^2 = 0.38$) at the seed harvest site where pasture grew longer prior to harvest. At a harvested grassland in Germany, Gebler et al. (2015) found reductions in leaf area caused a significant reduction in evaporation (up to 2.1 mm day^{-1}). It is likely that in an aerodynamically smooth temperate pasture, where soil water is often non-limiting and evaporation is largely controlled by R_n , leaf area has relatively minor control over evaporation. In contrast, when soil moisture becomes limiting and dry soil surfaces restrict soil water evaporation while plants are still able to access deeper soil moisture, reductions in leaf area are likely to increase bulk surface resistance and therefore reduce evaporation. Research to test this hypothesis would be useful to help answer the current debate in the literature.

2.4.4 Evaporation models

Measuring evaporation is difficult and expensive and consequently models are often used when estimates of water vapour fluxes are needed for water balance and Earth system modelling. Many evaporation models exist (see below) but most of the commonly used approaches are informed by seminal work done by Penman (1948) and Monteith (1965). Separately, Thornthwaite (1948) had developed an approach where potential evaporation could be estimated from mean monthly temperature and day length. The Thornthwaite approach was unsuitable for short term estimation (less than monthly) where temperature lags energy input by solar radiation. Penman recognised the limitations of the Thornthwaite

approach and the importance of a vapour pressure gradient to maintain evaporation rates. Consequently, he developed a semi-empirical reference surface approach which combined both energy budget and mass transfer by including the vapour pressure deficit and wind run. While ignoring crop water status, advection, and heat storage, this approach worked relatively well under non-water limiting conditions and has been widely applied, often with an empirical crop coefficient to account for variation in plant physiology. Monteith (1965) is credited with making an important breakthrough in the quantification of evaporation by introducing canopy resistance to the Penman equation in addition to aerodynamic and boundary layer conductance (Dolman et al., 2014). This approach combined energy budget, mass transfer, and surface conductance into one equation. The resultant Penman-Monteith equation has been widely used and shown to be accurate across a wide range of situations (Howell and Evett, 2004).

Since the development of the Penman-Monteith equation a number of other surface dependent models have been developed with varying complexity and parameter requirements including Priestley and Taylor (1972), McNaughton and Black (1973), and the multiple layer model of Shuttleworth and Wallace (1985). This range of methods, with varying complexity, has resulted in inconsistencies in evaporation estimation approaches. To encourage a more consistent approach Allen et al. (1998b) parameterised the Penman-Monteith equation to reduce parameter requirements by assuming a fixed bulk surface resistance (70 s m^{-1}) for crops well supplied with water and estimated the aerodynamic resistance as an inverse function of wind speed. The Food and Agriculture Organisation of the United Nations (FAO) recommend this parameterised version of the Penman-Monteith equation as the sole standard method for estimating evaporation and the development of this equation was considered a significant milestone in developing consistent evaporation estimation methodology (Howell and Evett, 2004). The model has since been used widely to estimate evaporation (Steduto et al., 2003) in larger and more complex water balance, ecosystem, and Earth system models and is therefore important for the evaluation of water resources, management and monitoring crop water requirements, drought forecasting and the study of climate change (Zhao et al., 2013).

However, the FAO-PM equation was developed to predict evaporation from a well-watered reference crop (E_o) and hence was designed to model evaporation under non water limiting conditions (Allen et al., 1998a). After calculating E_o a suitable crop coefficient (K_c) must be applied for the site to account for differences in crop height, albedo, canopy resistance, aerodynamic resistance, and soil water evaporation between the reference surface (E_o) and the modelled crop. The crop coefficient therefore assumes linear scaling of these factors which is a somewhat problematic assumption and consequently the further K_c deviates from 1 the more problematic its application. Under water limited conditions Allen et al. (1998b) prescribe a method to adjust reference surface evaporation by including a crop stress factor (K_s). Such adjustments require estimates of field capacity and permanent wilting point to calculate daily profile available water which is used to constrain daily estimates of evaporation.

A number of comparisons between measured and modelled evaporation have been published since the inception of this thesis (2014) including work from this thesis presented in Chapter 3. Graham et al. (2016) found strong agreement between FAO-PM modelled E_o and measured evaporation at daily to annual time scales when water was not limiting. However, when soil moisture declined following the cessation of irrigation (at the seed harvest site) modelled E_o was considerably higher than measured evaporation. In contrast, Kirschbaum et al. (2015) modelled evaporation using the detailed ecosystem model CenW and compared modelled output to evaporation measurements made by eddy covariance over a ryegrass-clover pasture in Waikato, New Zealand. The components of total evaporation (E_T , E_S , E_I) were modelled individually using the full Penman-Monteith equation. Agreement between the model and measurements was strong (model efficiency of 0.96 for weekly averaged values) and using this more detailed approach Kirschbaum et al. (2015) was able to accurately model E during periods of soil moisture limitation. These findings suggest that when soil moisture is non-limiting the FAO-PM approach is suitable for ryegrass-clover pastures in New Zealand. However, when soil moisture becomes limiting the full Penman-Monteith approach (using appropriate surface resistances) is likely more appropriate.

2.4.5 Evaporation measurements from grazed grasslands

Prior to the inception of this PhD research (2014) published E measurements from grasslands were largely from arid and Mediterranean climates that were not grazed (Burba and Verma, 2005; Chen et al., 2009; Hao et al., 2007; Kurc and Small, 2007; Wever et al., 2002), or were lightly grazed because of their low productivity (Aires et al., 2008; Baldocchi et al., 2004; Bowling et al., 2010; Hunt et al., 2002; Krishnan et al., 2012; Li et al., 2007; Li et al., 2006; Ryu et al., 2008). Given evaporation is strongly limited by available water in these arid and Mediterranean grasslands the seasonal progression of control is likely very different compared to temperate grasslands. For example, Aires et al. (2008) found that in a Mediterranean grassland soil water availability restricted grass growth and evaporation when energy availability was high whereas energy was limiting when water was freely available. In contrast, in temperate pastures, where rainfall is spread more evenly throughout the year, evaporation is less controlled by water availability and more by the seasonal progression of available energy. The focus of this review is on intensively grazed temperate pastures and consequently the following discussion will review ecosystem scale evaporation measurements from managed temperate grasslands.

Prior to the widespread use of eddy covariance to measure E continuously, Green et al. (1984) used the Bowen ratio method to measure E from a ryegrass, clover, and prairie grass pasture grazed by dairy cows in New Zealand. Bowen ratio measurements were compared to weighing lysimeter measurements and Priestley-Taylor estimates over the autumn and spring when moisture stress was minimal. Measured spring E was 101 mm and autumn E was 66 mm, which were 10-20% higher than measurements from the weighing lysimeter. Agreement was better for rain free days. Agreement with Priestley-Taylor estimates were also reasonable suggesting evaporation was largely weather driven. One of the few other studies measuring E from temperate grazed pastures was conducted by Harding et al. (2000). Eddy covariance was used to measure E from intermittently grazed *Lolium perenne-Cynosauriis cristatus* pasture over three consecutive years in the south of England to inform long-term modelling (partitioning of net radiation at the land surface). At this site, 80% of available energy was partitioned toward the latent heat flux (LE) suggesting water was not limiting for most of each year.

During summer, partitioning toward LE declined towards 70% of available energy when soil moisture deficits developed and more energy was partitioned toward H . Unfortunately, the eddy covariance data were not gap filled and therefore no information on seasonal or annual evaporation totals were presented. Prior to the inception of this thesis little other work had been published from intensively managed temperate grasslands.

Since 2014, there have been a number of publications that include E measurement from rotationally grazed temperate pastures both within New Zealand and internationally. Work from this thesis, Pronger et al. (2016), published some of the first replicated paddock scale E measurements and is the subject of Chapter 3. Graham et al. (2016) compared evaporation between an irrigated intensively grazed ryegrass-clover dairy pasture and nearby irrigated ryegrass seed crop in the South Island of New Zealand. Daily to annual E totals were very similar between sites and both sites responded to variation in R_n , air temperature, and atmospheric vapour pressure deficit (VPD) in a similar manner. However, when irrigation ceased at the seed harvest site, evaporation declined at the harvested site relative to the pasture site as water became limiting. In agreement with earlier work by Green et al. (1984) and Harding et al. (2000) the finding of Graham et al. (2016) suggested E was strongly controlled by radiation during most of each year and consequently sudden reductions in leaf area post-grazing had little effect on evaporation.

Annual E totals for intensively managed New Zealand grasslands from more recent studies suggest differences between sites are relatively small. Graham et al. (2016) reported annual total E of 791 in year 1 and 819 mm in year 2 for the aforementioned irrigated South Island pasture site. Duncan et al. (2016) estimated annual E to be 792 mm based on water balance residuals from drainage lysimeters spread across three different South Island pasture sites. A similar annual total of 818 mm was calculated from daily mean E reported by Kirschbaum et al. (2015) for a non-irrigated ryegrass-clover pasture site in the Waikato Region of New Zealand. These totals were considerably higher than for European grasslands where a modelling synthesis study by Ma et al. (2015) reported an annual mean measured E of 450 mm across 12 managed European grasslands. Mean annual

rainfall and temperature were lower at all these European grasslands compared to New Zealand grasslands and this likely explains the large differences in annual totals. Ma et al. (2015) classified the sites using the relationship between the climatic water balance and the De Martonne-Gottman aridity index and the three sites with the most similar climate to New Zealand still had considerably lower annual E totals relative to the New Zealand. These sites were in Ireland (MAP 1271 mm, MAT 9.6 °C, annual E 547 mm (Byrne and Kiely, 2006)), Switzerland (MAP 1197 mm, MAT 9.3 °C, annual E 539 mm (Ammann et al., 2007)), and France (MAP 1072 mm, MAT 7.8 °C, annual E 665 mm (Klumpp et al., 2011)). Results from the PaSim model Ma et al., (2015) were calibrating were relatively good with mean simulated E of 415 mm year⁻¹ (compared to mean measured E of 450 mm year⁻¹). The largest differences between modelled and measure E occurred during periods of moisture limitation.

The seasonal progression of environmental forcing is also considerably different for many European grasslands compared to New Zealand. For example, at the German pasture site (MAP 1033 mm, MAT 7.7°C) studied by Gebler et al. (2015), the ryegrass and smooth meadow pasture was covered by snow during winter and as a result E almost ceased. Annual E totals at this German site were 488, 507, and 520 mm for eddy covariance, lysimeters, and Penman Monteith estimates respectively, similar to the mean value reported by Ma et al. (2015) for European grasslands. Close agreement between measured and modelled evaporation suggested E was largely driven by weather and not limited by water. Monthly E at the German grassland was highest in late summer (about 100 mm in August) (Gebler et al., 2015) which is similar to maximum monthly values during summer in New Zealand (Kuske, 2009). However, in contrast to many grasslands globally, in New Zealand evaporation continues year-round (no snow cover) and this likely explains the large differences in annual totals despite seemingly closer agreement during warm seasons.

2.5 Water use efficiency

The previous section focused on E at the ecosystem scale. However, in agricultural grasslands WUE is important and clearly, in an environment where soil water shortage occurs frequently, maximising carbon gain or biomass

production per unit of water used is important. Leaf theory suggests that WUE (defined in Section 2.5.1) is highest at low stomatal conductance (Osmond et al., 1980) and therefore higher productivity, associated with increased stomatal conductance, comes at the cost of lower WUE. However, in hot dry conditions so long as plants can access soil water, increased stomatal conductance and hence transpiration has a cooling affect that can enhance WUE by reducing leaf temperatures to more optimal values for photosynthesis. WUE then reaches a maximum at some intermediate conductance value (Osmond et al., 1980). This interaction suggests that increased WUE does not always occur at a cost to production. Review of ecosystem scale grassland research (see Section 2.5.2) suggests that WUE is often higher in more productive ecosystems and during more productive seasons and review of controlling factors (see Section 2.5.5) shows WUE can vary between species and because of variation in the nutrient supply and the environment.

This section will begin by defining WUE and how the definition can change depending on purpose and scale of interest. Attention will then turn to paddock and ecosystem scale WUE literature with a focus on intensively grazed pastures. However, because of the scarcity of literature in this area measurements from extensively managed grasslands are also included where appropriate. Finally, focus will turn to factors which control WUE. While WUE literature from other grasslands has been included for context where appropriate the review will not include discussion regarding WUE from native grasslands (e.g. Flanagan and Farquhar, 2014; Ponton et al., 2006) or grasslands managed for other purposes such as switchgrass for biofuel production (e.g. Eichelmann et al., 2016; Wagle and Kakani, 2014).

2.5.1 Water use efficiency definitions

There are a number of methods used to measure primary production and consequently there are a number of definitions of WUE (see Table 2.1). Commonly used definitions of WUE are leaf scale WUE, ecosystem scale WUE (EWUE), and harvest WUE (HWUE). At the leaf scale, photosynthetic WUE is defined as the ratio of carbon fixed during photosynthesis (A) to water lost during transpiration (E_T). However, at the ecosystem scale, EWUE is typically defined

as the ratio of gross primary production (GPP) to total evaporation (E) and therefore ecosystem scale measurements include evaporation of soil water and leaf interception (Yang et al., 2010). In agronomy literature HWUE is defined as the ratio of above ground harvestable dry matter (DM) to water use because above ground production is ultimately what drives farm productivity and hence profit. Generally, there is a good correlation between EWUE and HWUE (Lambers et al., 2008) despite the HWUE not accounting for below ground production.

Other specialist definitions of WUE are also used. For example, in plant physiology literature intrinsic WUE (WUE_i) is defined as the ratio of A to stomatal conductance (g_s). At an ecosystem scale an equivalent measure is the ratio of GPP to surface conductance (G_s). Biome WUE (BWUE) is occasionally used in ecological literature and is calculated as the ratio of net ecosystem productivity (NEP) to E and therefore accounts for soil and plant respiration. Inherent WUE (IWUE) is also used occasionally (e.g. Beer et al., 2009) to compare WUE between different ecosystems while normalising for differences in the VPD (because increasing VPD disproportionality increases E relative to carbon fixation – see Section 2.5.5). IWUE is calculated as the ratio of GPP to E/VPD .

Table 2.1 Definitions of leaf, ecosystem, and harvest WUE (Ponton et al., 2006; Eichelmann et al., 2016) compared to intrinsic (ratio of carbon assimilation to stomatal or bulk surface conductance, (Farquhar et al 1989)) and inherent WUE (ratio of carbon assimilation to E/VPD , (Beer et al., 2009; Brown et al., 2005; Zhou et al., 2014))

Scale of interest	Water use efficiency (WUE)	Intrinsic water use efficiency (WUE_i)	Inherent water use efficiency (IWUE)
Leaf (WUE)	A/E_T	A/g_s	
Ecosystem (EWUE)	GPP/E	GPP/G_s	$GPP*VPD/E$
Harvest (HWUE)	DM/E		$DM*VPD/E$
Biome (BWUE)	NEP/E		

A = leaf carbon assimilation (μmol or g m^{-2}), E_T = transpiration (mm, kg or moles), g_s = stomatal conductance, G_s = surface conductance, GPP = gross primary production (μmol or g m^{-2}), E = ecosystem evaporation (mm, kg, moles), VPD = vapour pressure deficit.

2.5.2 Ecosystem WUE in grazed grassland ecosystems

At a global scale, mean EWUE estimates vary from about $1.80 \text{ g C (kg H}_2\text{O)}^{-1}$ based on MODIS to $2.6 \text{ g C (kg H}_2\text{O)}^{-1}$ from global EC tower based measurements (Zhang et al., 2016). At grazed grassland sites mean annual EWUE values vary widely across the globe with reported annual values ranging from $0.40 \text{ g C (kg H}_2\text{O)}^{-1}$ for short sparse vegetation on sandy soils with low rainfall in China (Hu et al., 2008) up to $3.79 \text{ g C (kg H}_2\text{O)}^{-1}$ for a temperate mountain valley grassland site in Austria (Wohlfahrt et al., 2008b). The focus of this research is on temperate grasslands and EWUE for temperate systems are typically toward the upper end of this global range.

In New Zealand, Kirschbaum et al. (2015) modelled carbon and water exchange over an intensively grazed non-irrigated dairy pasture and compared modelled values to measurements made by eddy covariance. Using their mean annual measured values, EWUE was $2.6 \text{ g C (kg H}_2\text{O)}^{-1}$ at an annual scale. During this study, a severe 1 in 100-year drought occurred which likely decreased annual scale EWUE. Graham et al. (2016) contrasted EWUE between an irrigated ryegrass-clover pasture and ryegrass pasture harvested for seed production in Canterbury, New Zealand. Annual scale EWUE was higher than in the Waikato (Kirschbaum et al., 2015) but similar at both irrigated sites (3.09 and $3.25 \text{ g C (kg H}_2\text{O)}^{-1}$ for the pasture and seed harvest site respectively). Also in Canterbury, EWUE calculated from GPP and E measurements over ryegrass-clover pastures reported by Hunt et al. (2016) varied from $2.37 \text{ g C m}^{-2} \text{ (kg H}_2\text{O)}^{-1}$ for a non-irrigated ryegrass-clover pasture up to $3.41 \text{ g C m}^{-2} \text{ (kg H}_2\text{O)}^{-1}$ for an adjacent irrigated ryegrass-clover pasture. These values from New Zealand are similar to mean values calculated for managed grasslands across Europe. Ma et al. (2015) compared measured to modelled water and carbon fluxes across multiple managed European grasslands and from their measured data calculated mean EWUE was $2.73 \text{ g C (kg H}_2\text{O)}^{-1}$ across all sites. Across these European sites, there was no relationship between EWUE and the aridity index or the climatic water balance. Management intensity was contrasted at one of the sites used by Ma et al. (2015). At this site in France (Klumpp et al., 2011), EWUE was higher under intensive management ($2.08 \text{ g C (kg H}_2\text{O)}^{-1}$) compared to an extensive management. Both

total E and GPP were higher under intensive management but the increase in GPP was relatively higher than for E .

2.5.3 Seasonal and inter-annual variation in EWUE

EWUE varies inter-annually and seasonally largely because of climatic forcing of environmental factors that control photosynthesis and evaporation such as soil water content, VPD, leaf area, and light quality (Yang et al., 2010). In general, drought decreases EWUE. Hu et al. (2008) measured EWUE over three consecutive years at four different sites on the Qinghai-Tibet Plateau in China. At the native grassland site EWUE was 0.95 and 1.15 g C (kg H₂O)⁻¹ for 2003 and 2004 but reduced to 0.31 g C (kg H₂O)⁻¹ in 2005 during a severe drought year where soil water deficits reduced leaf area and living biomass while the VPD increased. Hussain et al. (2011) also reported lower EWUE at an extensively managed grassland site (harvested for hay 2 – 3 times per year) during a drought year (2.39 g C kg⁻¹ H₂O) compared to a more normal year (2.82 g C kg⁻¹ H₂O). Evaporation totals were similar for both years despite the higher VPD during the drought year (reflecting interactions between soil water deficit and tight stomatal control) but GPP was considerably lower during the drought year and hence EWUE was lower. These studies show that drought typically decreases EWUE because photosynthesis ceases while very small amounts of E continue.

A number of studies have reported strong seasonal variation in EWUE. Hu et al. (2008) found EWUE was highest at the peak of the growing season (July-August) tracking closely with patterns in GPP. Further analysis suggested that variation in WUE and GPP were closely related to leaf area, probably because leaf area controls potential carbon assimilation and the ratio of transpiration to total evaporation (i.e. the proportion of non-productive water loss from soil). Hu et al. (2008) showed that EWUE and GPP were tightly coupled until soil moisture deficits became extreme (temperate steppe site during 2005 drought) resulting in the cessation of photosynthesis but continued evaporation (at a very low rate) and consequently a strong decline in EWUE and the de-coupling of EWUE and GPP. In a native Canadian grassland, Flanagan and Farquhar (2014) also found periods of increased EWUE were correlated with periods of increased GPP in contrast to decreased E . EWUE was highest at the peak of the growing season (May – June)

and lowest in March. Similar seasonal variation in EWUE was also reported by Schapendonk et al. (1997) for perennial ryegrass in the Netherlands where EWUE peaked in May and June. Minimum EWUE occurred in summer (mid-August) because the VPD was at its maximum and because photosynthesis became light saturated so was unable to respond to further increases in irradiance while transpiration increased more linearly with irradiance (Schapendonk et al., 1997). Seasonal variation in EWUE appears to be most tightly linked to variation in GPP which is often mediated by leaf area. In contrast, changes in leaf area have a smaller relative effect on E and consequently reductions in leaf area typically decrease EWUE because of the resulting reduction in GPP.

2.5.4 Synthesis studies of EWUE

Law et al. (2002) carried out one of the first synthesis studies that compared fluxes of carbon and water across a range of biomes (deciduous and evergreen forest, grasslands, crops, and tundra) from early global FLUXNET data. In contrast to later research, their data synthesis suggested EWUE was similar across a wide range of biomes when averaged across an entire year and therefore they concluded that physiological processes controlling EWUE probably reached a sustainable balance when averaged over a year. Interestingly, grasslands (only two sites) had marginally higher annual EWUE than any other ecosystem. A later synthesis by Beer et al. (2009) analysed IWUE (see Table 2.1 for definition) across a wide range of plant functional types and climatic conditions and found that grasslands typically had lower IWUE compared to forest systems. In contrast to much of the ecosystem scale work, Beer et al. (2009) found that IWUE generally increased during short term moderate drought. This was likely because the metric they used (IWUE) minimises the influence of increased VPD during drought (similar to leaf level intrinsic WUE). Beer et al. (2009) also found that canopy light interception (which sets the upper limit for canopy photosynthesis) explained half the variance in IWUE for herbaceous ecosystems. Xiao et al. (2013) analysed the magnitude, spatial patterns, and controlling factors of EWUE for major terrestrial ecosystems across China. Across all ecosystems there was a non-linear positive correlation between EWUE and annual precipitation, GPP, and growing season length. Higher productivity ecosystems (forests and coastal wetlands) had higher EWUE than lower productivity ecosystems (grasslands and

croplands). The findings of Xiao et al. (2013) and Beer et al. (2009) again suggest leaf area is a strong determinate of EWUE and IWUE because of the influence of leaf area on light interception and GPP and lesser effect on E .

2.5.5 Factors influencing variation in EWUE in grazed pastures

Variation in grassland EWUE is regulated by interactions and feedbacks among multiple forcing variables including temperature, VPD, leaf area, soil water content, light quantity and quality, soil and leaf nitrogen content, plant phenology and physiology (Yang et al., 2010) and pastoral management. Separating the effect of these factors on EWUE is problematic because of interdependencies. For example, when soil water deficits occur, air temperature and the VPD simultaneously increase and plant productivity declines resulting in less green leaf area and lower light interception. The combined effect is usually a decline in EWUE. The following section discusses each of these factors individually but it is important to appreciate that they are often interlinked and therefore somewhat inseparable.

2.5.5.1 Saturation vapour pressure deficit

Plants typically respond to increasing VPD by closing stomata which theoretically increases WUE at the leaf level because stomatal closure reduces transpiration more than CO_2 uptake. This differential effect on CO_2 uptake and water vapour loss largely occurs because the total resistance to CO_2 diffusivity is less affected because of the additional presence of leaf internal resistance to CO_2 diffusion whereas water vapour is only controlled by stomatal and leaf boundary layer resistance (Chapin et al., 2012). However, at the ecosystem scale other sources of water loss cannot be constrained by plants and the VPD is often reported as the overriding control at ecosystem scales with high VPD correlated low EWUE (Beer et al., 2009; Ponton et al., 2006; Scanlon and Albertson, 2004). For grasslands and crops, Law et al. (2002) found the overriding control of VPD was limited to the summer growing season for temperate vegetation. In colder climates and during the rest of the year (excluding summer), temperature and phenology were more important than VPD. Law et al. (2002) found high VPD reduced EWUE in grasslands and croplands more than in forests. However, during summer all sites reached consistently low EWUE when the VPD exceeded

~ 1.5 kPa. Hu et al. (2008) claimed that the dependence on atmospheric VPD is often only dominant for short term studies (not whole growing seasons) because variation in the atmospheric VPD can be high relative to small changes in leaf area over short time periods. This suggests the dominant control on EWUE changes depending on the timescale investigated and the magnitude of change in other factors such as leaf area.

2.5.5.2 Leaf area

Leaf area controls the potential for photosynthesis and the partitioning of evaporation but may have smaller relative influence on total ecosystem evaporation (see Section 2.4.4). When leaf area is high, most water lost is via transpiration (a 'productive' use of water) compared to soil evaporation (non-productive loss of water). Hu et al. (2008) found that leaf area was the dominant factor controlling seasonal variation in EWUE. EWUE was largely regulated by the effect of leaf area on carbon assimilation and the ratio of transpiration to total evaporation. Wohlfahrt et al. (2008b) also found that environmental drivers of NEE were largely obscured by the strong response of GPP to variation in the green leaf area in an alpine grassland. The seasonal effect of environmental drivers was mediated by cutting events which decoupled the seasonal course of environmental drivers. These studies suggest EWUE and leaf area are positively correlated because of the positive relationship between leaf area and GPP. In contrast, total E is less sensitive to leaf area because decreased E_T maybe compensated for by increased E_S as a result of more radiation reaching the soil surface (Beer et al., 2009). However, short-term variation in factors such as atmospheric VPD, light quality, and water availability can reduce photosynthesis below the potential of the leaf area present. Long-term, soil water availability, and hence climate, largely controls leaf area (Law et al., 2002) and consequently EWUE is likely higher in temperate compared to arid environments.

2.5.5.3 Light quality

Intercepted shortwave radiation controls the energy available for photosynthesis and is therefore an important plant growth determinant that sets the upper limit for canopy-scale photosynthesis (Beer et al., 2009; Lambers et al., 2008). Light interception is in turn largely dependent on leaf area (Lambers et al., 2008).

Hussain et al. (2011) found that photosynthetically active radiation (PAR) explained 80% of the daytime variation in GPP during grassland canopy development over a growing season. Light use efficiency was found to decline during drought, most likely because plant senescence reduced the green leaf area thus reducing the capacity for CO₂ assimilation (Hussain et al., 2011). The proportion of diffuse to direct photosynthetically active radiation also has potential to control EWUE of grasslands especially when the leaf area is high. Under more diffuse light conditions, leaf temperatures and the VPD are reduced and light is distributed throughout the canopy more efficiently enhancing photosynthesis (Law et al., 2002). Rocha et al. (2004) investigated the effect of the proportion of diffuse to total PAR on EWUE using eddy covariance and meteorological data at an aspen dominated hardwood forest. They found that midday EWUE increased as the proportion of diffuse light increased which was typically associated with increasing cloud cover. However, because evaporation reduced under more diffuse conditions (presumably associated with a lower VPD and leaf transpiration rates) determining the mechanism or combination of mechanisms responsible for the observed increase in EWUE under diffuse conditions was problematic (Rocha et al., 2004).

2.5.5.4 Temperature

Biochemical processes controlling photosynthesis, plant growth, and WUE are affected by temperature and different plants have evolved different optima and tolerances to temperature extremes (Lambers et al., 2008). In C3 plants high temperatures (>25°C) increase photorespiration and simultaneously reduces photosynthesis because more O₂ molecules displace CO₂ at the receptor sites on Rubisco (Lambers et al., 2008). Therefore, excessively high temperatures are likely to reduce photosynthesis while simultaneously increasing *E* and reducing EWUE. For example, the optimum temperature range for ryegrass (5 – 18°C) is lower than for white clover (8 - 23°C) (DairyNZ, 2006) and consequently if temperature was the only varying factor EWUE should be higher for clover compared to ryegrass at temperatures between 18 – 23°C.

2.5.5.5 Soil water deficit

Plant transpiration must be replaced by soil water uptake which is dependent on plant root distribution, soil water content, and hydraulic conductivity within the soil matrix (Beer et al., 2009). When water loss from transpiration exceeds soil water recharge (over extended periods) soil water deficits develop and in response plants will typically reduce stomatal conductance in order to reduce transpiration. Leaf scale theory suggests reduced stomatal conductance should increase WUE (Osmond et al., 1980). However, reduced transpiration increases the sensible heat flux driving increased air temperature and VPD both of which usually reduce EWUE. When soil water deficits are prolonged, the photosynthetic apparatus may be impaired through photo inhibition, reduced mesophyll conductance, and loss of turgor resulting in plant senescence and reduced green leaf area and thus light interception (Yang et al., 2010) all of which reduce EWUE. Therefore, at the ecosystem scale, high soil water deficits likely reduce EWUE. However, differences in plant traits such as rooting depth and thus access to water will cause variation in response between species and locations.

2.5.5.6 Biotic adaptations including photosynthetic pathways

Large differences occur in the ratio of intercellular to atmospheric CO₂ concentration (C_i/C_a) and WUE between plants with different photosynthetic pathways (C3, C4, CAM) (Farquhar et al., 1989) and different life histories or functional groups (Ponton et al., 2006; Smedley et al., 1991). WUE is higher in plants that use the CAM ($4 - 20 \text{ mmol C mol}^{-1} \text{ H}_2\text{O}$) and C4 ($4 - 12 \text{ mmol C mol}^{-1} \text{ H}_2\text{O}$) photosynthetic pathways while woody C3 plants ($2 - 11 \text{ mmol C mol}^{-1} \text{ H}_2\text{O}$) are usually more efficient than herbaceous C3 plants ($2 - 5 \text{ mmol C mol}^{-1} \text{ H}_2\text{O}$) with smaller differences within species of the same pathway (Lambers et al., 2008). Many of the plant species used in grazed pastures are C3 and differences in a number of biochemical, physiological, and morphological plant traits can result in different gas exchange characteristics (Comstock and Ehleringer, 1992). Therefore, different plant functional groups (i.e. grasses compared to herbs) can show systematic variation in their gas exchange characteristics leading to variation in WUE (Ponton et al., 2006; Smedley et al., 1991). Smedley et al. (1991) showed that carbon isotope discrimination was usually lower in grasses

than herbs, and lower in perennials than annuals, indicating WUE was higher in grasses relative to herbs and perennials relative to annuals.

Many plants have adapted strategies to cope with drought (leaf shedding, leaf rolling, drought dormancy, or the CAM photosynthetic pathway (Lambers et al., 2008)) which are not desirable in a agronomic systems where continued production is required. In production systems strategies to increase WUE or access to water are desirable with deeper roots being an obvious advantage in deep soils or where shallow ground water can be accessed.

2.5.5.7 Nitrogen

Leaf nitrogen content strongly influences the CO₂ carboxylation rate (Hussain et al., 2011) with typically large proportions (50 – 80%) of plant N being allocated for the synthesis of photosynthetic protein (Evans, 1989). Therefore, high leaf nitrogen content will likely enhance the photosynthetic rate with little increase in transpiration and hence increase WUE. Moot et al. (2008) found that HWUE was significantly higher for N fertilised cocksfoot pasture (38 kg DM ha⁻¹ mm⁻¹) compared to a non-fertilised control (17 kg DM ha⁻¹ mm⁻¹) suggesting integration of legumes and strategic use of N will provide higher WUE. Ripullone et al. (2004) found EWUE and HWUE both increased as leaf N content increased in douglas fir and poplar trees. Leaf N content had no effect on the transpiration rate or stomatal conductance and thus the mechanism underlying the observed increase in WUE was related to increased photosynthetic rates (Ripullone et al., 2004).

2.5.5.8 Grazing management

The frequency, timing, and duration of grazing and cutting events in managed grasslands increase seasonal and inter-annual variation in GPP (Wohlfahrt et al., 2008b) with ensuing consequences for ecosystem scale calculations of EWUE. In the past grazing was viewed as a predation-like plant-herbivore relationship which was detrimental to plant growth (De Mazancourt et al., 1998). However, more recently it has been recognised that grazing could increase plant production depending on the complex interaction between a number of functional processes controlled by grazing (Leriche et al., 2001). For example, biomass reduction via grazing may reduce the intensity and duration of water stress for plants (Archer

and Detling, 1986), reduce self-shading (Jameson, 1963), accelerate nutrient cycling (De Mazancourt et al., 1998; Odriozola et al., 2014), and modify the allocation of assimilates within the plant increasing photosynthetic rates within remaining plant tissue (Caldwell et al., 1981; Leriche et al., 2001). Explanation of the observed variability in recovery from cutting/grazing events has received little attention and current soil-vegetation-atmosphere-transfer (SVAT) models struggle to simulate the recovery period following grazing/cutting. Improving our understanding of this recovery period is required to assess potential benefits of altered grassland management practices (timing, frequency, and intensity of grazing events) (Wohlfahrt et al., 2008b). It is likely that increasing post-grazing pasture residuals, and consequently reducing non-productive water loss, would increase EWUE.

2.5.6 Harvest WUE of pastoral systems

Harvest WUE is a measure of the amount of above ground utilisable DM grown per unit water used and is therefore more directly linked to agronomic productivity and consequently farm profit. In contrast to EWUE, water consumption is often measured as the change in soil moisture content and therefore HWUE often excludes E_T . In some cases, E_S is also removed by modelling E_S and subtracting this from water use as estimated from changes in soil moisture content (e.g. Brown 2004). While such approaches are useful for examining differences in plant level WUE when the objective is to improve efficiency of water use at the ecosystem scale it is preferable to measure all sources of evaporation.

In New Zealand, under irrigation, Nobilly (2015) compared HWUE between standard ryegrass-clover pasture and a range of more diverse mixtures which included legumes, herbs, and grasses. HWUE was higher in more diverse pasture mixes over the summer and early autumn months when additional legumes were included compared to simple grass mixes and mixes that contained herbs. These findings suggested increases in HWUE were attributable to lucerne which has deeper roots and higher leaf N content. Brown et al. (2005) compared production and soil water extraction among pure red clover, chicory, and lucerne crops over a six-year period. Measurements suggested lucerne had higher HWUE but it was

suggested that this was likely because of water extraction from below the maximum depth of measurement (2.3 m). Skinner (2008) found that adding chicory to ryegrass-clover mixes increased early summer production significantly during the first year after establishment but had little effect in the second year. The increased productivity in the first year was attributed to increased HWUE as opposed to increased water use.

Moot et al. (2008) brought together water use and production data from a number of studies in the South Island of New Zealand to examine HWUE. Under dryland conditions, annual HWUE varied considerably from 6.7 kg DM (kg H₂O)⁻¹ for a cocksfoot pasture, to 18 kg DM (kg H₂O)⁻¹ for perennial ryegrass, and up to 40 kg DM (kg H₂O)⁻¹ for lucerne all on the same soil type. Seasonally HWUE varied strongly (between 3 and 22 kg DM (kg H₂O)⁻¹ for ryegrass) and was low during periods of high soil moisture deficits and atmospheric vapour pressure deficits. N inputs were also found to influence HWUE. Under non-water limiting conditions pastures that included legumes and those with high N fertiliser application had higher HWUE. For example, N fertilised cocksfoot pasture produced 38 kg DM (kg H₂O)⁻¹ compared to a non-fertilised cocksfoot control that produced 17 kg DM (kg H₂O)⁻¹.

Results from the studies discussed above suggest increasing diversity is likely to result in increased HWUE. Increased water extraction via deep rooted species will not only benefit the deep rooted plant but is likely to indirectly improve production and HWUE in co-existing shallow rooted species via a hydraulic redistribution and reduction in the atmospheric VPD. An increase in HWUE associated with increased diversity is also likely related to the inclusion of more legumes with increased leaf N content leading to increased photosynthetic efficiency. Therefore, including a range of legumes (for example white clover, red clover, and lucerne) and managing grazing rotations to maximise legume growth and persistence is likely advantageous. The strategic use of fertiliser N will also likely improve HWUE.

2.6 Carbon isotopes and WUE

The carbon isotope composition of C3 plant biomass can provide a time-integrated measure of WUE at both plant and canopy scales that would be difficult or impossible to gain from direct physiological measurements (Lambers et al., 2008).

2.6.1 Carbon isotopes

Atmospheric CO₂ is comprised of two stable isotopes (¹²CO₂ ~99% and ¹³CO₂ ~1%). Plants discriminate against the heavier isotope (¹³CO₂) during photosynthesis and the extent of the discrimination can be used to make time-integrated measurements of photosynthetic performance including WUE. Discrimination against ¹³CO₂ occurs largely because of the biochemical properties of Rubisco which reacts more readily with the lighter isotope (¹²CO₂). To a lesser extent, discrimination against ¹³CO₂ also occurs because of the slower diffusion of ¹³CO₂ in air relative to ¹²CO₂ (Farquhar et al., 1989).

The ¹³C content of organic matter is commonly presented in the literature using both upper and lower case delta – carbon isotope *composition* ($\delta^{13}\text{C}$) and carbon isotope *discrimination* ($\Delta^{13}\text{C}$). Carbon isotope *composition* ($\delta^{13}\text{C}$) represents the ratio of ¹³C/¹²C in a sample with respect to ratio in the internationally accepted standard Vienna Pee Dee Belemite (VPDB). In contrast, carbon isotope *discrimination* ($\Delta^{13}\text{C}$) is the ratio of ¹³C/¹²C in plant material relative to the atmosphere that plants are accessing their C from (e.g. the relative abundance of ¹³C between the source and the product) (Condon et al., 2002). Isotope discrimination can be a more useful descriptor because it is relative to the source and hence provides mechanistic insight into biological processes.

2.6.2 Relationship between discrimination and WUE

The carbon isotope composition of C3 plant biomass can be used to measure WUE because both WUE and $\delta^{13}\text{C}$ share a dependence on the ratio of intercellular (C_i) to atmospheric (C_a) CO₂ concentration (Farquhar et al., 1982). The intercellular partial pressure of CO₂ reflects stomatal conductance (g_s) relative to photosynthetic activity (A) and the ratio of A to g_s is known as intrinsic WUE (WUE_i). When stomatal conductance is high relative to the assimilation rate of

CO₂ the ratio of C_i/C_a approaches 1 and a larger proportion of the ¹³CO₂ discriminated against by Rubisco is able to diffuse back to the atmosphere. The high relative exchange with the atmosphere reduces the fixation of the heavier isotope resulting in the plant isotope composition migrating toward the upper limits of fractionation for C3 plants ($\delta^{13}\text{C}$ approaches -35‰ \approx low WUE). Conversely, when the ratio of C_i/C_a is low because stomatal conductance is small relative to photosynthetic activity, CO₂ drawdown inside the leaf maintains a stronger diffusion gradient into the leaf reducing the opportunity for ¹³CO₂ diffusion out of the leaf. Under such circumstances discrimination against ¹³CO₂ is low and $\delta^{13}\text{C}$ migrates toward the lower limit for C3 plants ($\delta^{13}\text{C}$ approaches -20‰ \approx high WUE) (Ehleringer and Rundel, 1989; Lambers et al., 2008). An increase in WUE can be driven by either a relative increase in C assimilation or a decrease in transpiration. Therefore, in agricultural systems $\delta^{13}\text{C}$ measurements need to be used in conjunction with production measures to avoid selection for plants with high transpiration efficiency but low productivity.

2.6.3 Isotope analysis

Isotope discrimination measurements for investigating WUE can be analysed on bulk leaf biomass or different fractions of the leaf biomass, for example cellulose and carbohydrates. Bulk leaf biomass is the most common approach largely because it is cost effective and is a measure of integrated WUE over the period of leaf growth. In contrast, a more labour intensive approach is to extract carbohydrates (leaf sugars) or cellulose. Leaf carbohydrates typically integrate WUE over a few days prior to harvesting the leaf biomass (Bowling et al., 2008). Moreno-Gutierrez et al. (2012) suggest that when using isotope discrimination to compare WUE between plant species in a homogeneous environment it is advantageous to analyse the stable isotope composition of the leaf cellulose or carbohydrates in contrast to that of bulk organic matter. Measuring the stable isotope composition of leaf cellulose should reduce noise in the relationship because of the absence of species specific organic compounds (oils, waxes, resins, tannins) (Moreno-Gutierrez et al., 2012). In addition to leaf material the isotope composition of respired CO₂ has also been used to measure WUE (e.g. Ponton et al., 2006; Scartazza et al., 2014). Klumpp et al. (2005) showed that although root respiration was depleted and shoot respiration was enriched relative to the whole

plant when respiration sources were combined there was no significant difference between isotope composition of the plant compared to that of respired CO₂. The bulk leaf approach is quick and cost effective and ultimately must be balanced against the advantages of extracting leaf cellulose or carbohydrates.

Importantly, to make inferences about WUE based on carbon isotope discrimination it is important plants compared are subject to an equivalent VPD (Smedley et al., 1991) or that differences in the VPD are accounted for using approaches similar to those developed by Farquhar et al. (1982). Additionally, it is also important that stomatal conductance and internal conductance (mesophyll conductance) are strongly positively correlated (Flexas et al., 2012). There is still uncertainty about how strong the correlation is between stomatal and mesophyll conductance but there is evidence that a positive correlation does occur (Barbour et al., 2010; Evans, 1999) and mesophyll conductance is generally higher in species like grasses and herbs (Flexas et al., 2012).

2.6.4 Isotope WUE and grasslands

Strong correlation between $\delta^{13}\text{C}$ and WUE has been demonstrated for many crops including beans (*Phaseolus vulgaris*) (Ehleringer, 1990), peanuts (*Arachis hypogea*) (Wright et al., 1992), and cowpeas (*Vigna unguiculata*) (Ismail and Hall, 1992) in addition to those from the grass family including wheat (*Triticum aestivum*) (Condon et al., 1990), barley (*Hordeum vulgare*) (Anyia et al., 2007) and range grass (*Agropyron desertorum*) (Ehleringer et al., 1990). However, literature from production grasslands is scarce and the few isotopic studies that have been done focus on natural grassland communities. Smedley et al. (1991) followed the seasonal course of isotopic discrimination in 42 species in an arid southwestern North American grassland. In general, all species increased WUE in response to decreased soil moisture and increased evaporative demand. Short season annuals tended to use water less efficiently than perennials, and herbs less efficiently than grasses. Evidence was also presented that supported the theory that plants that use the same water source (e.g. similar rooting depth) have similar water use patterns. The research of Smedley et al. (1991) demonstrated that WUE varies considerably between co-existing grassland species and that stable carbon isotope composition of leaves was useful for examining this variation.

Later work by Flanagan and Farquhar (2014) compared leaf and ecosystem scale WUE in a non-grazed native Canadian grassland using isotope discrimination and eddy covariance methods. Leaf scale isotope WUE was found to be 2-3 times higher than ecosystem scale WUE and this difference was largely attributed to isotope measurements not accounting for carbon loss through root respiration and water loss through soil water evaporation. In the drier year, WUE was higher using both approaches suggesting that, despite the 2 to 3-fold difference, the methods were correlated and therefore $\delta^{13}\text{C}$ measurement might be suitable for detecting differences in WUE at a paddock scale. However, while isotope WUE was measured at multiple points through each growing season Flanagan and Farquhar (2014) did not present seasonal correlations between the two methods. Scartazza et al. (2014) compared WUE measured using isotopic analysis of bulk leaf, soluble leaf sugars, and respired CO_2 to EC measured EWUE at a Mediterranean shrub land and abandoned agricultural site. Closest agreement was between EWUE and isotope WUE calculated from soluble leaf sugars but all methods worked relatively well. For example, at the abandoned agricultural site EWUE was $3.8 \pm 0.4 \text{ mmol C mol}^{-1} \text{ H}_2\text{O}$ compared to 4.1, 3.3, and 4.7 $\text{mmol C mol}^{-1} \text{ H}_2\text{O}$ for the soluble leaf sugars, bulk leaf, and composition of respired CO_2 .

Due to the scarcity of comparison to ecosystem scale measurements it is still uncertain whether leaf scale $\delta^{13}\text{C}$ is correlated with EWUE and consequently whether $\delta^{13}\text{C}$ can be used as a cost effective method for determining EWUE at paddock scales.

2.7 Summary and identification of research gaps

Dry summer periods present an ongoing challenge for pastoral farmers which may worsen in the future if drought frequency increases as a result of climate change. Review of literature suggests increasing pasture species diversity improves summer pasture growth during both wet and dry summers and this has been suggested to be as a result of both deeper roots and increased WUE. However, few researchers have compared WUE of traditional ryegrass-clover pastures to more diverse mixtures and comparisons at the ecosystem scale are absent. This

review has focused on three areas where there appears to be scope for further novel research and these areas of interest are briefly summarised below.

2.7.1 Evaporation from grazed pastures

Evaporation measurements from intensively managed temperate grasslands were scarce prior to the inception of this thesis (2014). This led to many unanswered questions including the magnitude of annual and seasonal fluxes, spatial variation, controls, and the influence of intensive grazing events. These unknowns contributed to uncertainty in selecting appropriate modelling methods to estimate water vapour fluxes from intensively managed pastures. Since 2014 a number of studies have been published both internationally and within New Zealand that help answer these questions including research from this thesis (see Chapter 3). Graham et al. (2016) compared evaporation at two irrigated grasslands and showed that spatial variation in evaporation was likely low. Additionally, evaporation was insensitive to large changes in leaf area following intensive grazing events. Agreement between measurements and modelled FAO-PM E_o was good during most of each year when water was not limiting. However, agreement between measured and modelled evaporation was poor when irrigation ceased and more complex modelling approaches are likely needed when soil moisture is limiting. Kirschbaum et al. (2015) showed that using the full Penman-Monteith approach and modelling each component (E_T , E_I , E_S) individually maybe more appropriate under water limitation and further work is required in this area. In contrast to the findings of Graham et al. (2016), Gebler et al. (2015) found large reductions in leaf area following harvest events resulted in strong reductions in E . Further work is required to determine the conditions under which leaf area is important. Differences are likely related to the combination of the proportion of biomass removed, rooting depth, and soil moisture content at depth and on the surface. Future experimental design should attempt to disentangle these factors.

2.7.2 Ecosystem WUE in grazed pastures

Given drought frequency is predicted to increase in many locations globally and conflict over water resources is increasing it is critical we evaluate the potential of alternative pasture species to improve dry season production. The inclusion of

species with different life histories and consequently divergent environmental advantages may alter seasonal growth dynamics and potentially improve EWUE and dry season production. Review of EWUE literature found little research has been done in intensively managed pastures. Literature from less intensively managed grasslands showed that annual scale EWUE varies considerably from about $0.40 \text{ g C (kg H}_2\text{O)}^{-1}$ (dry sparse grassland) to $3.79 \text{ g C (kg H}_2\text{O)}^{-1}$ (humid temperate grassland). Inter-annual and seasonal variability in WUE was also significant and generally declines during drought. EWUE appears to be coupled to gross primary production and leaf area as opposed to evaporation which is less sensitive to changes in leaf area. From the review of literature, it was not possible to disentangle the relative effect of pasture species on variation in EWUE because no side-by-side experiments have been done. Comparative, side-by-side study of pasture systems, at the paddock scale using the eddy covariance method is a novel approach to investigating EWUE in pastoral systems. The ability to work from anywhere between half hourly up to annual scales provides the capability to delve into controlling factors and thus improve understanding of these production systems that is not possible using the agronomic approach to measuring HWUE.

2.7.3 Using the natural abundance of carbon isotopes as an indicator of paddock scale WUE

While the eddy covariance technique is well suited for measuring paddock scale WUE and controls it is technically demanding and expensive limiting scope for replication. Seminal work on the relationship between carbon isotope discrimination and WUE was done by Farquhar et al. (1982). The method has been used widely by plant physiologists as a leaf scale indicator of WUE because of the dual dependency of WUE and $\delta^{13}\text{C}$ on the ratio of leaf internal to external CO_2 concentration. To date little work has been done comparing these plant leaf scale measurements to EWUE measurements. The few comparisons that have been done were in natural grasslands (Flanagan and Farquhar, 2014), shrublands (Scartazza et al., 2014), and forest systems (Hu et al., 2010). These studies suggest that the two methods are correlated but the magnitude of absolute agreement varies. For example, in a natural grassland Flanagan and Farquhar (2014) found $\delta^{13}\text{C}$ measurements were 2 to 3 time higher than EWUE while Scartazza et al. (2014) found strong absolute agreement in shrub and abandoned

agricultural landscapes. Using $\delta^{13}\text{C}$ is attractive because the method does not modify the measurement environment, integrates over useful time scales, and is a quick, simple, and cost effective procedure and therefore is suitable for replicated studies ranging from small scale randomised block designs to large scale paired site studies.

2.8 References

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Chapter Three

3 Low spatial and inter-annual variability in evaporation from a year-round intensively grazed temperate pasture system

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The contributions of authors were (also see Appendix D):

Project conception and experimental design was a collaboration between all co-authors. Eddy-covariance measurements, filtering and gap filling were a collaboration between Suusanna Rutledge, Aaron Wall, (who both worked on an aligned net ecosystem carbon balance project) and Jack Pronger with advice from Dave Campbell. Data analysis and writing of the manuscript was done by Jack Pronger. Louis Schipper and Dave Campbell were the primary reviewers of this chapter with Mike Clearwater, Susanna Rutledge, and Aaron Wall providing additional feedback to improve the manuscript.

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3.1 Abstract

Ecosystem scale measurements of evaporation (E) from intensively managed pasture systems are important for informing water resource decision making and validation of hydrologic models and remote sensing methods. We measured E from a year round intensively grazed temperate pasture system in New Zealand using the eddy covariance method for three years (2012 – 2014). Evaporation varied by less than 3% both spatially (770 – 783 mm) and temporally (759 – 776 mm) at an annual scale. The low spatial and temporal variation largely occurred because E was strongly controlled by net radiation ($r^2 = 0.81$, $p < 0.01$, daytime, half-hourly), which did not vary much between sites and years. However, E was strongly limited when volumetric moisture content (VMC) declined below permanent wilting point causing a strong reduction in the decoupling coefficient and an increase in the Bowen ratio. Grazing events appeared to have no effect on E during autumn and winter but reduced E by up to 5% during summer and spring while complete removal of vegetation during autumn herbicide application reduced E by ~30%. This implied that over the pasture regrowth period soil water evaporation (E_S) could provide up to 70% of E relative to a vegetated site (during autumn) and, given that grazing events removed about 60% of leaf area, these findings suggest E_S was likely able to compensate for decreased transpiration post-grazing. Agreement between measured E (E_{EC}) and FAO-56 reference crop E (E_o) was good when soil moisture limitation was not occurring. However, during periods of soil moisture limitation E_o exceeded E_{EC} and a correction factor was needed. We trialled the water stress coefficient (K_s) and a simple three bin VMC correction factor (K_{VMC}) and found the K_{VMC} approach worked better at a daily and monthly scale while both approaches worked well at an annual scale.

Keywords: evaporation, evapotranspiration, eddy covariance, grassland, grazing, FAO-56 Penman-Monteith

3.2 Introduction

Grazed pastures cover about 26% of the global ice free land area (Steinfeld *et al.*, 2006) occupying a larger area than any other land use (Asner *et al.*, 2004). In many parts of the world more intensive rotational grazing of these systems is

occurring to increase global food production largely supported by increased fertiliser use (Tilman *et al.*, 2002; Woodford, 2006). A major constraint to pasture production is the availability of water and this availability is largely controlled by the balance between precipitation and evaporation (E). Measuring E is difficult and expensive and therefore published measurements of E from pastoral systems are scarce. Evaporation measurements are fundamental for understanding hydrological processes, land-atmosphere interactions and terrestrial ecosystem function (Kelliher *et al.*, 1993) and the relative scarcity of measurements is a limitation to the development and validation of Earth system models, primary production models, and remote sensing methods for grassland systems (Seaquist *et al.*, 2003).

Grasslands are generally poorly coupled to the atmosphere (McNaughton and Jarvis, 1991) and therefore the dominant controls on evaporation are typically available energy and water (e.g. Brümmer *et al.*, 2012). Available energy and water are highly variable between grassland sites because of differences in climate resulting in large variation in evaporation patterns and annual totals (Krishnan *et al.*, 2012). Globally, there is a strong correlation between total annual precipitation and evaporation (Zhang *et al.*, 1999; Zhang *et al.*, 2001) demonstrating that variation in annual precipitation patterns and totals will drive variation in E among grasslands. Other factors including turbulent transport (or aerodynamic conductance) and vegetation diversity, density, and structure can also cause variation in E among grasslands (Frank, 2003). Ultimately, evaporation from grasslands is controlled by a combination of meteorological factors (net radiation, air temperature, humidity, and wind speed), crop characteristics (stomatal control, plant height, rooting depth, leaf area, roughness and albedo), and land management and other environmental factors (soil moisture content, soil physical structure, and nutrient availability) (Allen *et al.*, 1998).

Intensive rotational grazing of pasture systems causes regular and rapid reductions in leaf area and this biomass removal, followed by regrowth, could affect E . Evaporative water loss occurs via three pathways including direct evaporation of soil water (E_S), intercepted liquid water from plant surfaces (E_I), and through the plant system via transpiration (E_T). The partitioning among these three pathways

is largely dependent on leaf area (Kelliher *et al.*, 1995; Allen *et al.*, 1998). A reduction in leaf area usually reduces E_T and E_I but can lead to increased E_S (soil compensation) because more radiation and rainfall will reach bare soil surfaces (Bremer *et al.*, 2001; Frank, 2003; Wang *et al.*, 2012). Reduction in leaf area through grazing has, in some cases, been shown to reduce total E by up to 40% on daily time scale (Bremer *et al.*, 2001), and 6-7% on annual time scale (e.g. Day and Detling, 1994; Bremer *et al.*, 2001; Frank, 2003) while in other circumstances grazing appears to have had no effect (Shuttleworth *et al.*, 1989; Stewart and Verma, 1992; Wang *et al.*, 2012). Currently, we do not know whether grazing reduces total E largely because of uncertainty around the degree to which increases in E_S compensate for reductions in E_T (see Falge *et al.*, 2005).

Models are often used to predict E because of the difficulty and expense involved in direct measurement (Green *et al.*, 1984; Fisher *et al.*, 2005). A number of models have been developed for this purpose including by Penman (1948), Penman-Monteith (Monteith, 1965), Priestley and Taylor (1972), McNaughton and Black (1973), and Shuttleworth and Wallace (1985). In the past, Priestley and Taylor (1972) has often been used and recommended in pasture systems (e.g. Green *et al.*, 1984) largely because it required minimal input data (net radiation and temperature). However, more recently Allen *et al.* (1998) have parameterised the Penman-Monteith model and the Food and Agriculture Organisation of the United Nations (FAO) now recommend this parameterised version (FAO-56) as the standard method for estimating E for crops and pasture. The development of this equation was considered a significant milestone in enabling a consistent E estimation methodology (Howell and Evett, 2004). FAO-56 has since been widely used to estimate E (Steduto *et al.*, 2003) in larger and more complex water balance, ecosystem, and Earth system models and is therefore important for the evaluation of water resources, management and monitoring crop water requirements, drought forecasting, and the study of climate change (Zhao *et al.*, 2013). Despite the recognised importance and wide-spread use of the FAO-56 version of Penman-Monteith there have been few comparisons between the model and field scale measurements for intensively grazed temperate pastures.

In their 1993 review, Kelliher *et al.* (1993) highlighted the need for more ecosystem scale measurements of E from grassland systems, and surprisingly, more than 20 years later there is still a paucity of data from intensively grazed pasture systems. For example, a recent modelling study by Ma *et al.* (2015) included measurements of E from multiple grazed pasture systems across Europe. While some of the sites were identified as intensively grazed the mean stocking rate was 0.68 dairy cows ha⁻¹ which is low compared to intensively grazed systems in New Zealand which often exceed 3 dairy cows ha⁻¹. Other grassland E research has largely focused on un-grazed systems (e.g. Wever *et al.*, 2002; Burba and Verma, 2005; Hao *et al.*, 2007; Kurc and Small, 2007; Chen *et al.*, 2009) or very lightly grazed, low productivity systems without year round-grazing (e.g. Hunt *et al.*, 2002; Baldocchi *et al.*, 2004; Li *et al.*, 2006; Li *et al.*, 2007; Aires *et al.*, 2008; Ryu *et al.*, 2008; Bowling *et al.*, 2010; Krishnan *et al.*, 2012). Furthermore, most E studies have been conducted in low rainfall climates, for example, in a summary of E studies using the water balance approach (Zhang *et al.*, 1999) over 80% of sites experienced annual rainfall of less than 1000 mm and none were from year-round rotationally grazed systems.

New Zealand farming systems provide an excellent opportunity to measure E from high intensity grazing operations that are likely to increase globally as other countries in the temperate zone increase production (e.g. South America). In New Zealand, high producing exotic grasslands cover ~ 22% of the land surface (5.8 million ha) and about 90% of this area is not irrigated (Ministry for the Environment, 2009) and therefore reliant on rainfall for growth (Moot *et al.*, 2009). Traditionally, New Zealand pasture systems have been dominated by perennial ryegrass (*Lolium perenne*) and white clover (*Trifolium repens*) with the temperate maritime climate generally being well suited to dryland ryegrass pastures. These high producing ryegrass and clover pastures typically grow between 14,000 kg and 18,000 kg of dry matter (DM) ha⁻¹ year⁻¹ (Tozer *et al.*, 2013). Farms are usually subdivided into paddocks of area 2-3 ha and are rotationally grazed year-round with large herds of cows (~200) grazing for short periods of time (often 12 – 24 hours) resulting in sudden and large reductions in pasture biomass and leaf area.

The primary objective of this study was to quantify the magnitude and temporal and spatial variability in E from an intensively grazed ryegrass and clover pasture system. A secondary objective was to investigate the relationships between E and grazing events to determine whether modelling approaches need to account for grazing to predict E from pastoral systems. Finally, measured E was compared to modeled E using the FAO-56 method. We used three eddy covariance (EC) systems installed on one farm to measure spatial variability and measurements were extended over three years at one site to determine inter-annual variability.

3.3 Methods

3.3.1 Site description

The research site was located on a commercial dairy farm in the Waikato region of New Zealand. The climate is temperate with a strong oceanic influence which moderates extremes, however, extended dry periods often occur in late summer and early autumn (Moot *et al.*, 2009). The 30 year (1981-2010) mean annual rainfall and temperature were 1249 mm and 13.3°C at the nearest climate station 13 km SW of the study sites (NIWA 2010). The farm was ~ 207 ha with paddock sizes generally between 2 and 3 ha and grazed by two herds of dairy cows at a stocking rate of ~ 3.3 lactating dairy cattle ha⁻¹. Pasture species were dominated by perennial ryegrass and white clover. Paddocks were grazed 11 to 12 times per year. Grazing rotations varied from about 21 days in spring when growth rates reached 70 kg dry matter (DM) ha⁻¹ day⁻¹ up to 90 days in mid-winter when growth rates declined to about 15-20 kg DM ha⁻¹ day⁻¹.

The study area was located within a relatively flat alluvial landscape with gently undulating ridges and swales – remnants of ancestral river channels and levees (McLeod, 1992). Since the river abandoned this path and alluvial deposition ceased some 20,000 years BP (before present) the land surface has been covered by a thin mantle of mainly rhyolitic volcanic ashes up to 0.50 m thick. Variation in sedimentation and drainage on the alluvial surface has resulted in the formation of a complex of four different soil types including the Waihou and Piarere (Typic Orthic Allophanic Soils, Hewitt 1998), the Te Puinga (Mottled Orthic Allophanic Soil, Hewitt 1998), and the Waitoa (Typic Orthic Gley Soil, Hewitt

1998). The Te Puninga soil was dominant and had no significant barriers to roots and high profile available water content (243 mm) within the upper 1 m. In the upper 0.3 m permanent wilting point (PWP) was $0.25 \text{ m}^3 \text{ m}^{-3}$, field capacity (FC) was $0.50 \text{ m}^3 \text{ m}^{-3}$ (Landcare Research, 2015) and the lower limit of readily available water content (RAW) was calculated to be $0.35 \text{ m}^3 \text{ m}^{-3}$ using the approach of Allen *et al.* (1998).

Replicate EC systems were installed at three sites across the farm where there was suitable upwind fetch, minimal land surface slope, and few trees to create flow disturbance. Site 1 was located at $37^{\circ}45'55.23''$ S, $175^{\circ}48'04.67''$ E, Site 2 ($37^{\circ}46'07.34''$ S, $175^{\circ}48'08.65''$ E) was located 385 m south of Site 1, and Site 3 ($37^{\circ}45'42.61''$ S, $175^{\circ}47'30.04''$ E) was located 945 m northwest of Site 1. Flux footprints were modelled for each half hour period using the analytical footprint model of Kormann and Meixner (2001) following the method described in Campbell *et al.* (2015) and footprint analysis showed there was no overlap between sites. Measurement started in late 2011 at sites 1 and 2 and in October 2012 at Site 3. Measurements continued until the end of 2014 at Site 1 but stopped at Site 2 and 3 in April 2013 when sites 2 and 3 were sprayed with herbicide (glyphosate) to allow subsequent pasture renewal (a common practice in NZ to increase pasture production (Tozer *et al.*, 2013)). In the current paper we use the period up until April 2013 to investigate spatial variability in E and then focus on Site 1 for three full years to investigate seasonal and inter-annual variation in E .

3.3.2 Data collection

Paddock scale E and net ecosystem CO_2 exchange (NEE) were measured using the eddy covariance (EC) technique. Each EC system included a 3D sonic anemometer (CSAT3, Campbell Scientific Inc., Logan, UT, USA) and an enclosed path gas analyser (LI-7200, LI-COR Inc., Lincoln, NE, USA), both mounted at 1.55 m, to measure water vapour and CO_2 density. Both instruments were operating at 20 Hz. The enclosed path gas analysers were calibrated 6-monthly using CO_2 free air and a known CO_2 standard to set the span and offset following manufacturer's directions. Continuous measurements of meteorological and environmental variables were collected at each site to provide explanatory

data and to aid gap filling of the EC measurements. Air temperature and relative humidity were measured at a height of 1.55 m using a HMP45a (Vaisala Inc., Helsinki, Finland) at Sites 2 and 3 and at Site 1 a HMP45a was used prior to March 2014 and a HMP155 (Vaisala Inc., Helsinki, Finland) post March 2014. Incoming and outgoing shortwave and longwave radiation were measured at a height of 1 m using 4-component net radiometers (NR01, Hukseflux Thermal Sensors, Delft, Netherlands), with net radiation (R_n) calculated as their sum. Soil heat flux (G) was measured using pairs of soil heat flux plates (HFT3, Hukseflux) inserted at 80 mm depth and corrected for heat storage calculated from 30-minute temperature changes in the 0–80 mm soil layer using averaging thermocouples (TCAV, CSI) and volumetric soil moisture content. Thermistors were used to measure soil temperature at 50, 100, and 200 mm depth and soil moisture was measured at the same depths using Campbell Scientific CS616 soil moisture probes (Campbell Scientific Ltd., Shepshed, UK). Precipitation was measured using a tipping bucket rain gauge (TB5, Hydrological Services). All site data were measured by CR3000 and CR1000 dataloggers (Campbell Scientific Inc., Logan, UT, USA) as half hourly values.

3.3.3 Data processing and gap filling

EddyPro software (Version 5.2, LICOR Inc.) was used to compute half hourly mean latent heat flux (LE) and sensible heat flux (H) from high frequency measurements. In addition to the EddyPro quality control flags (Mauder and Foken, 2004) fluxes were filtered for turbulent intensity using the standard deviation of vertical velocity fluctuations (Acevedo *et al.*, 2009) with all periods with velocity $< 0.11 \text{ m s}^{-1}$ being rejected, fluxes sourced from behind the CSAT, additional instrument malfunctions not removed by the EddyPro quality control flags, and periods when cows were grazing more than 5% of the EC footprint. The turbulence threshold was determined following Reichstein *et al.* (2005). Briefly the data set was split into 6 soil temperature classes of the same sample size. Each temperature class was split into 20 turbulence classes. The threshold value was then determined by selecting the turbulence class where the night time flux reached more than 95% of the average flux compared to higher turbulence classes. After filtering about 50% high quality flux data remained with about 70% of gaps occurring at night. Gaps were filled using an artificial neural network

(ANN) following a similar approach to Papale and Valentini (2003). ANNs have generally been found to be superior to other gap filling techniques (Moffat *et al.*, 2007). Input variables to the ANN included net radiation, vapour pressure deficit (VPD), wind speed, air temperature, soil moisture content at 100 mm depth and the phytomass index (see Section 3.3.7). The ANN was constructed with 4 hidden nodes and run in two monthly periods. Day and night time periods were gap filled separately and then combined providing a continuous half hourly means of evaporation for each site.

3.3.4 Improvements in energy balance closure through time

Energy balance closure increased incrementally through time as EC system improvements were implemented. Improvements included increasing air flow through the enclosed path gas analysers from 15 to 18 l m⁻¹ (Jan 2012), heating of the intake tube of the enclosed path gas analysers to reduce relative humidity and hence the incidence of condensation within the tube (June 2012), reducing intake tube diameter to promote turbulent plug flow (February 2013), and the addition of smaller sample tube inlet rain caps to minimise flow distortion (March 2014). The annual energy balance ratio ($EBR = (LE + H) / (R_n - G)$) consequently increased over time from 0.79 for 2012, 0.80 for 2013, and 0.84 for 2014. All system enhancements were aimed at improving measurements of the water vapour covariance and hence increased energy balance closure was attributed to improved measurement of LE . To facilitate inter-annual comparison, we accounted for system improvements by correcting all half hourly measurements of LE relative to the last period after the smaller sample tube inlet rain caps were fitted (we did not force energy balance closure). Corrections were made on a half hourly time step using the change in slope and offset of the relationship between available energy and the convective sum relative to the final period after the smaller sample tube inlet rain caps were installed. The same correction factors were applied at all three sites to avoid any possibility of artificially creating differences between sites. Using this approach, the relative increase in closure was maintained between sites but closure at each site was not forced to be the same. This approach resulted in four correction periods (prior to flow increase, prior to heating the intake tube, prior to reducing tube diameter, and prior to small rain caps) which were each split into day and night and separate corrections applied prior to recombining all

data. On an annual scale these corrections increased Site 1 total E by 6% in 2012, 2% in 2013, and 1% in 2014.

3.3.5 Decoupling coefficient

The decoupling coefficient (Ω) is an index (0 -1) of the decoupling between the vegetation surface and the atmosphere (Jarvis and McNaughton, 1986) and is often used to differentiate between periods when E is controlled by available energy (Ω approaching 1) or by surface conductance and the vapour pressure deficit (Ω approaching 0) (e.g. Hirano *et al.*, 2015). The decoupling coefficient is calculated as follows:

$$\Omega = \frac{\varepsilon+1}{\varepsilon+1+\frac{g_a}{g_s}} \quad (1)$$

where ε is s/γ , s is the slope of the relationship between saturation vapour pressure and temperature (kPa K^{-1}), γ is the psychrometric constant (0.067 kPa K^{-1}), g_a (m s^{-1}) is bulk aerodynamic conductance, and g_s is surface conductance (m s^{-1}). Aerodynamic conductance was calculated from the inverse of aerodynamic resistance to momentum transfer as follows:

$$g_a = \left[\frac{2}{ku_*} \left(\frac{d_h}{d_v} \right)^{\frac{2}{3}} + \frac{U}{u_*^2} \right]^{-1} \quad (2)$$

where k is the von Karman constant (0.4), d_h is thermal diffusivity, d_v is molecular diffusivity of water vapour (d_h/d_v is ~ 0.89 at 20°C (Humphreys *et al.*, 2006)), U is mean wind speed (m s^{-1}), and u_* is friction velocity (m s^{-1}). Surface conductance (g_s) is an integration of individual leaf stomatal conductance (transpiration) and surface wetness for evaporation and was calculated using a rearrangement of the Penman-Monteith equation as follows:

$$\frac{1}{g_s} = \frac{1}{g_a} \left[\frac{\varepsilon R_a + \rho C_p g_a \frac{D}{\gamma}}{LE} - \varepsilon - 1 \right] \quad (3)$$

where available energy, R_a , was approximated as the sum of H and LE , ρ is air density (kg m^{-3}), C_p is the specific heat of air at constant pressure ($1007 \text{ J kg}^{-1} \text{ K}^{-1}$) and D is the vapour pressure deficit (Humphreys *et al.*, 2006; Hirano *et al.*, 2015).

3.3.6 FAO-56 reference crop evaporation

FAO-56 reference crop evaporation (E_o) (Allen *et al.*, 1998) was calculated at a daily timescale as:

$$E_o = \frac{0.408s(R_n - G) + \gamma \frac{900}{T + 273} UD}{s + \gamma(1 + 0.34U)} \quad (4)$$

where R_n and G were measured at each site and are in units of $\text{MJ m}^{-2} \text{ d}^{-1}$, T is air temperature measured at 2 m height ($^{\circ}\text{C}$). U was calculated as the horizontal component of wind speed from the 3D sonic anemometer mounted at 1.55 m height and adjusted to 2 m height using the approach outlined in Allen *et al.* (1998).

The FAO-56 crop coefficient (K_c) was then determined by dividing measured E by E_o when VMC was above the lower limit of RAW to prevent VMC limitations effecting K_c . During periods of soil moisture limitation (when VMC was below RAW) we calculated the soil water stress coefficient (K_s) following the method of Allen *et al.* (1998) to adjust E_o . See supplementary material for further detail.

3.3.7 Analysing the effect of grazing

To determine whether grazing events reduced LE we examined the relationship between LE from the grazed site and LE from a paired un-grazed site (defined below). Relationships were compared using filtered data (not gap-filled). Data were filtered so that 60% or more of the flux footprint (section 2.1) came from the paddock of interest. The phytomass index (PI) was used to compare standing living biomass between sites prior to grazing and to determine the relative magnitude of biomass removal during grazing. PI is a dimensionless index (0 – 1) that is calculated on a daily basis as the difference between average night-time and daytime NEE during non-light limiting conditions normalised to unity at the peak of the growing season (Lohila *et al.*, 2004). The method is likely a better

indicator of primary production than intermittently measured above ground dry matter or leaf area (Lohila *et al.*, 2004; Campbell *et al.*, 2015) and at our site sudden reductions in PI aligned with the timing of grazing events across all time periods investigated. Analysis was constrained to the period prior to April 2013 when Sites 2 and 3 were available for comparison. Selection of grazing events was limited by the requirement that both sites were within 0.1 PI units prior to grazing and the paired site was not grazed within 5 days of the grazed site to allow sufficient half hourly data for comparison. Net radiation and soil moisture content did not vary much between sites and therefore by using the paired site approach we were able to control for changes in drivers over the pre- and post-grazing period.

3.3.8 Statistical analysis

All statistical analyses was carried out using the MATLAB Statistics toolbox (MathWorks Inc., R2012a). One-way analysis of covariance (ANOCOVA) models were used to determine if differences in regression slopes were significant. A p-value of less than 0.05 was considered a significant difference. Linear regression statistics (coefficient of determination and RMSE) were calculated using the LinearModel.fit function.

3.4 Results

3.4.1 Seasonal variation in meteorological and environmental drivers of evaporation

Seasonal patterns of incoming shortwave radiation (K_{\downarrow}) and air temperatures were relatively similar across years and did not vary much from the 30 year normal (Figure 3.1a). Rainfall patterns varied between years with very low mid and late summer rainfall in 2013 and low late summer and early autumn rainfall in 2014. Summer rainfall over 2013 and 2014 was much lower than the 30 year normal (Figure 3.1b). Low summer rainfall in 2013 and 2014 resulted in VMC remaining below PWP for about 54 days during the late summer of 2013 and about 29 days during late summer and autumn of 2014 and PWP was not reached in 2012 (Figure 3.1c). The low rainfall and subsequent depletion of soil moisture during the late summer and early autumn of 2013, and autumn of 2014 resulted in very high VPD in 2013 and high VPD in 2014 relative to the summer and autumn of

2012 (Figure 3.1d). R_n followed a similar pattern to $K\downarrow$ with only minor differences between years. Generally, LE followed the seasonal patterns in R_n reaching peak values in mid-summer and minimums in mid-winter. However, LE decoupled from $K\downarrow$ and R_n in late-summer and early-autumn of both 2013 and 2014 when the lack of available soil moisture drove a peak in H . Therefore, the seasonal peak in H lagged the seasonal peak in R_n . G was typically slightly positive during summer when radiation was heating the soil and slightly negative in winter when the soil was providing energy back to the atmosphere (Figure 3.1e). The annual energy balance ratios at our sites improved through time (see Section 2.4) but were between 0.79 and 0.84, which is in the typical range of EC studies (Wilson *et al.*, 2002).

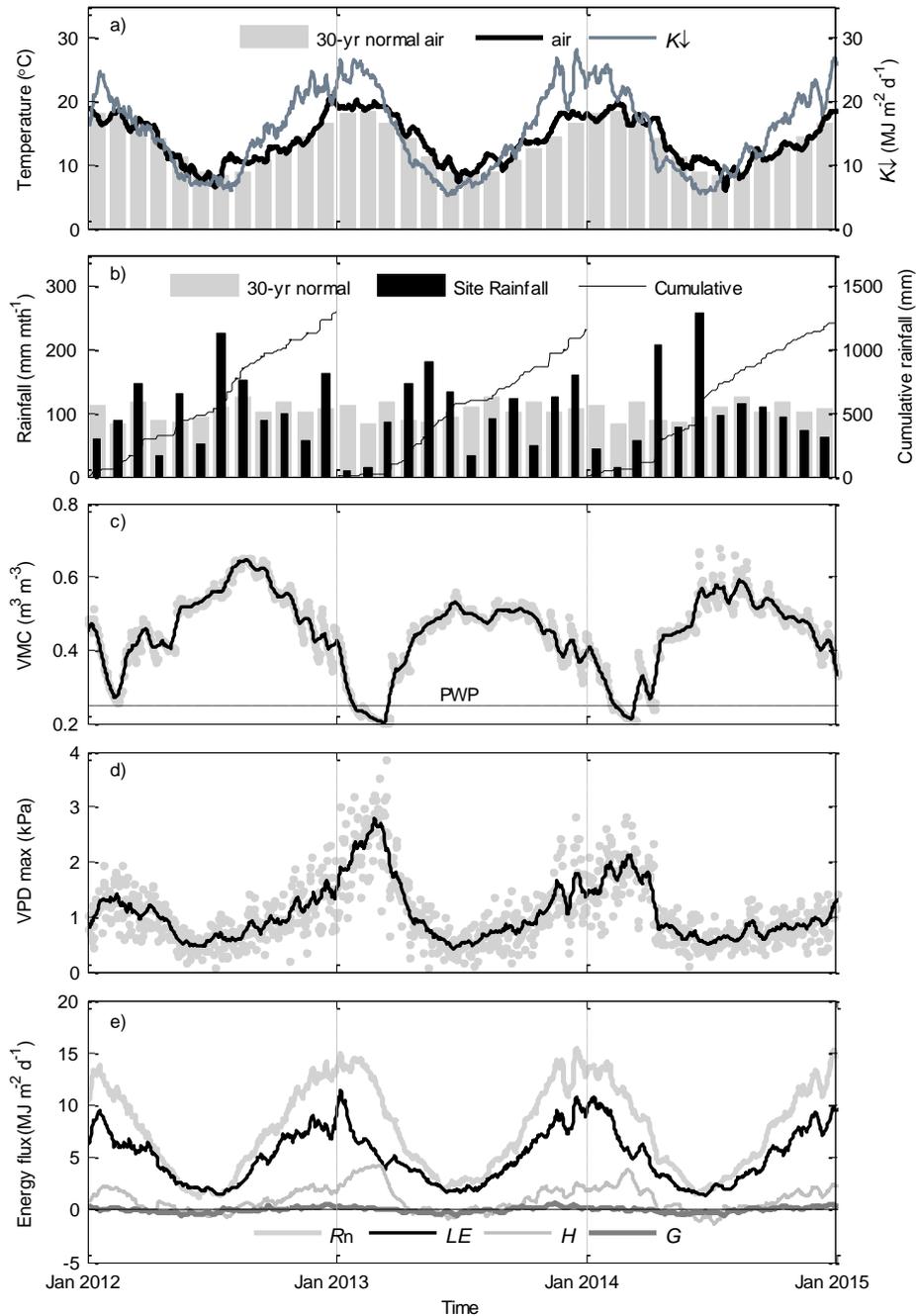


Figure 3.1. Variation in environmental conditions at Site 1 across the 3 site years; (a) monthly normal air temperature (1981 – 2010) and 15 day running mean of measured incoming shortwave radiation and mean daily air temperature; (b) monthly normal rainfall (1981 – 2010) and monthly measured rainfall and cumulative rainfall for each year; (c) daily (grey dots) and 15 day running mean of volumetric soil moisture content at 10 cm depth (dashed line indicates permanent wilting point); (d) daily (grey dots) and 15 day running mean of maximum daily vapour pressure deficit; (e) 15 day running means of 24 hour energy budget terms (net radiation (R_n), latent heat flux (LE), sensible heat flux (H), and the soil heat flux (G)).

3.4.2 Seasonal, annual and inter annual variation in evaporation

Inter-annual variation in E was less than 3% with annual totals at Site 1 of 770 mm in 2012, 757 mm in 2013, and 776 mm in 2014 and mean annual E over the three-year period was 768 ± 11 mm (95% CI). Figure 3.2 shows that differences in daily smoothed E were larger during the December to April period when variation in soil moisture content between years was higher. Lower total annual E in 2013 was largely related to variation in summer rainfall – combined January and February rainfall in 2013 was 25 mm compared to 148 mm in 2012 and 58 mm in 2014 (the 30 year normal was 196 mm over the same two-month period). Consequently, total E over the first quarter of 2013 (when VMC remained below PWP for about 54 days) was 239 mm compared to 260 mm in 2012 and 279 mm in 2014 and this reduction in E occurred despite this first quarter of 2013 receiving the highest sum of R_n (1092 MJ m^{-2}) compared to 2012 (961 MJ m^{-2}) and 2014 (1036 MJ m^{-2}). These were the largest differences in E measured between sites with total E over the first quarter of 2013 being 9% less than 2012 and 17% less than 2014. Over the three years mean seasonal contribution to E was the largest in summer (296 mm or 39%) and spring (237 mm or 31%) and lowest in autumn (149 mm or 19%) and winter (86 mm or 11%).

Spatial variation in daily and seasonal E between sites was also low (Figure 3.3). Mean daily E for 2012 was 2.09 mm at Site 1 and 2.11 mm at Site 2. The coefficient of determination between these two sites at a daily timescale was 0.89 and the RMSE was 0.39 mm. Over the shorter period between October 2012 and March 2013, when all three sites were operating together, mean daily E was 2.87 mm at Site 1, 2.79 mm at Site 2, and 2.78 mm at Site 3. The coefficient of determination between site 1 and 2 over this shorter period was 0.89 and the RMSE was 0.39 mm. The coefficient of determination between sites 2 and 3 over this shorter period was 0.95 and the RMSE was 0.25 mm. Over 2012, annual E totals for Sites 1 and 2 were within 3% of each other when we measured 770 mm from site 1 and 783 mm from Site 2. Total E was also within 3% across all three sites over the period 1st October 2012 until the end of March 2013 with 520 mm at Site 1, 505 mm at Site 2, and 504 mm at Site 3. In general, the only periods where daily and seasonal E varied substantially between sites was during the late

summer and early autumn dry periods (February and March 2013) when maximum differences between sites were about 0.50 mm day^{-1} . Maximum rates of E occurred in early summer when R_n was high but soil moisture limitations had not yet developed. During this period, maximum daily E was about 6 mm (Figure 3.2 is smoothed) and maximum hourly rates were about 0.8 mm.

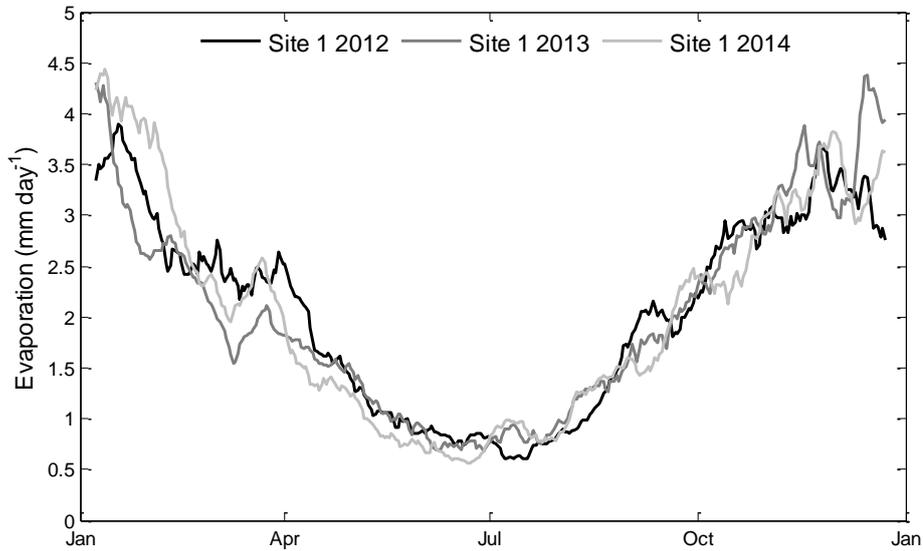


Figure 3.2. Daily smoothed E (15-day running mean) for Site 1 over the three years (2012 – 2014)

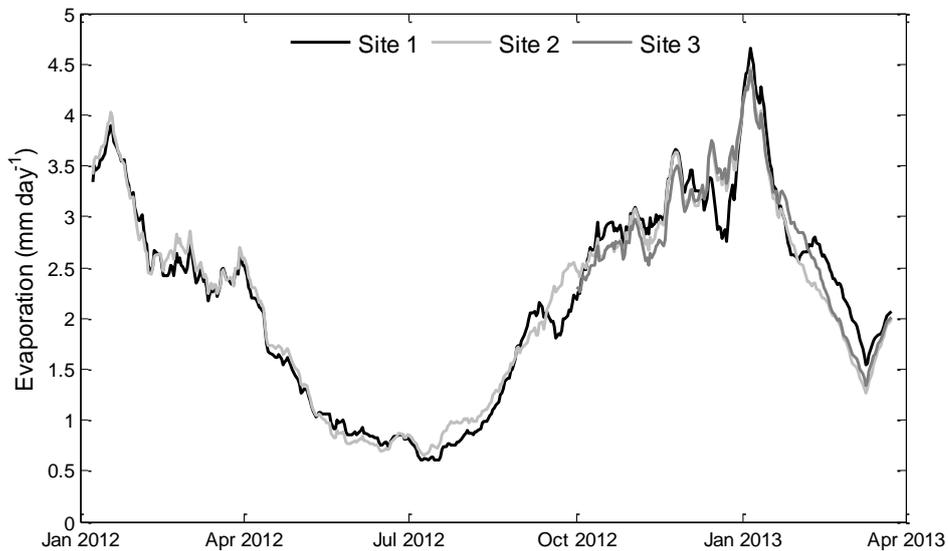


Figure 3.3. Daily smoothed E (15-day running mean) for Site 1 and 2 between January 2012 and end of March 2013 and site 3 from October 2012 until end of March 2013

3.4.3 Controls of evaporation

During most of each year the decoupling coefficient (Ω) was close to 0.8 (Figure 3.4) indicating available energy was the dominant driver of E . However, during the dry period of both 2013 and 2014, Ω declined sharply when soil moisture approached PWP ($0.25 \text{ m}^3 \text{ m}^{-3}$). As VMC approached PWP, Ω and VMC began to vary in unison suggesting surface conductance restrictions (both soil surface and plant stomata) were strongly regulating E . The partitioning of R_n between H and LE also changed as VMC approached PWP. When VMC was above the lower limit of RAW, available energy was predominantly partitioned toward LE . However, when soil moisture reached some threshold value between the lower limit of RAW and PWP more energy was partitioned toward H and consequently the Bowen ratio ($\beta = H/LE$) approached and exceeded 1 during the dry periods of early 2013 and 2014 (Figure 3.4). In agreement with expectations based on Ω being around 0.8 for most of each year, R_n explained greater than 80% of the variation in half hourly measurements of LE across all soil moisture conditions for the three consecutive years at Site 1 ($r^2 = 0.81$, $p < 0.01$). In order to highlight the co-dependence of LE on R_n and soil moisture Figure 3.5 shows the relationship between R_n and LE for daytime data (between 1000 and 1400 hrs) when soil moisture was not limiting ($VMC > FC$) compared to when soil moisture was between the lower limit of RAW and FC (Figure. 3.5a), between PWP and the lower limit of RAW (Figure 3.5b), and below PWP (Figure 3.5c). Soil moisture did not appear to limit E until VMC declined below PWP. When PWP was reached LE reduced relative to R_n , especially at higher radiation levels, and consequently a significant difference ($p < 0.01$) in the slope of the relationship between LE and R_n occurred (slope reduced from 0.51 to 0.25) (Figure 3.5c).

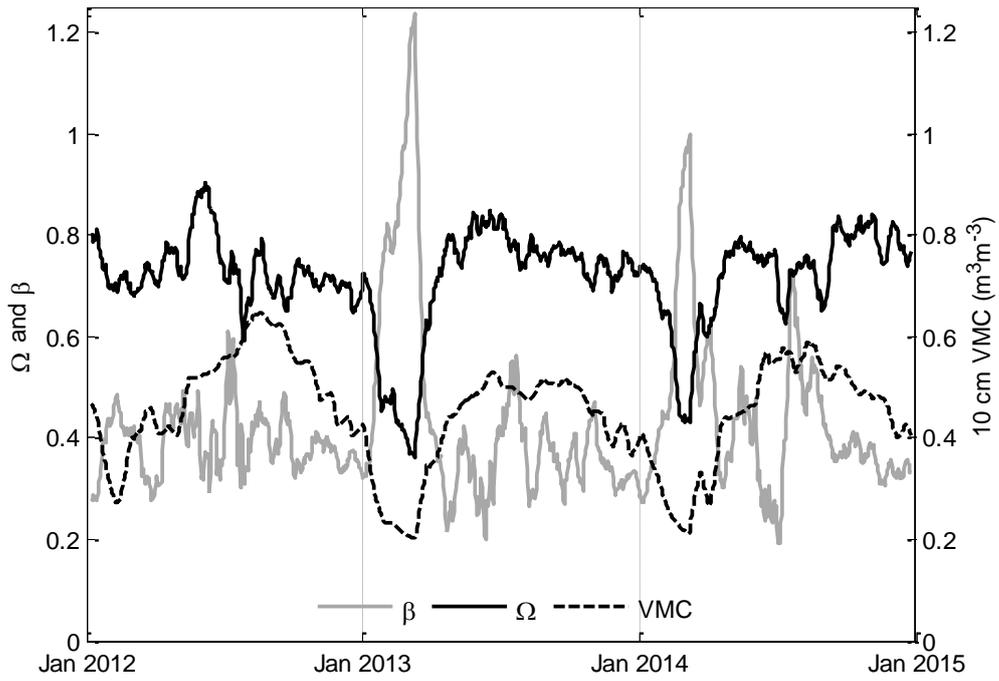


Figure 3.4. 15-day running mean of the decoupling coefficient (Ω) and the Bowen ratio (β) during the middle of the day (10 am – 2 pm) and 10 cm depth VMC across the 3 years at Site 1.

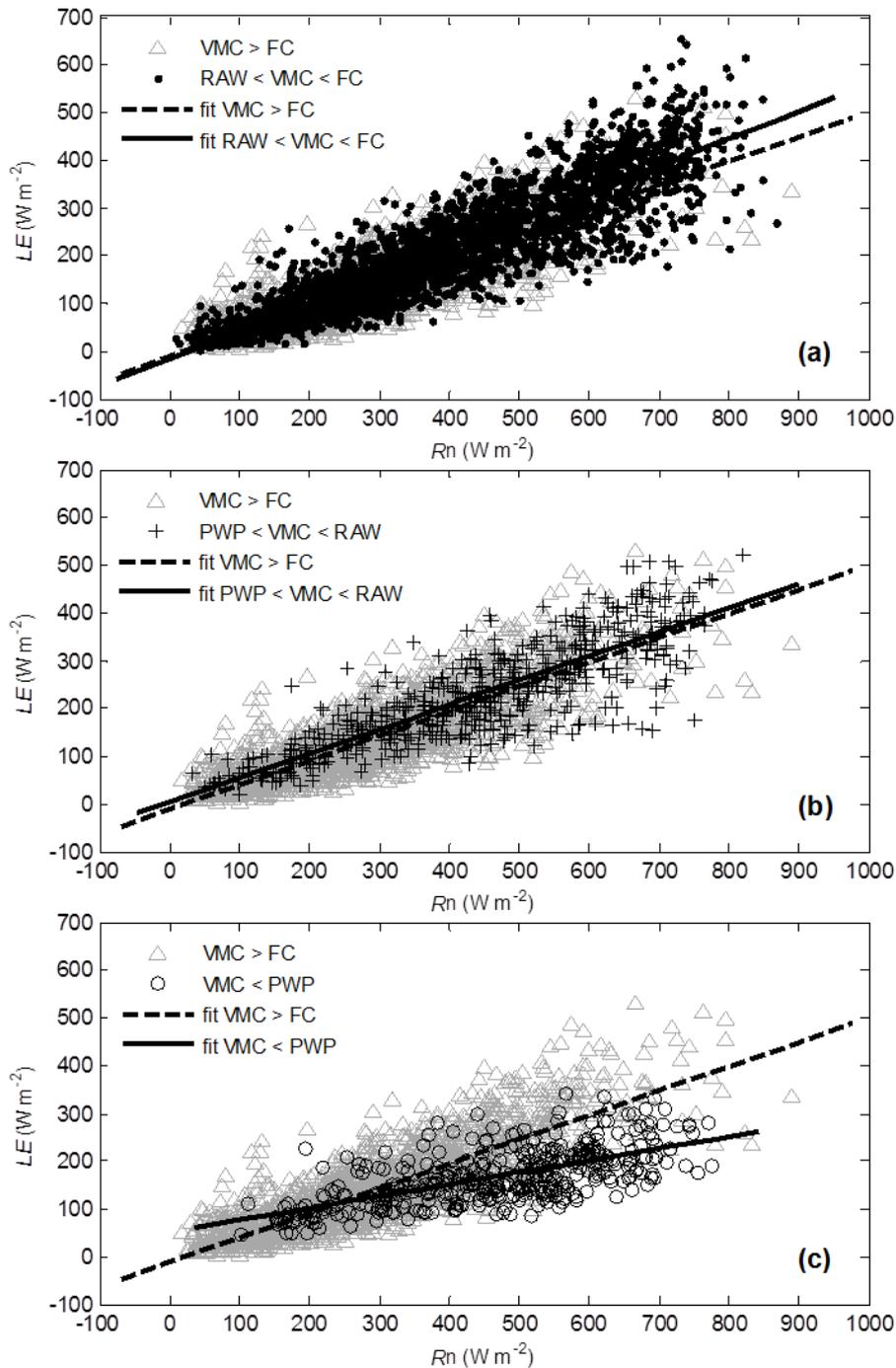


Figure 3.5. Relationship between half hourly R_n and LE between 10 am and 2 pm across the three years at Site 1 when; (a) soil moisture was $> FC$ compared to when soil moisture was between FC and RAW ; (b) soil moisture was $> FC$ compared to when soil moisture was between PWP and RAW ; and (c) soil moisture was $> FC$ compared to when soil moisture was below PWP . Significant difference in slope ($p < 0.01$) only occurred when soil moisture was below PWP .

In addition to R_n and soil moisture we looked at other potential controls of E including the VPD, air temperature, and wind speed. Using a linear regression approach each variable was able to explain some of the variation in E . However, disentangling the relative importance of radiation, air temperature and the VPD is difficult because increased radiation increases air temperature which in turn increases the VPD (Sturman and Tapper, 2006). For completeness we report the explanatory power (using the coefficient of determination) of these other factors but acknowledge the limitations of this approach. When soil moisture was not limiting ($VMC > 0.35 \text{ m}^3 \text{ m}^{-3}$) for daytime periods when R_n was $> 200 \text{ W m}^{-2}$ (across the 3 years at Site 1) the VPD had the most additional explanatory power ($r^2 = 0.40$) followed by air temperature ($r^2 = 0.32$) and wind speed ($r^2 = 0.16$). In an attempt to disentangle the correlations between R_n , air temperature and the VPD we also looked at the relationships between each variable and E after standardising for R_n by dividing LE by R_n . Following such an approach wind speed had the most additional explanatory power ($r^2 = 0.28$) and the explanatory power of the VPD ($r^2 = 0.16$) and air temperature ($r^2 = 0.14$) decreased.

3.4.4 Effect of grazing and vegetation senescence on evaporation

The relationships between LE from grazed and non-grazed sites prior to and following 12 grazing events spread evenly across seasons were analysed. Representative grazing events for spring, summer, autumn and winter are shown in Figure 3.6 with the grazed site always shown on the x -axis and the non-grazed site on the y -axis. While we do not have measured leaf area data for the period when all sites were in ryegrass and clover pasture we have done post-hoc work to show that grazing results in a significant reduction in leaf area throughout the year (see Appendix A, Figure A2). During the period that leaf area was monitored, on average LAI declined from $3.55 \pm 0.32 \text{ m}^2 \text{ m}^{-2}$ to $1.40 \pm 0.21 \text{ m}^2 \text{ m}^{-2}$, a 61% reduction in leaf area. However, this reduction did vary through the year with largest reductions in leaf area during winter (74%) and lowest reductions during summer (42%). Pasture management and grazing rotation lengths were similar for the period that the grazing effect was measured and the later period when LAI measurements were made (September 2015 to May 2016). For all winter and autumn grazing events there were no significant differences ($p > 0.05$) in the

slopes or offsets between LE from the grazed and non-grazed sites prior to and following grazing. However, for two out of three grazing events in the summer, and one out of three grazing events in the spring, there was a significant change in slope of the relationship between the grazed and non-grazed site ($p < 0.05$) between pre- and post-grazing. For the grazing events that reduced LE we found LE was typically similar pre- and post-grazing when LE was $< 200 \text{ W m}^{-2}$ but appeared to decrease at the grazed site compared to the non-grazed when LE was $> 200 \text{ W m}^{-2}$. No significant differences in LE were observed between sites when grazing had not occurred at either site. We calculated the daily reduction in E using the differences between the slopes of summer pre- and post-grazing events (and their 95% CI bounds) for mean summer and spring conditions (see supplementary material). The differences in slopes amounted to relatively small reductions in E post grazing of $0.2 \pm 0.2 \text{ mm day}^{-1}$ for the summer grazing event and $0.18 \pm 0.01 \text{ mm day}^{-1}$ for the spring grazing event which was about a 5% reduction in daily E .

In autumn 2013 (April) herbicide was applied at Site 2 and then the site was heavily grazed. We used this management event to compare E between a surface with sparse dead vegetation (Site 2) and a paired live vegetated site (Site 1). There was a significant ($p < 0.01$) reduction in slope of the relationship between LE from the herbicide treated and vegetated sites pre- and post-vegetation senescence (Figure 3.6). After senescence LE was similar between sites when LE was low ($< 100 \text{ W m}^{-2}$) but during conditions of high R_n , LE from the non-vegetated site reduced relative to the vegetated site. For an average April day, the reduction in LE was calculated to be $0.48 \pm 0.07 \text{ mm of } E$ or about 27% relative to the vegetated site (see Appendix A). This result demonstrated that senescence of vegetation reduced evaporation, especially when R_n was high. This differed from grazing where about 50% of the vegetation leaf area remained post grazing and therefore continued to draw water from lower in the soil profile.

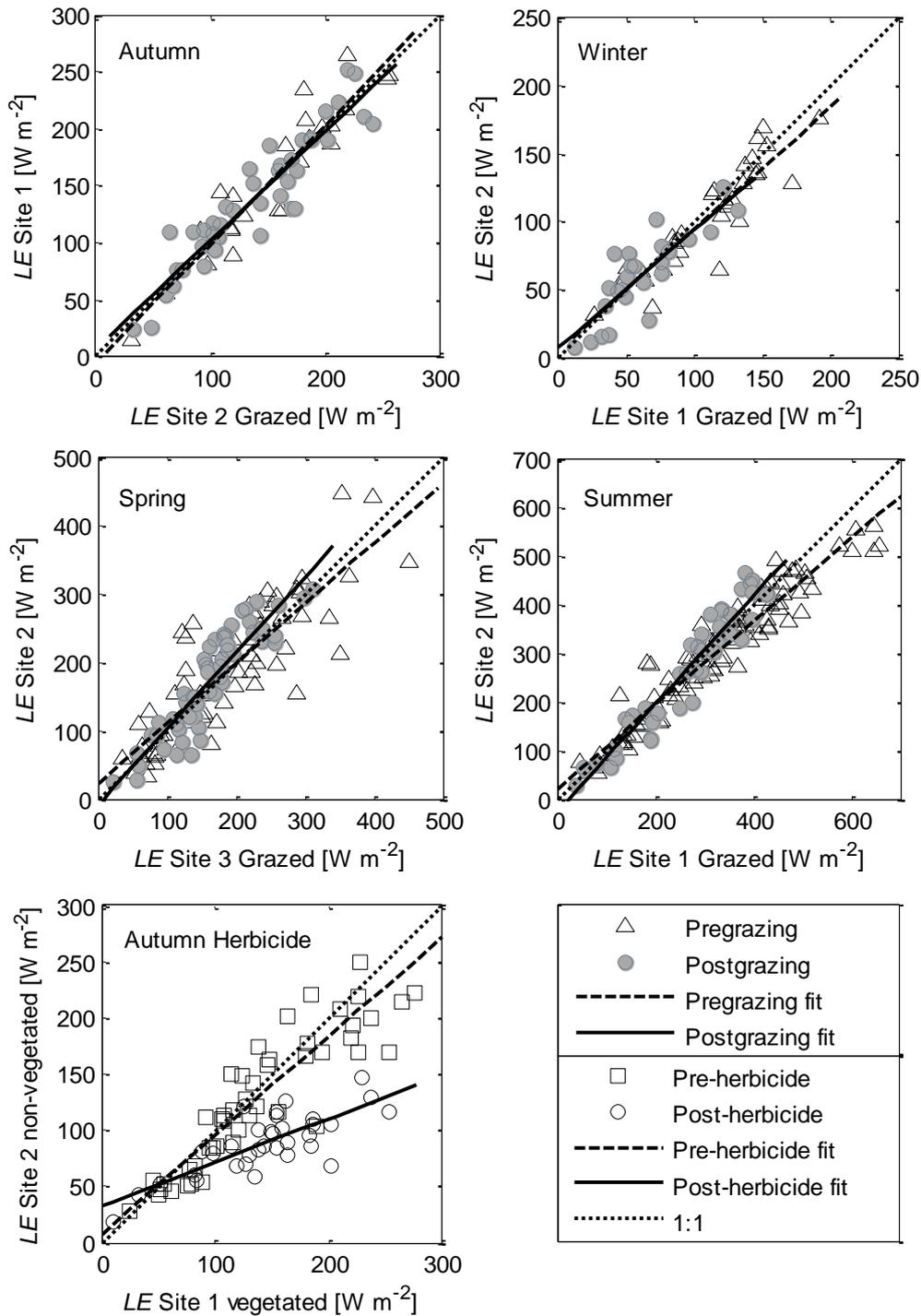


Figure 3.6. Relationship between half hourly *LE* of grazed (x axis) and non-grazed (y axis) sites for representative summer, autumn, winter, and spring grazing events and relationship between *LE* of a vegetated and a non-vegetated site after herbicide application in autumn. Significant differences in slope occurred in spring ($p = 0.04$) and summer ($p < 0.01$) and following herbicide application ($p < 0.01$) (see supplemental material for full analysis of slope statistics).

3.4.5 Comparison to the Penman-Monteith FAO-56 modelled evaporation

There was a strong relationship between daily (Figure 3.7, $r^2 = 0.91$, $p < 0.01$) and monthly ($r^2 = 0.98$, $p < 0.01$) measured and modelled E when VMC was above the lower limit of RAW across all seasons and for all three years. Over the three-year period, K_c was calculated to be 0.99 by dividing total E_{EC} by total E_o for all days when VMC was above the lower limit of RAW. On an annual basis, K_c varied slightly between years with an annual value of 0.98 in 2012 and 2013 and 1.02 in 2014 when VMC was above the lower limit of RAW. Seasonally, there was some variation in K_c (Figure 3.7) but this variability was not consistent between years. For example, during the summer months, K_c was 0.98 in 2012, 0.90 in the 2013 (a very dry summer), and 1.00 in 2014 while during the winter months K_c was 1.01 in 2012, 0.95 in 2013, and 0.93 in 2014. We opted to use a K_c value of 1.0 because seasonal variation was not consistent and annually K_c varied between 0.98 and 1.02.

When soil moisture declined below RAW modelled E_o often exceeded measured E_{EC} (Figure 3.7). Therefore, to model E during periods when VMC was below the lower limit of RAW it was necessary to scale E_o based on VMC. We tried two different methods. Firstly, we followed the approach of Allen *et al.* (1998) (see Appendix A) where a water balance model was used to calculate a daily soil moisture stress factor (K_s) that was then multiplied by E_o . On a daily, monthly, and annual basis agreement between measured and modelled E improved (Table 3.1) after applying K_s but a lag in model response resulted in over prediction early in dry periods (e.g. January 2013) and under prediction late in the dry period (March 2013) (Figure 3.8). Using this water balance approach, we found K_s was sensitive to the values of PWP and rooting depth used in the model. For example, in 2013 annual modelled totals varied between 743 mm (PWP of 0.30) and 825 mm (PWP 0.20) within a plausible range of PWP thresholds and between 746 mm (rooting depth of 0.8 m) and 822 mm (rooting depth of 1.2 m) within a plausible range of rooting depths. We used a rooting depth of 1 m based on mid-range rooting depth for ryegrass pasture systems (Hayman and Stocker, 1983; Parfitt *et al.*, 1985) and the PWP of our dominant soil type (Te Puninga).

Secondly, we trialled a method whereby E_o was adjusted using a three step correction factor based on the ratio of E_{EC} to E_o in three VMC classes (K_{VMC}). Over the three years at Site 1, when VMC was below PWP, the ratio of E_{EC} to E_o was 0.57, when VMC was between PWP and the lower limit of RAW the ratio was 0.87, and when VMC was at or above RAW the ratio was 1.0 and no correction was applied. This simple three step approach performed well with better r^2 and root mean squared errors (RMSE) compared to the water balance model on both a daily and monthly time step (Table 3.1). At an annual scale both methods worked well with modelled totals always within 5% using the water balance approach and within 4% using the K_{VMC} approach. The lag in model response using the water balance approach was not evident at an annual scale because under-prediction early in dry periods was accounted for by over-prediction late in dry periods.

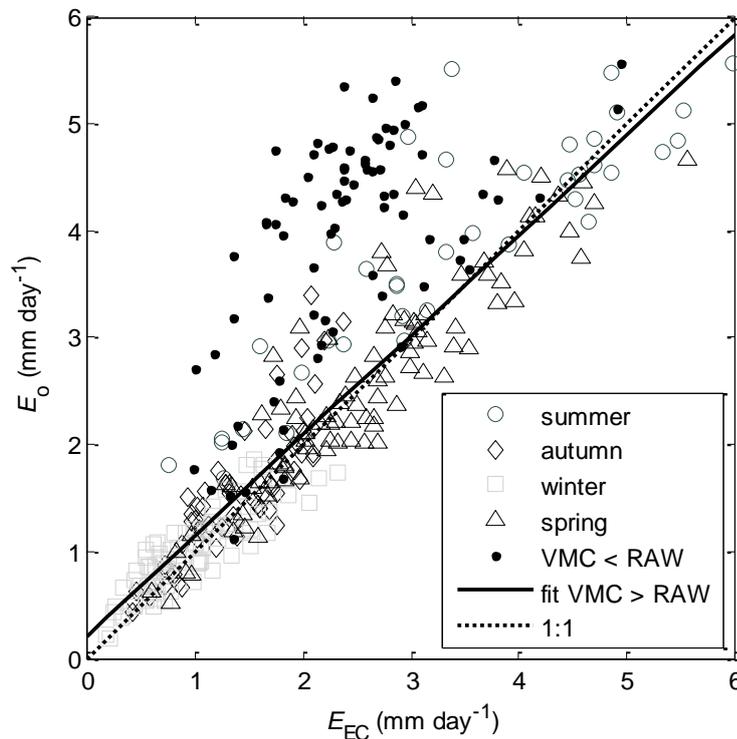


Figure 3.7. Relationship between daily sums of measured E (E_{EC}) and reference crop E (E_o) separated by season and VMC below RAW ($0.35 \text{ m}^3 \text{ m}^{-3}$) for 2013 at Site 1 (VMC > RAW $r^2 = 0.89$)

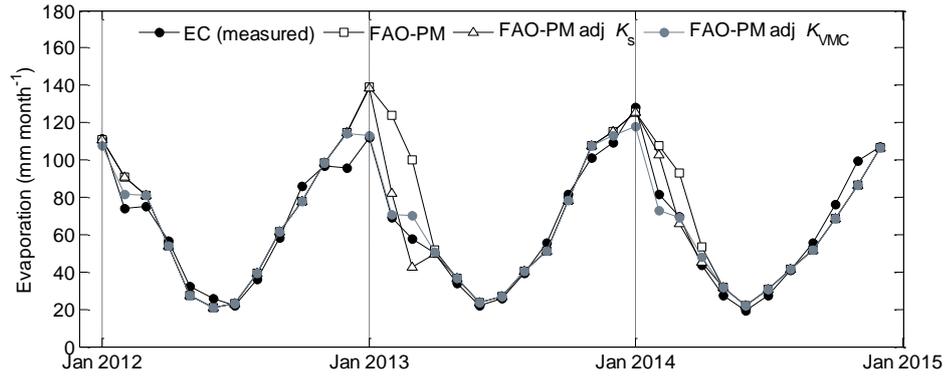


Figure 3.8. Measured monthly evaporation (solid black circles) compared to modelled reference crop evaporation (open squares) and adjusted reference crop evaporation using a daily variable water stress factor (open triangles) and adjusted reference crop evaporation using a simple three bin VMC correction factor (grey circles).

Table 3.1. Daily and monthly model fit values for measured E compared to reference crop evaporation (E_o), adjusted reference crop evaporation using a daily variable water stress factor ($E_o \text{ adj } K_s$), and adjusted reference crop evaporation using a simple three bin VMC correction factor ($E_o \text{ adj } K_{vmc}$) and annual totals (mm) for each approach.

	Slope	Offset	R ²	RMSE
Daily				
$E_{EC} \text{ vs } E_o$	0.93	0.34	0.77	0.64
$E_{EC} \text{ vs } E_o \text{ adj } K_s$	0.93	0.20	0.84	0.52
$E_{EC} \text{ vs } E_o \text{ adj } K_{vmc}$	0.88	0.26	0.89	0.40
Monthly				
$E_{EC} \text{ vs } E_o$	1.05	2.8	0.85	14.10
$E_{EC} \text{ vs } E_o \text{ adj } K_s$	1.05	-1.29	0.94	8.68
$E_{EC} \text{ vs } E_o \text{ adj } K_{vmc}$	0.97	2.49	0.96	6.11
Annual E Totals (mm)				
	E measured	E_o	$E_o \text{ adj } K_s$	$E_o \text{ adj } K_{vmc}$
2012	770	800	800	786
2013	757	894	792	782
2014	776	820	779	748

3.5 Discussion

3.5.1 Magnitude of spatial and temporal variation in evaporation

We made replicated measurements from up to three EC systems and E varied by less than 3% between sites. Low spatial variation likely occurred because E was strongly controlled by available energy and water supply, which is typical for a short grassland which is poorly coupled to the atmosphere (Jarvis and McNaughton, 1986), and these main drivers did not vary much between sites. Inter-annual variation in E was less than 3% during the three consecutive years at

Site 1 (770 mm in 2012, 757 mm in 2013, and 776 mm in 2014). Low inter-annual variation in E occurred despite variation in summer rainfall and soil moisture content between years. The largest differences in E between years were observed during the dry period – typically between January and March. Total E during the first quarter of 2013 was about 20 mm lower (9%) than the same period in 2012 and about 40 mm (17%) lower than 2014. However, at an annual timescale, when slight variation between other seasons was included, these differences scaled to less than 3% between years. This suggests that these relatively short periods of variation in VMC between years had only a minor impact at an annual scale and the overriding control of radiation was more important at this site.

Annual mean E at Site 1 of 768 mm over the three years was generally similar to EC measurements at other intensively grazed pasture sites. However, direct comparisons to other sites is difficult because climatic variation strongly regulates the availability of water and energy. Using the relationship between the climatic water balance and the De Martonne-Gottman aridity index (Ma *et al.*, 2015), we selected three grazed grassland sites with the closest climate regimes to our site. These sites were in Ireland (MAP 1271 mm, MAT 9.6 °C, annual E 547 mm (Byrne and Kiely, 2006)), Switzerland (MAP 1197 mm, MAT 9.3 °C, annual E 539 mm (Ammann *et al.*, 2007)), and France (MAP 1072 mm, MAT 7.8 °C, annual E 665 mm (Klumpp *et al.*, 2011)). Annual E totals at these sites were lower than at our sites but this could be expected given the lower MAT.

Review of catchment scale estimates of E using the water balance approach demonstrated that there was a good relationship between E and annual precipitation at the catchment scale across the globe (Zhang *et al.*, 1999; Zhang *et al.*, 2001). Using the pasture system equation from Zhang *et al.* (2001) and measured MAP from Site 1, their model predicted annual E of 700 mm which was about 10% less than the annual mean at Site 1 of 768 mm. Most of the sites used to develop their model had lower rainfall than our site and hence the model was likely best suited to lower rainfall climates. Only two other pasture sites (both located in Australia) from Zhang *et al.* (2001) had similar annual rainfall to our site and E totals from these two sites were very similar our site. The Wights

catchment had MAP of 1260 mm and average annual E of 757 mm (Silberstein *et al.*, 1999) while the Tumut catchment had MAP of 1180 mm and an average annual E of 768 mm (Jolly *et al.*, 1997). A water balance approach at Site 1 would suggest about 480 mm of precipitation drains to ground water annually (1250 mm MAP – 768 mm E) and moves beyond the root zone. This estimate of drainage is consistent with other research done at this location (Sparling *et al.*, 2016) and research done in other New Zealand pastoral systems with similar annual rainfall (e.g. Parfitt *et al.*, 2010). Overall, our measured annual E was generally higher than the other EC studies where MAT was cooler than at our site but consistent with water balance studies where MAP was similar to our site.

The largest fraction of annual evaporation in our pasture system occurred during the spring (~31%) and summer (~39%) periods. Measured maximum evaporation rates of 6 mm day⁻¹ and 0.8 mm hour⁻¹ were similar to maximum daily and hourly rates presented in a review by Kelliher *et al.* (1993) (4.1 - 6.2 mm day⁻¹ and 0.44 - 0.75 mm hour⁻¹) but higher than the maximum rate of 4.5 mm day⁻¹ reported in a temperate grassland in Canada (Wever *et al.*, 2002). The maximum hourly rate of E typically occurred just after midday when solar radiation was near its peak. This is typical of grassland systems, in contrast to forest systems where maximum E typically occurs later in the afternoon when the vapour pressure deficit is at its daily peak (Kelliher *et al.*, 1993).

3.5.2 Climatic controls of evaporation

Extended dry periods occurred in both 2013 and 2014 with pastures reaching PWP in late summer through early autumn. The decline in soil moisture correlated with a decline in Ω and an increase in the Bowen ratio as the system moved from radiation controlled E to soil moisture limited E (see Section 3.3). This transition between energy and water limited evaporation has been previously identified in grasslands during drought periods (e.g. Alfieri *et al.*, 2007; Aires *et al.*, 2008). Linear regression analysis confirmed that for most of the year, when soil moisture was not limiting, radiation explained most of the variation in E . A strong relationship between R_n and E is typical for grassland systems (e.g. Brümmer *et al.*, 2012) because such systems are generally poorly coupled to the atmosphere (Jarvis and McNaughton, 1986). Furthermore, the shallow root structure of managed grasslands means seasonal restriction of E from a lack of

soil moisture is typical and largely explains why annual evaporation totals are usually larger from forests under similar annual rainfall regimes (Zhang *et al.*, 2001). Other controlling factors including air temperature, VPD, and wind speed explained a much lower proportion of the variation in E and were likely confounded by R_n . For example, increases in R_n increase air temperature which in-turn increases VPD. Consequently, there are strong positive correlations between R_n , air temperature, and VPD and it is difficult to separate the direct and indirect effect of these controlling factors.

3.5.3 Evaporation following grazing and removal of vegetation

We did not find evidence for a strong reduction in E following grazing in agreement with a number of studies (Shuttleworth *et al.*, 1989; Stewart and Verma, 1992; Wang *et al.*, 2012). However, we did observe a reduction in daily E of about 5% directly following some spring and summer grazing events. Others have reported larger decreases in E following grazing. For example, Bremer *et al.* (2001) found cattle grazing reduced annual evaporation by 6.1% in a tallgrass prairie compared to non-grazed prairie and that daily evaporation could be reduced by up to 40%. Frank (2003) reported 7% lower annual evaporation from a cattle-grazed prairie in the semi-arid Northern Great Plains (USA). In a harvested system in Germany, Gebler *et al.* (2015) found grass cutting reduced E by up to 2.1 mm day^{-1} . However, in this harvested system cutting occurred when pasture was between 0.5 and 0.7 m high which was much taller than at our sites where grazing typically occurred when pasture height was between 0.2 and 0.3 m and leaf area likely declined from about $3.5 \text{ m}^2 \text{ m}^{-2}$ pre-grazing to $1.4 \text{ m}^2 \text{ m}^{-2}$ post-grazing.

The inconsistent effect of grazing on E reported in the literature is likely related to differences in soil moisture regimes, rooting depths, and biomass removal at the time of grazing. The partitioning of E is largely determined by leaf area (Allen *et al.*, 1998). Prior to grazing, when a pasture system has high leaf area, total E is likely dominated by E_T . Following grazing, more bare soil is likely exposed and therefore increases in E_S may compensate for decreases in E_T (Falge *et al.*, 2005; Wang *et al.*, 2012). Therefore, we hypothesised that the largest differences between grazed and non-grazed sites would occur during summer when R_n was

high and the soil surface was unable to supply enough water to meet the available energy supply. Our data supports this hypothesis with no differences observed between the grazed and non-grazed sites in autumn and winter and small reductions in total E (about 5%) from the grazed sites during spring and summer.

The magnitude of the reduction in E following grazing is likely dependent on the ability of the soil to supply water to the surface to meet the atmospheric demand. Evaporation from soil is generally accepted to follow a three-stage sequence. During stage 1, when surface soil water is non-limiting, E_S is only limited by available energy. Stage 2 begins when VMC in the upper soil layer declines below a critical threshold and E_S is limited by soil hydraulic properties that control the movement of liquid water and vapour to the soil surface (Ventura *et al.*, 2006). Wilson *et al.* (1997) showed that the critical threshold at which stage 1 ended was typically about -3.0 MPa, well below PWP (-1.5 MPa). Stage 3 begins when there is little liquid water movement in the soil and the rate of evaporation from the soil surface becomes negligible (Ventura *et al.*, 2006). Experimental work from bare soil columns (Wilson *et al.*, 1991) suggested stage 1 could persist for about 4 days with a rapid decline in E_S between days 4 - 11. We found that on a daily scale (during autumn 2013), the non-vegetated site could supply about 70% of total E relative to the vegetated site. This is similar to the difference in daily E reported by Kerr and McPherson (1978) where daily E initially declined from 4.7 mm day⁻¹ over pasture (LAI > 3.0) to 3.4 mm day⁻¹ over bare soil. Also similar to Kerr and McPherson (1978), differences between the vegetated and non-vegetated site largely occurred during midday conditions when R_n was high suggesting that during these conditions E_S from Site 2 was limited by soil hydraulic properties (stage 2). The small reduction in total E following grazing (about 5%) compared to the larger reduction following vegetation senescence (about 30%) suggests that biomass remaining post grazing was still transpiring a large fraction of total E . Although heavy grazing is typically avoided because of its detrimental effects on pasture regrowth it is possible that heavier grazing and consequently larger changes in LAI pre- and post-grazing may result in larger reductions in total E than we observed.

3.5.4 Measured E compared to FAO-56 modelled E

During most of each year when VMC was above the lower limit of RAW modelled and measured E agreed well on a daily ($r^2 = 0.91$) and monthly ($r^2 = 0.98$) time scale without applying a crop coefficient (K_c). Typically, reference crop E is multiplied by K_c to account for differences between the hypothetical grass reference surface and the modelled system (Allen *et al.*, 1998). At an annual scale, our site-derived K_c varied between 0.98 to 1.02 over the three years of measurement suggesting a value of 1.0 is likely suitable in our system and was in the range recommended by Allen *et al.* (1998) (0.85 to 1.05). We did find some variation in K_c between seasons but this variation was not consistent between years. For example, in 2012, K_c was relatively consistent across the seasons (0.98 to 1.01) while in the dry summer of 2013 and autumn of 2014 K_c reduced to 0.90 despite filtering out all periods when VMC was below RAW. We speculate that low K_c values during these periods was linked to the overriding influence of these unusually dry periods. We suggest a fixed K_c is defensible in intensively grazed temperate pasture systems because regular grazing ensures active growth occurs year-round and we have shown that E is strongly controlled by R_n .

However, during dry summer and autumn periods, we found modelled E consistently exceeded measured E on a daily and monthly timescale. These exceedances likely occurred because the model was developed for use over well-watered crops (Allen *et al.*, 1998). In systems where water does become limiting a correction factor is needed to scale E_o based on soil moisture. We compared two different approaches. Firstly, we used a daily water balance model following Allen *et al.* (1998) to calculate a daily soil moisture stress factor (K_s) between 0 (no available soil moisture) and 1 (no water limitation) which was multiplied by E_o . After applying K_s annual performance improved (Table 3.1) but a lag in model response resulted in over-prediction of E early in dry periods and under-prediction late in dry periods (Figure 3.8). At an annual scale over-predictions were corrected for by the following under-prediction and therefore modelled totals were within 5% of measured totals.

Secondly, we used a three step VMC correction factor based on the ratio of E_{EC} to E_o in three VMC bins (see Section 3.4.5). This approach worked well with better daily and monthly model fit parameters than the water balance model (Table 3.1). However, because VMC correction ratios were calculated from site measurements further comparison at other sites is needed to determine whether the ratios we used correlate to similar soil moisture contents at other sites. Notably, this approach did not suffer from the lag in response that occurred in the water balance model and eliminating this lag is important for modelling E at sub-annual timescales.

Ecosystem scale measurement of E is inherently difficult and there is uncertainty in ecosystem scale flux measurements that must be considered when making direct comparisons with model estimates. For E measurement the apparent energy imbalance ($R_n - G > LE + H$) maybe of most concern. However, the proportion of this imbalance attributable to under-measurement of E is difficult to quantify and the EC community has yet to reach an accepted solution. Energy imbalance is thought to be associated with multiple factors including, but not limited to, measurement and data processing error and the potential mismatch between the flux footprint and point measurements of R_n and energy storage terms (Wilson *et al.*, 2002, Leuning *et al.* 2012). Energy balance closure around 80% is typical for EC measurements (Wilson *et al.*, 2002). Leuning *et al.* (2012) concluded closure could be improved substantially with careful attention to all sources of measurement and data processing error. They suggested phase lags and incorrect estimates of energy storage terms were likely responsible for a large proportion of the remaining energy imbalance. Over time we attempted to minimise system limitations which may have led to under measurement of E and corrected for these limitations (see section 2.4). Following these improvements closure improved at our sites by up to 5% resulting in a mean EBR of about 85%. We suspect the remaining imbalance is largely attributable to the mismatch between the EC footprint and the point measurements of R_n and energy storage terms. We are confident the proportion of remaining energy imbalance attributable to under measurement of E at our sites is $< 5\%$ and therefore our finding with respect to modelling E using FAO-56 have a similar range of uncertainty.

3.6 Conclusions

Spatial and temporal variation in E from these year-round intensively grazed grassland sites was low. This occurred because E was primarily controlled by available energy (R_n) during most of each year. However, soil moisture strongly limited E when VMC declined below PWP. Comparison of measured E and FAO-56 reference crop E showed very good agreement at daily and monthly timescales when VMC was not limiting. During dry periods a correction factor was required and we found a simple approach based on the ratio of E_{EC} to E_o in three VMC bins worked better than a water balance model which suffered due to lag in response. Intensive grazing events caused, at most, only very minor decreases in total E while complete vegetation senescence, following herbicide application in autumn, reduced E by about 30%. This implied that, at least for short periods, E_S could supply about 70% of total E relative to a paired vegetated site. Therefore, reductions in E_T post-grazing were likely compensated for by increased E_S and it follows that when modelling total E , grazing events are likely unimportant. Low spatial and temporal variation in E combined with the strong overriding control of R_n suggests that the FAO-56 model can be used with confidence in year-round grazed temperate pasture systems but further comparisons to measured E are needed to verify the best approach when soil moisture is limiting.

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Chapter Four

4 Contrasting water use and water use efficiency in simple and diverse pasture mixes grazed by dairy cows

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The contributions of authors were (also see Appendix D):

Project conception and experimental design was a collaboration between all co-authors. Field sampling was done by Jack Pronger and Aaron Wall. Eddy-covariance measurements, filtering and gap filling were a collaboration between Susanna Rutledge, Aaron Wall, (who both worked on an aligned project) and Jack Pronger with advice from Dave Campbell. Data analysis and writing of the manuscript was done by Jack Pronger. Louis Schipper was the primary reviewer of this chapter with Dave Campbell, Mike Clearwater, Susanna Rutledge, and Aaron Wall providing additional feedback to improve the manuscript.

4.1 Abstract

Grazed pastures cover a larger area than any other land use globally and water shortage is one of the primary factors limiting their productivity. Consequently, the efficient use of soil water is of utmost importance. The objective of this study was to compare water use efficiency (WUE) between a traditional ryegrass and clover pasture (New Rye) and a more diverse pasture (New Mix) which included multiple grasses, legumes, and herbs. It was hypothesised that the more diverse pasture, which included deeper rooting species, would be more productive during dry summers because of increased access to soil water and increased WUE associated with the inclusion of more legumes. Carbon exchange and evaporation (E) was measured for 3.5 years using a paired eddy covariance (EC) experimental design with a 6-month pre-treatment period to identify any pre-existing site differences. New pastures were established following herbicide application by the direct drill method. Above ground harvestable dry matter (DM) production was also measured over the post-treatment period. Gross primary production (GPP) was higher at New Mix during both dry (4.0%) and wet (8.8%) summer conditions and these increases were supported by DM production measurements. Evaporation rates were not significantly different and consequently both ecosystem WUE (EWUE) and harvest WUE (HWUE) were higher in the New Mix during summer conditions. No differences in production (GPP and DM) were found during shoulder season conditions, while E was significantly lower at New Mix (5.8%) resulting in higher EWUE. Hypothetically, higher EWUE during spring may conserve soil moisture increasing available soil moisture during summer. Both GPP and DM production were lower at New Mix during cool winter conditions while E was not different resulting in lower cool season EWUE and HWUE at New Mix. Seasonal production differences between swards suggested that the increased diversity at New Mix was advantageous during warm months while traditional ryegrass and clover pastures were superior during cool months. At an annual scale there were no differences in production between treatments because the seasonal differences compensated one another.

4.2 Introduction

Grazed pastures cover about 26% of Earth's ice free land area and these grasslands are important for supplying an increasing global demand for food (Steinfeld et al., 2006). Despite water being the most abundant molecule at the Earth's surface its irregular or unpredictable distribution strongly restricts plant production in grasslands (Law et al., 2002; Martensson et al., 2017) leading to production and economic losses. In addition, the frequency and severity of water shortages are predicted to increase with climate change (IPCC, 2014) and consequently production losses may also increase in the future. Therefore, approaches to improve pastoral production through higher WUE are needed to increase or maintain food security as water supply potentially becomes more limiting (White and Snow, 2012).

Intensively managed pastures cover about one third of New Zealand (Ministry for the Environment, 2009) and efficient production and profitability is tightly linked to year-round pasture growth (Pembleton et al., 2015). Traditionally, these intensively managed pastures have been dominated by a simple binary mix of ryegrass and white clover because of the ease of establishment and management, and the high production of quality herbage that is grown throughout most of the year (Kemp et al., 1999). However, at high temperatures and under dry conditions, ryegrass and clover pastures are low yielding and herbage quality declines (Charlton and Stewart, 1999). Given the increasing incidence of extended dry periods (Clark et al., 2011) more farmers are exploring the potential of pastoral diversity to improve dry season production by including more legumes, herbs, and grasses (Pembleton et al., 2015).

The idea of using a diverse range of species to increase production is not new. Darwin (1859) famously postulated that plants that were more distantly related would be more productive when grown together, compared to more closely related plants, because they would likely occupy different niches such that they would complement one another as opposed to competing. These ideas have more recently been embraced by forage and grazing researchers and increasing plant diversity in pastures is now recognised as one approach to improve the sustainability of pastoral farming (Sanderson et al., 2006; Skinner and Dell, 2016).

Pasture production has generally been found to be equivalent or better for more diverse mixtures, both in New Zealand (Edwards, 2013; Nobilly et al., 2013; Ruz-Jerez et al., 1991), and internationally (Kirwan et al., 2007; Sanderson et al., 2006; Skinner et al., 2004). Increases in dry season production have in some cases been attributed to the deeper rooting species accessing more water (Skinner et al., 2004) or alternatively increased water use efficiency (Skinner, 2008). In addition to the potential for increased herbage production, there is evidence that more diverse pastures can reduce nitrate concentrations in urine (Edwards, 2013) and associated nitrate leaching, may improve animal health, and because of the inclusion of deeper rooted species, may increase carbon inputs to deeper soil layers where there is potential to store atmospheric carbon (McNally et al., 2015).

In pastoral grasslands, root mass generally declines exponentially with depth while water extraction declines linearly (Woodward et al., 2001) suggesting even small amounts of root biomass at depth improves plant access to water. Deep rooting species commonly used in more diverse pastures in New Zealand include the herbs, chicory (*Cichorium intybus*) and plantain (*Plantago major*), and the legumes, lucerne (*Medicago sativa*) (also known as alfalfa) and red clover (*Trifolium pratense*). Both species traits and interactions between co-existing species likely improve production in more diverse pastures. For example, Hoekstra et al. (2014) demonstrated that interactions between species resulted in plantain accessing deeper soil moisture in mixes compared to when grown in monoculture. Furthermore, deep rooted legumes that fix atmospheric N resulting in higher leaf N content have been shown to increase WUE (e.g. Moot et al., 2008; Ripullone et al., 2004). Increased WUE likely occurs because the photosynthetic rate is largely determined by the amount of nitrogen invested in photosynthetic apparatus (Gilbert et al., 2011) resulting in higher rates of photosynthesis at equivalent stomatal conductance (Ripullone et al., 2004). Therefore, the combination of multiple plant traits, particularly deep roots and the ability to fix atmospheric N, and plasticity in traits may result in improved access to water and higher WUE.

At the leaf scale, WUE can be measured as the ratio of carbon fixed during photosynthesis to water lost via transpiration. However, it is difficult to

accurately extrapolate these plant scale measurements to the ecosystem (Ponton et al., 2006) and therefore direct ecosystem level measurements are needed.

Ecosystem WUE (EWUE) is typically defined as the ratio of gross primary production (GPP) to evaporation (E) (Yang et al., 2010) where E is defined as the process where liquid water is transformed to the gaseous phase and is synonymous with the term 'evapotranspiration' (Srinivasan et al., 2016).

Therefore, EWUE measurements include E from plant transpiration (E_T) in addition to evaporation of soil water (E_S) and evaporation of intercepted water off plant surfaces (E_I). EWUE therefore accounts for all evaporative pathways and consequently provides a more complete picture of pastoral system efficiency, with EWUE being recognised as an important productivity metric (Wagle and Kakani, 2014) which until the more recent proliferation of eddy covariance method was very difficult to measure. These ecosystem level estimates of WUE are the foundation for many global scale coupled carbon and water (CCW) models used to predict CO₂ and water vapour exchange and therefore accurate quantification of EWUE is critical to improve Earth system modelling (Zhang et al., 2016).

However, EWUE has not been well quantified for many ecosystems including intensively grazed pasture systems.

Considerable variation in annual scale EWUE and seasonal variation has been observed among eddy covariance studies over grazed grasslands globally. In New Zealand (Canterbury) EWUE calculated from GPP and E reported by Hunt et al. (2016) varied from 2.37 g C m⁻² (kg H₂O)⁻¹ for a non-irrigated ryegrass-clover pasture up to 3.41 g C m⁻² (kg H₂O)⁻¹ for an adjacent irrigated ryegrass-clover pasture. Also in Canterbury, Graham et al. (2016) reported EWUE values of 3.1 g C m⁻² (kg H₂O)⁻¹ for a temperate irrigated ryegrass-clover pasture and higher values (3.79 g C m⁻² (kg H₂O)⁻¹) were reported for a temperate mountain grassland in Austria (Beer et al., 2009; Wohlfahrt et al., 2008). In contrast, much lower values (0.40 – 1.38 g C m⁻² (kg H₂O)⁻¹) were reported for arid grazed grasslands in China (Hu et al., 2008) while Jongen et al. (2011) reported annual EWUE of 2.53 g C m⁻² (kg H₂O)⁻¹ for a Mediterranean grassland in Portugal. Variation in EWUE also occurs seasonally because of climatic forcing of environmental factors that control the ratio of photosynthesis to E including soil water content, vapour pressure deficit (VPD), leaf area, and light quality (Yang et

al., 2010). VPD is often reported as the dominant control on EWUE because an increase in VPD increases E with no increase in GPP. High VPD is typically associated with dry hot conditions and low EWUE (Beer et al., 2009; Ponton et al., 2006; Scanlon and Albertson, 2004). This strong link between VPD and EWUE led Beer et al. (2009) to propose a modified “inherent” WUE (IWUE) where GPP is multiplied by the VPD prior to dividing by E . This approach generally results in stronger correlations between GPP and E and is useful when comparing WUE between different ecosystems (see Beer et al., 2009; Zhang et al., 2016; Zhou et al., 2014).

From an agronomic perspective, the WUE of above ground harvestable dry matter (DM) production is important and harvest WUE (HWUE) is defined as harvested DM divided by water consumption. This agricultural approach to measuring WUE differs from EWUE because only harvestable DM is measured rather than GPP which includes below ground production. Also, because of the difficulty in directly measuring E , water use is often determined by measuring changes in soil moisture storage in studies that examine HWUE. Despite these differences, correlations between EWUE and HWUE are generally strong (Lambers et al., 2008). HWUE varies depending on species (Martin et al., 2006) and changes in environmental conditions (Brown et al., 2005). HWUE under non-water limiting conditions for ryegrass and clover pastures in New Zealand is generally about 20 kg DM ha⁻¹ mm⁻¹ (Martin et al., 2006) but varies seasonally and between different pasture mixes. Nobilly (2015) found HWUE was higher for more diverse pasture mixes compared to ryegrass and clover swards, and increased productivity and HWUE were linked to the inclusion of the deep rooted legumes.

As demand for global water resources increases, research is required to identify pastoral plant species and mixes that use water more efficiently while maintaining production (White and Snow, 2012). The objective of this study was to compare evaporation and WUE of traditional ryegrass and clover pasture with that of a more diverse pasture sward with a focus on dry summer growing conditions when ryegrass performance is often poor because of shallow roots and air temperatures that are often above optimal for ryegrass. The more diverse pasture included deeper rooting herbs (chicory and plantain) and lucerne in addition to ryegrass and

clover. It was hypothesized that both access to water and WUE would be higher from the mixed sward during dry summer periods because of the inclusion of deeper roots and more legumes. Evaporation and WUE was measured at the paddock scale using a paired eddy covariance experimental design. Additionally, we also monitored pasture DM production to compare GPP to DM production and EWUE to HWUE. Meteorological variables that control WUE at daily to annual scales were also measured at each site.

4.3 Methods

4.3.1 Site

The experimental site has previously been described by Pronger et al. (2016) and Rutledge et al. (2017). Briefly, we established three eddy covariance (EC) towers on a commercial dairy farm in the Waikato region of New Zealand (37°46'07 S, 175°48'07 E). The 30 year mean annual rainfall and temperature were 1249 mm and 13.3°C respectively. The farm was grazed by two herds of dairy cows at a stocking rate of 3.3 lactating dairy cows per hectare. The 207 ha farm was divided into paddocks of between 2 and 3 hectares in size and each paddock was grazed up to twelve times per year. Grazing rotation length varied seasonally with short rotation lengths in spring (about 21 days) when pasture growth rates were high and longer rotation lengths in winter (up to 90 days) when growth rates were low.

4.3.2 CO₂ and H₂O fluxes

Two EC systems were installed on the farm in late 2011 and a third EC system was installed in August 2012 with all three systems established over the existing ryegrass (*Lolium perenne*) and white clover (*Trifolium repens*) pastures until April 2013 to determine pre-treatment differences. In April 2013 glyphosate herbicide was applied at sites 2 and 3 to kill the existing ryegrass-clover pasture. At Site 2 a new mixed sward pasture (New Mix, Fluxnet code NZ-Tr2) was direct drilled which included the broadleaf herbs chicory and plantain, the legumes lucerne (also commonly known as alfalfa), and white clover, and multiple grass species including ryegrass, timothy (*Phleum pratense*), cockfoot (*Dactylis glomerata*), and prairie grass (*Bromus willdenowii*). At Site 3, a new ryegrass and white clover

pasture (New Rye, Fluxnet code NZ-Tr3) was direct drilled. The current research focus is on the comparison between the two new pasture swards and therefore from here onward Site 1 (Old Rye, Fluxnet code NZ-Tr1) will not be discussed (pasture was not renewed). The New Rye and New Mix EC systems measured CO₂ exchange and evaporation in a paired site approach over the existing established old ryegrass and clover pasture at both sites from 1st September 2012 until 31 March 2013. This 7-month period gave us a base line to compare the relationship between the two sites prior to imposing treatments and importantly included the spring and summer periods.

Both EC systems had identical instrumentation including a 3D sonic anemometer (CSAT3, Campbell Scientific Inc., Logan, UT, USA) and an enclosed path gas analyser (LI-7200, LI-COR Inc., Lincoln, NE, USA (CSI)) measuring at 1.55 m height above the soil surface. High frequency data were collected at 20 Hz and stored on a datalogger (CR3000, CSI). Continuous measurement of meteorological and environmental variables were collected at both sites including air temperature and relative humidity (HMP155, Vaisala Inc., Helsinki, Finland), net radiation (NR01, Hukseflux Thermal Sensors, Delft, Netherlands), soil heat flux (HFT3, Hukseflux, Delft, Netherlands), soil temperature at 50, 100, and 200 mm depth (107 probes, CSI), soil moisture at 100 and 200 mm depth (CS616, CSI) and precipitation (tipping bucket rain gauge, TB5, Hydrological Services, NSW, Australia). All ancillary data were stored on dataloggers (CR3000, CR1000, CSI) as half hourly values.

High frequency EC data were processed using EddyPro software (Version 5.2, LICOR Inc.) and the resultant half hourly fluxes were then filtered and gap filled to permit calculation of daily, monthly, and annual totals. In addition to EddyPro quality control flags (Mauder and Foken, 2004) half hourly fluxes were filtered for periods with developed turbulent intensity below 0.11 m s⁻¹ (using standard deviation of vertical velocity fluctuations (Acevedo et al., 2009), the turbulent intensity threshold was determined following Reichstein et al. (2005)), periods of instrument malfunction, periods when cows were grazing more than 5% of the EC footprint, and periods when fluxes were sourced from behind the sonic anemometer (filtered based on wind direction). Gaps were filled using an

artificial neural network (ANN) described in Pronger et al. (2016) and Rutledge et al. (2017). The ANN was run in two monthly windows with day and night time periods filled separately. The modelled response of night time net ecosystem production (NEP) was then used to calculate day time ecosystem respiration (ER) which was added to daytime NEP to determine GPP. We acknowledge that some bias can be introduced to our estimates of day time GPP using this approach (Oikawaa et al., 2017; Reichstein et al., 2005) but at present there is not a better routine method to partition these fluxes.

4.3.3 Pasture production and species composition

Following establishment of the treatments in April 2013, we measured pasture production and changes in species composition using grazing exclusion cages. Ten cages (85 × 55 cm) were randomly placed within the footprint of both EC systems to exclude cattle grazing. At a similar frequency to the grazing rotation 45 × 45 cm quadrats frames were used to clip biomass to mean grazing height (about 40 mm) from within the grazing exclusion cages. New cage locations were randomly selected and pre-trimmed to 40 mm (reflective of post-grazing pasture height) and exclusion cages were shifted to the new location for the next period. Cage relocation was necessary to ensure cuts were representative of the grazed paddock because grazing cattle influence species composition and growth through grazing behaviour and excretory returns. Herbage harvested from the 10 replicates from each treatment were then oven dried at 95°C for 24 hours and dry weights were used to calculate above grazing height dry matter (DM) production. Four times per year, the harvests that best aligned with the end of each season were subsampled in the lab (about 400 pieces per quadrat) and sorted to determine the contribution of each pasture species, weeds, and dead material to the sward.

4.3.4 Data analysis

To examine seasonal differences between treatments, daily data were divided into environmental conditions representative of seasonal boundaries of interest based on changes in soil temperature and moisture. Briefly, mean daily 10 cm soil temperature from site measurements from the beginning of 2012 to the end of 2016 (5 years) were smoothed (15-day running mean) and examined in relation to dates of seasonal boundaries. On average 10 cm soil temperatures were greater

than 18°C during summer months, between 12°C and 18°C during shoulder seasons (spring and autumn) and less than 12°C during winter months and therefore these boundaries were used to divide the data set into representative seasonal conditions. During summer data were further divided into warm-dry (daily mean 10 cm soil volumetric moisture content (VMC) < permanent wilting point (PWP, $\text{VMC} < 0.25 \text{ m}^3 \text{ m}^{-3}$)), warm-intermediate (PWP < VMC < lower limit of readily available water (RAW, $\text{VMC} > 0.35 \text{ m}^3 \text{ m}^{-3}$), and warm-wet (10 cm $\text{VMC} >$ lower limit of RAW). During shoulder and winter season conditions soil moisture limitations were rare at this temperate site. For example, over cool season conditions no days were below the lower limit of RAW while during shoulder season conditions only four days were below the lower limit of RAW. Monitoring of species composition showed that pasture diversity declined sharply at New Mix in the final 12-month period from June 2015 to June 2016 (see Appendix B, Figure B1). Therefore, we excluded this final 12-month period from analysis leaving two full years of data post-treatment and the 7-month pre-treatment. For warm-dry conditions there were 50 days pre- and 100 days post-treatment. For warm-intermediate conditions there were 28 days pre- and 87 post-treatment. For warm-wet conditions there were 59 days pre- and 296 post-treatment. For shoulder season conditions there were 71 days pre- and 250 post-treatment. For winter conditions there were 179 days in the post-treatment period but during the 7-month pre-treatment period there were only 5 days that met winter criteria.

While Pronger et al. (2016) showed between-site variability in E was very small at these sites (< 3%), Rutledge et al. (2017) demonstrated that there were important pre-treatment differences in net ecosystem production (NEP) and its component fluxes gross primary production (GPP) and ecosystem respiration (ER). To account for pre-treatment differences measured over the period prior to imposing treatments, statistical analysis examined the relationship between the paired sites prior to imposing treatments compared to after imposing treatments. One-way analysis of covariance (ANOCOVA) models, that combine traditional ANOVA techniques with regression methods, were used to test for differences in regression slope between the paired sites pre- and post-treatment where a significant change in slope indicates a treatment effect (Loftis et al., 2001). A p-value of 0.05 or less

was considered a significant difference. Due to the absence of pre-treatment winter conditions, statistical comparison of winter conditions was limited and the post-treatment annual analysis (Table 4.1) excluded winter conditions from both pre- and post-treatment data. All statistical analyses were carried out with the MATLAB statistics tool box (Mathworks Inc., R2012a).

To aid visualisation of site differences interpolated surfaces were generated from measured data which show the changes in *E*, GPP, and EWUE with changes in soil temperature and moisture. This interpolation was carried out using the Matlab “griddata” function (Matlab 2012a) with a soil temperature-moisture matrix where soil moisture varied from 20% to 60% in 2% increments and soil temperature from 6°C to 24°C in 1°C increments.

4.4 Results

4.4.1 Meteorology and annual and inter-annual variation in fluxes

This section describes general trends in monthly to annual rainfall, soil moisture, *E*, GPP, EWUE, DM production, and HWUE for the pre-treatment period (September 2012 to March 2013) and post-treatment period (June 2013 – June 2016) (Figure 4.1 (a – f)). The purpose of this description is to provide a broad overview of the environment and flux magnitudes prior to analysing differences between treatments in the following sections (4.4.2 – 4.4.5). The current section includes all three years post treatment; however, because herb abundance declined sharply in the third year (Appendix B, Figure B1) this final year was not included in the subsequent sections where we analyse the effect of pasture diversity on WUE.

For the post-treatment period, annual rainfall totals were lower than the 30-year normal (1249 mm) across all years and varied between 1078 mm (2015) and 1221 mm (2014). The lower than normal annual totals were largely a result of lower rainfall over the late summer and early autumn periods (Jan – Mar) when totals for the 2013 to 2015 time-period were between 110 and 131 mm compared to a mean of 270 mm over the past 30-years. During the dry summers, VMC dropped below wilting point for extended periods in years 1 and 3 (Figure 4.1a). The final summer (2015-2016) was wetter than normal (rainfall 344 mm for Jan-Mar).

Mean annual air temperatures varied between 13.3°C (2015) and 14.0°C (2013) over the study period compared to the 30 year normal of 13.3°C. The seasonal pattern in air temperatures were similar between years and similar to the 30 year mean monthly trend (data not shown). Overall the study period experienced considerably drier summers, lower annual rainfall, and higher mean annual temperatures relative to the 30-year normal.

Mean daily E , GPP, EWUE, DM production, and HWUE for each month are shown in Figure 4.1 (b – f respectively). Annual variation in E was similar between years and followed the annual trend in radiation (data not shown) except for periods of soil water limitation during late summer (Figure 4.1a). Mean daily E was about 1 mm day⁻¹ during mid-winter (June) increasing to maximum of about 4 mm day⁻¹ in mid-summer. Pre-treatment (1 Sep 2012 – 31 Mar 2013) E totals were 509 mm at New Rye and 518 mm at New Mix while post-treatment (Jun 2013 – Jun 2016) mean annual E totals were 739 mm at New Rye and 719 mm at New Mix. Seasonal variation in GPP was similar to E following seasonal trends in available energy. However, compared to E , pre-treatment differences in GPP between treatments was larger. Pre-treatment (1 Sep 2012 – 31 Mar 2013) GPP totals were 1482 g C m⁻² at New Rye and 1397 g C m⁻² at New Mix while post-treatment (Jun 2013 – Jun 2016) mean annual GPP was 2346 g C m⁻² at New Rye and 2286 g C m⁻² at New Mix. Annual trends in EWUE were opposite to E and GPP with maximum EWUE occurring in mid-winter when radiation was low and VPD and air temperature were at annual minimums. Lowest EWUE occurred during dry late summer periods when radiation, air temperature, and VPD were high and GPP was restricted by available soil water. Pasture DM production was only measured post-treatment but followed similar annual trends to GPP. Post treatment mean annual DM production was 14705 kg DM ha⁻¹ at New Rye and 15027 kg DM ha⁻¹ at New Mix. HWUE followed similar annual trends in EWUE but with some apparent differences between swards largely as a result of seasonal differences in pasture DM production. For example, during winter 2014, pasture growth was considerably higher at New Rye and consequently HWUE was also higher at New Rye.

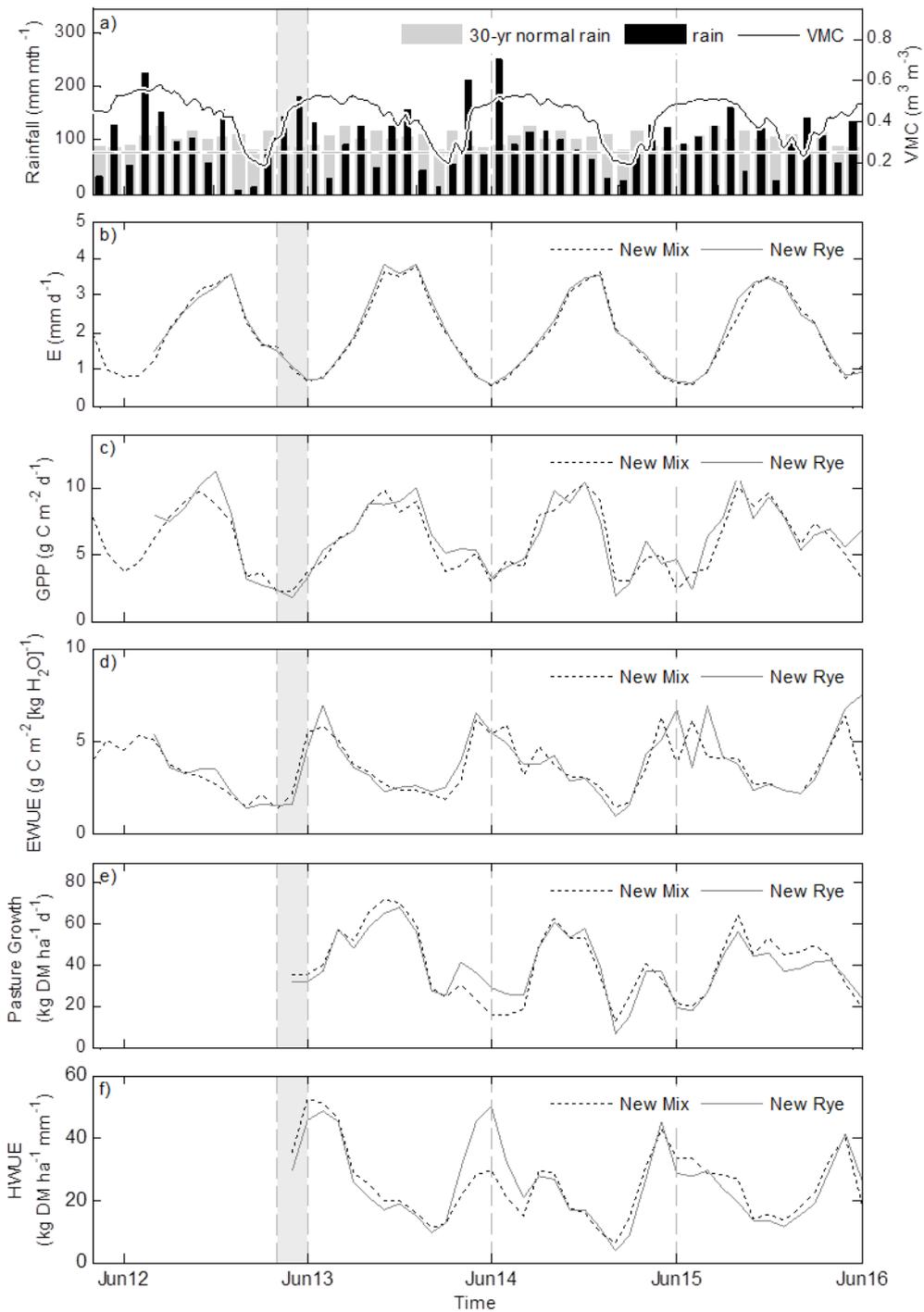


Figure 4.1. Monthly (a) total measured rainfall compared to normal rainfall (1980 – 2010) and 15-day running mean 10 cm depth VMC (horizontal dashed line is wilting point); (b) mean daily E ; (c) mean daily GPP; (d) mean daily EWUE; (e) mean daily pasture growth rate; (f) mean daily HWUE. The vertical grey bar (April to June 2013) is the period of pasture renewal.

4.4.2 Annual Comparison

Visual comparison of both sites post-treatment and the differences between them are shown in Figure 4.2 based on variation in soil temperature and moisture (numerical comparisons presented in Table 4.1). These interpolated surfaces show data for the first two years post-treatment (June 2013 – June 2014) for *E*, GPP, and EWUE prior to the reduction in pasture diversity at New Mix in the final year. During this final year differences between sites weakened as diversity at New Mix declined.

Evaporation varied strongly with soil temperature and was at maximum rates of close to 5 mm day⁻¹ when soil temperatures were above 20°C (Figure 4.2a and b). Evaporation did not appear to be restricted by soil moisture until VMC declined below wilting point. Differences in *E* between sites were small (Figure 4.2c) except for when soil temperatures exceeded 23°C when *E* was about 0.5 to 1 mm day⁻¹ higher at New Mix. GPP was also highest when soil temperatures were high but peak GPP shifted further right suggesting that GPP was being constrained when soil moisture declined below RAW (Figure 4.2e and f). Differences in GPP between sites (Figure 4.2f) were generally small. However, at soil temperatures > 23°C, GPP was up to 5 g C m⁻² day⁻¹ higher at New Mix. Also, when soil temperatures were about 15°C and VMC was between about 30% and 40% (approximately the lower limit of RAW), GPP was about 2 g C m⁻² day⁻¹ higher at New Mix. In contrast to *E* and GPP, EWUE was highest when soil temperatures were low and VMC was high corresponding to winter conditions when the VPD was low (Figure 4.2g and h). Differences in EWUE between treatments were small for most of the growing conditions (Figure 4.2i). However, similar to GPP, EWUE was higher when soil temperatures were about 15°C and VMC was around the lower limit of RAW and also when soil temperatures were high and moisture contents were low. Figure 4.2 suggests that the New Mix site may have had small production and EWUE advantages during intermediate soil moisture and temperatures and again when soil temperatures were very high and soil moisture was low (this is further explored in Section 4.4.3).

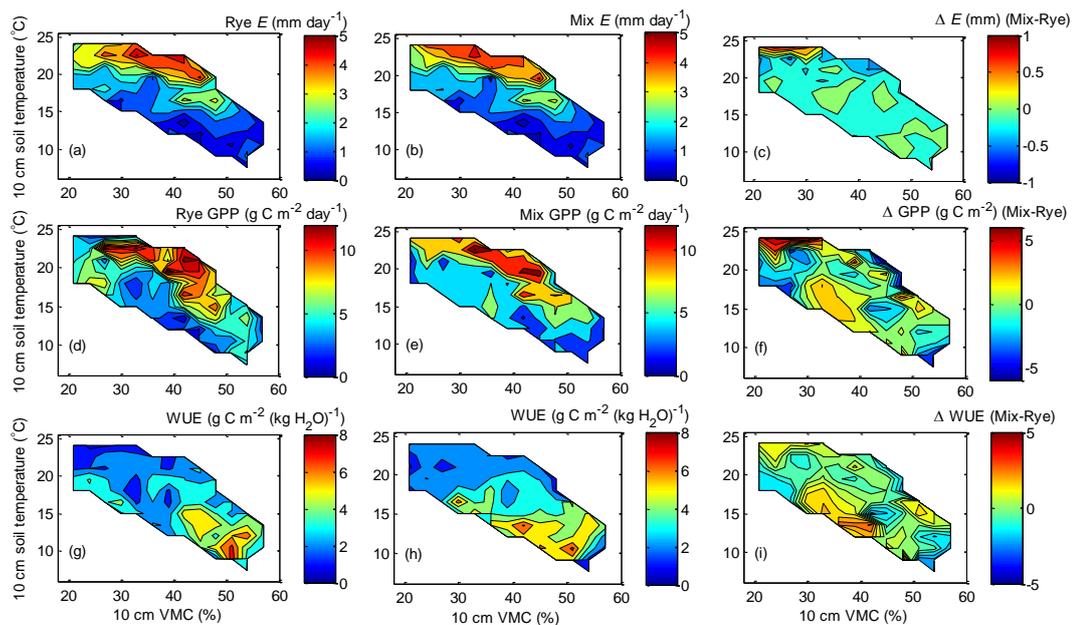


Figure 4.2. Interpolated surface of E , GPP, and EWUE for New Rye and New Mix gridded by soil moisture and soil temperature for the post treatment period (June 2013 – June 2015). The top row shows E at New Rye (left) and New Mix (middle) and the difference between sites (right) where a positive difference is a higher flux at New Mix (New Mix - New Rye). The middle row is the same sequence for GPP and bottom row shows EWUE.

4.4.3 Numerical annual comparison

Annual mean daily E were similar between sites pre-treatment (within 1%) and post treatment (within 2%) as shown in Table 4.1. However, taking into account the pre-treatment difference, slope analysis revealed there was a small but significant change in the relationship between the two sites post-treatment. Post-treatment, E rates at New Mix were slightly lower relative to New Rye when daily E rates were high (i.e. during warmer conditions). Accounting for pre-treatment differences, GPP was about 3% higher at New Mix but this difference was not significant. Accounting for pre-treatment differences, EWUE was about 6% higher at New Mix but again this difference was not significant. DM production and HWUE were both very similar between treatments at an annual scale. The absence of a difference in DM production and HWUE suggested the measured non-significant increases in GPP and EWUE at New Mix were not real treatment effects and therefore at an annual scale there was no real difference in WUE between swards despite some evidence of a small reduction in E at New Mix.

Table 4.1. Evaporation, GPP, EWUE, DM production, and HWUE for the pre-treatment (1 Sep 2012 – 31 Mar 2013) and post treatment period (1 Jun 2013 – 31 May 2015). Positive differences indicate higher values at New Mix. Differences between sites for both the pre- and post-treatment period are shown in the site difference column. The treatment difference column takes into account any pre-treatment differences when calculating the post treatment difference. For description of statistical approach see Section 4.3.4.

Annual		New Rye mean	SEM	New Mix mean	SEM	Site difference (%)	Treatment difference (%)
E (mm day ⁻¹)	<i>Pre-treat</i>	2.62	0.07	2.65	0.07	1.0	
	<i>Post-treat</i>	2.16	0.05	2.11	0.05	-2.0	-3.0*
GPP (g C m ⁻² day ⁻¹)	<i>Pre-treat</i>	7.37	0.26	7.12	0.23	-3.5	
	<i>Post-treat</i>	6.47	0.14	6.46	0.13	-0.2	3.3 ^{NS}
EWUE (g C m ⁻² (kg H ₂ O) ⁻¹)	<i>Pre-treat</i>	2.81		2.69		-4.4	
	<i>Post-treat</i>	3.00		3.06		1.8	6.2 ^{NS}
DM (kg ha ⁻¹ day ⁻¹)	<i>Post-treat</i>	43.17	0.76	42.90	0.79	-0.6	NA
HWUE (kg DM ha ⁻¹ (kg H ₂ O) ⁻¹)	<i>Post-treat</i>	20.02		20.29		1.4	NA

*indicates significant difference in relationship between pre- and post-treatment at 5% level, ^{NS} indicates no significant difference between sites pre- and post-treatment, NA indicates no pre-treatment data and consequently statistical comparison was limited, SEM is standard error of the mean.

4.4.4 Warm growing conditions

Warm summer conditions were divided into dry (VMC < PWP), intermediate (VMC < lower limit of RAW but > PWP) and wet (VMC > lower limit of RAW) to account for variation in soil moisture limitation during warm growing conditions.

During dry warm conditions (Table 4.2) measured *E* was up to 8.5% higher at New Mix but this difference did not reach statistical significance ($p = 0.09$). GPP was significantly higher at New Mix post-treatment (about 4%) during dry warm conditions. However, EWUE was significantly lower at New Mix post-treatment during dry warm conditions (because *E* was potentially 8.5% higher at New Mix). Supporting the significant increases in GPP, DM production was proportionally much higher at New Mix during dry warm conditions (about 20%) which resulted in higher HWUE (Table 4.2). These results suggest diversity increased

productivity during dry warm periods but this increase in productivity was likely attributable to increased water use rather than increased EWUE.

Table 4.2. Evaporation, GPP, EWUE, DM production, and HWUE for the pre-treatment (1 Sep 2012 – 31 Mar 2013) and post treatment period (1 Jun 2013 – 31 May 2015) for warm (10 cm soil temperature > 18°C) and dry (10 cm VMC < WP) growing conditions. Positive differences indicate higher values at New Mix. Differences between sites for both the pre- and post-treatment period are shown in the site difference column. The treatment difference column takes into account any pre-treatment differences when calculating the post treatment difference. For description of statistical approach see Section 4.3.4.

Warm dry		New Rye mean	SEM	New Mix mean	SEM	Site difference (%)	Treatment difference (%)
E (mm day ⁻¹)	<i>Pre-treat</i>	2.04	0.09	1.96	0.09	-4.2	8.5 ^{NS}
	<i>Post-treat</i>	2.02	0.08	2.11	0.08	4.3	
GPP (g C m ⁻² day ⁻¹)	<i>Pre-treat</i>	2.68	0.24	3.11	0.12	16.3	4.0*
	<i>Post-treat</i>	3.52	0.21	4.24	0.25	20.3	
EWUE (g C m ⁻² (kg H ₂ O) ⁻¹)	<i>Pre-treat</i>	1.31		1.59		21.4	-6.0*
	<i>Post-treat</i>	1.74		2.01		15.4	
DM (kg ha ⁻¹ day ⁻¹)	<i>Post-treat</i>	15.53	1.10	18.77	0.91	20.9	NA
HWUE (kg DM ha ⁻¹ (kg H ₂ O) ⁻¹)	<i>Post-treat</i>	7.67		8.89		15.9	NA

*indicates significant difference in relationship between pre- and post-treatment at 5% level, ^{NS} indicates no significant difference between sites pre- and post-treatment, NA indicates no pre-treatment data and consequently statistical comparison was limited, SEM is standard error of the mean.

During warm conditions when soil moisture was low but not below WP (Table 4.3) *E*, GPP, and EWUE were all marginally lower at New Mix but none of these differences were significant. However, DM production was marginally higher at New Mix (about 5%) resulting in potentially higher HWUE suggesting again (see previous paragraph) that under conditions of soil water limitation, diversity may have had a small positive effect on harvestable DM production.

Table 4.3. Evaporation, GPP, EWUE, DM production, and HWUE for the pre-treatment (1 Sep 2012 – 31 Mar 2013) and post treatment period (1 Jun 2013 – 31 May 2015) for warm (10 cm soil temperature > 18°C) intermediate soil moisture (10 cm VMC > WP & VMC < RAW). Positive differences indicate higher values at New Mix. Differences between sites for both the pre- and post-treatment period are shown in the site difference column. The treatment difference column takes into account any pre-treatment differences when calculating the post treatment difference. For description of statistical approach see Section 4.3.4.

Warm-intermediate		New Rye mean	SEM	New Mix mean	SEM	Site difference (%)	Treatment difference (%)
E (mm day ⁻¹)	Pre-treat	2.72	0.15	2.66	0.16	-2.0	-1.6 NS
	Post-treat	2.83	0.12	2.73	0.12	-3.6	
GPP (g C m ⁻² day ⁻¹)	Pre-treat	6.38	0.34	5.73	0.45	-10.2	-4.2 NS
	Post-treat	7.67	0.37	6.57	0.38	-14.4	
EWUE (g C m ⁻² (kg H ₂ O) ⁻¹)	Pre-treat	2.35		2.15		-8.4	-2.8 NS
	Post-treat	2.71		2.41		-11.2	
DM (kg ha ⁻¹ day ⁻¹)	Post-treat	39.67	1.95	41.67	1.42	5.0	NA
HWUE (kg DM ha ⁻¹ (kg H ₂ O) ⁻¹)	Post-treat	14.02		15.26		8.9	NA

*indicates significant difference in relationship between pre- and post-treatment at 5% level, ^{NS} indicates no significant difference between sites pre- and post-treatment, NA indicates no pre-treatment data and consequently statistical comparison was limited, SEM is standard error of the mean.

During warm wet growing conditions (Table 4.4), *E* measurements were slightly lower at New Mix (4.3%) but this difference was not significant. However, GPP was significantly higher at New Mix during warm wet conditions and this increase was large enough to be of importance (8.8%). The non-significant reduction in *E* together with the significant increase in GPP resulted in a significant increase in EWUE of 12.9%. Supporting the increased GPP, DM production measurements also showed an increase at New Mix post-treatment resulting in higher HWUE. These findings indicate diversity has a positive effect on production and WUE during warm wet conditions.

Table 4.4. Evaporation, GPP, EWUE, DM production, and HWUE for the pre-treatment (1 Sep 2012 – 31 Mar 2013) and post treatment period (1 Jun 2013 – 31 May 2015) for warm (10 cm soil temperature > 18°C) wet (10 cm VMC > lower limit of RAW) growing conditions. Positive differences indicate higher values at New Mix. Differences between sites for both the pre- and post-treatment period are shown in the site difference column. The treatment difference column takes into account any pre-treatment differences when calculating the post treatment difference. For description of statistical approach see Section 4.3.4.

Warm-wet		New Rye mean	SEM	New Mix mean	SEM	Site difference (%)	Treatment difference (%)
E (mm day ⁻¹)	Pre-treat	3.46	0.15	3.56	0.15	3.0	-4.3 NS
	Post-treat	2.82	0.07	2.78	0.07	-1.3	
GPP (g C m ⁻² day ⁻¹)	Pre-treat	10.48	0.43	9.60	0.37	-8.4	8.8*
	Post-treat	6.68	0.22	6.71	0.22	0.4	
EWUE (g C m ⁻² (kg H ₂ O) ⁻¹)	Pre-treat	3.03		2.69		-11.1	12.9*
	Post-treat	2.37		2.41		1.8	
DM (kg ha ⁻¹ day ⁻¹)	Post-treat	40.11	1.40	41.60	1.29	3.7	NA
HWUE (kg DM ha ⁻¹ (kg H ₂ O) ⁻¹)	Post-treat	14.23		14.96		5.1	NA

*indicates significant difference in relationship between pre- and post-treatment at 5% level, ^{NS} indicates no significant difference between sites pre- and post-treatment, NA indicates no pre-treatment data and consequently statistical comparison was limited, SEM is standard error of the mean.

4.4.5 Shoulder season growing conditions

Shoulder season growing conditions were defined as 10 cm soil temperatures of between 12°C and 18°C without soil moisture limitation (10 cm VMC > lower limit of RAW). During shoulder season conditions (Table 4.5), *E* was significantly lower at New Mix (5.8%) while GPP was similar between treatments. Consequently, EWUE was higher at New Mix but this difference was not significant. In agreement with post treatment GPP measurements, DM production was very similar between treatments along with HWUE. These results show production was very similar between treatments during shoulder season growing conditions while *E* was significantly lower. Therefore, at this site, in the absence of water stress, diversity may have increased EWUE through decreased water use rather than increased productivity.

Table 4.5. Evaporation, GPP, EWUE, DM production, and HWUE for the pre-treatment (1 Sep 2012 – 31 Mar 2013) and post treatment period (1 Jun 2013 – 31 May 2015) for shoulder season growing conditions (10 cm soil temperature between 12°C and 18°C and VMC > lower limit of RAW). Positive differences indicate higher values at New Mix. Differences between sites for both the pre- and post-treatment period are shown in the site difference column. The treatment difference column takes into account any pre-treatment differences when calculating the post treatment difference. For description of statistical approach see Section 4.3.4.

Shoulder season		New Rye mean	SEM	New Mix mean	SEM	Site difference (%)	Treatment difference (%)
E (mm day ⁻¹)	<i>Pre-treat</i>	2.31	0.09	2.37	0.11	2.4	-5.8*
	<i>Post-treat</i>	1.77	0.07	1.71	0.06	-3.4	
GPP (g C m ⁻² day ⁻¹)	<i>Pre-treat</i>	8.54	0.26	8.28	0.29	-3.0	1.7 ^{NS}
	<i>Post-treat</i>	6.82	0.21	6.73	0.18	-1.3	
EWUE (g C m ⁻² (kg H ₂ O) ⁻¹)	<i>Pre-treat</i>	3.69		3.50		-5.3	7.5 ^{NS}
	<i>Post-treat</i>	3.85		3.93		2.2	
DM (kg ha ⁻¹ day ⁻¹)	<i>Post-treat</i>	48.49	0.72	47.32	1.04	-2.4	NA
HWUE (kg DM ha ⁻¹ (kg H ₂ O) ⁻¹)	<i>Post-treat</i>	27.38		27.66		1.0	NA

*indicates significant difference in relationship between pre- and post-treatment at 5% level, ^{NS} indicates no significant difference between sites pre- and post-treatment, NA indicates no pre-treatment data and consequently analytical comparison was limited, SEM is standard error of the mean.

4.4.6 Cool winter growing conditions

Cool winter growing conditions were defined as periods when soil temperature was less than 12°C and soil moisture was not limiting. The pre-treatment period did not include winter growing conditions and therefore we were unable to account for pre-treatment differences. Therefore, this section is included for completeness but is of limited value for analytical comparison. During winter conditions *E* was the same at both sites; however, GPP was about 6% lower at New Mix resulting in lower EWUE. In agreement with GPP measurements, DM production was also lower at new Mix (10.9%) resulting in lower HWUE. Agreement between both measures of production strongly suggests increased diversity resulted in lower cool season productivity at this site.

Table 4.6. Evaporation, GPP, EWUE, DM production, and HWUE for the post treatment period (1 Jun 2013 – 31 May 2015) for cool winter growing conditions (10 cm soil temperature between $< 12^{\circ}\text{C VMC} >$ lower limit of RAW). Positive differences indicate higher values at New Mix. Differences between sites for both the pre- and post-treatment period are shown in the site difference column. The treatment difference column takes into account any pre-treatment differences when calculating the post treatment difference. For description of statistical approach see Section 4.3.4.

Winter		New Rye mean	SEM	New Mix mean	SEM	Site difference (%)	Treatment difference (%)
E (mm day ⁻¹)	Pre-treat	NA	NA	NA	NA	NA	NA
	Post-treat	0.88	0.04	0.88	0.03	-0.6	NA
GPP (g C m ⁻² day ⁻¹)	Pre-treat	NA	NA	NA	NA	NA	NA
	Post-treat	4.70	0.16	4.42	0.13	-6.0	NA
EWUE (g C m ⁻² (kg H ₂ O) ⁻¹)	Pre-treat	NA	NA	NA	NA	NA	NA
	Post-treat	5.32		5.03		-5.4	NA
DM (kg ha ⁻¹ day ⁻¹)	Post-treat	34.73	0.86	30.95	1.15	-10.9	NA
HWUE (kg DM ha ⁻¹ (kg H ₂ O) ⁻¹)	Post-treat	39.27		35.21		-10.4	NA

NA indicates no pre-treatment data and consequently statistical comparison was limited, SEM is standard error of the mean.

4.4.7 Seasonal summary

In general, diversity increased productivity (both GPP and DM production) during warm growing conditions (Table 4.7). Under dry warm conditions GPP was significantly higher (4%) likely because of increased access to water (non-significant increase in E at New Mix of 8.5%). However, during warm wet growing conditions GPP was significantly higher at New Mix (8.8%) while E rates were similar suggesting diversity increased EWUE. For shoulder season conditions productivity was similar but E was significantly lower at New Mix (5.8%), again suggesting diversity increased EWUE. These results indicate that species in the diverse pasture conserved water under non-water limiting growing conditions relative to ryegrass. During cool season growing conditions both GPP and DM production were higher at New Rye (5 - 10%) while E was similar between treatments indicating higher WUE at New Rye. At an annual scale production metrics were similar suggesting warm season gains at New Mix were compensated by cool season reductions relative to New Rye.

Table 4.7. Seasonal summary where New Mix is identified as M and New Rye as R. Warm season is 10 cm soil temperatures > 18°C, shoulder season is 10 cm soil temperatures between 12°C – 18°C, and cool season is 10 cm soil temperatures < 12°C. Warm season is further divided into dry (10 cm VMC < PWP), intermediate (PWP < 10 cm VMC < RAW), and wet (10 cm VMC > lower limit of RAW).

	Warm			Shoulder	Cool	Annual
	Dry	Intermediate	Wet			
E	M > R	M = R	M = R	M < R*	M = R	M < R*
GPP	M > R*	M = R	M > R*	M = R	M < R	M = R
EWUE	M < R*	M = R	M > R*	M > R	M < R	M > R
DM	M > R	M = R	M = R	M = R	M < R	M = R
HWUE	M > R	M = R	M > R	M = R	M < R	M = R

Significant treatment differences ($p < 0.05$) of any magnitude are identified with *. Differences greater than 5% that were not significant only use greater or less than symbols and non-significant differences less than 5% are shown using an equals sign.

4.5 Discussion

4.5.1 Gross Primary Production and above ground DM production

During dry warm growing conditions GPP was significantly higher (4.0%) at New Mix and this was strongly supported by DM production measurements. Under warm wet growing conditions GPP was again significantly higher at New Mix (8.8%) and DM production measurements supported the higher GPP measurements. These findings suggest diversity increased productivity during warm growing conditions. During shoulder season conditions there was no difference in GPP between treatments and this was supported by DM production measurements. For cool winter conditions, GPP was about 6% lower at New Mix and this was supported by DM production which was 10.9% lower at New Mix. Both GPP and DM production were very similar at annual scales and therefore higher warm season production at New Mix was compensated by higher cool season production at New Rye. Although many of these seasonal differences are not large, production increases of 5-10% will be important to farmers during dry periods.

Comparisons of GPP between traditional ryegrass-clover and more diverse pasture systems are scarce. However, a number of others have compared harvestable DM production between simple and more diverse pastures. In

agreement with our finding, increasing pasture diversity has generally been found to increase DM production under both dry warm and wet warm growing condition, both in New Zealand (Edwards, 2013; Nobilly et al., 2013; Ruz-Jerez et al., 1991), and globally (Kirwan et al., 2007; Sanderson et al., 2006; Skinner and Dell, 2016; Skinner et al., 2004). Skinner and Dell (2016) compared production between a grass clover mix and a more diverse mix which included additional herbs and legumes over a nine-year period. Despite diversity declining through time, in eight out of nine years the more diverse mix produced more biomass with a mean annual increase of 31%. Seasonal increases were 34% in spring, 30% in summer, and 26% in autumn and summer increases were larger during wet summers (Skinner and Dell 2016). The relative production advantages of diverse pastures over simple binary pastures have often been higher in wet summers compared to dry summers (e.g. Goh and Bruce, 2005; Nobilly, 2015; Skinner and Dell, 2016). For example, Nobilly (2015) found relative production advantages for more diverse pastures were larger under full irrigation during summer compared to deficit irrigation. In agreement with these studies our findings suggest diversity was beneficial in both warm and dry summer conditions but these gains were offset by low winter productivity indicating integration of both systems would be beneficial.

Our measured annual GPP at both sites ($\sim 2300 \text{ g C m}^{-2}$) was within the range typically reported for New Zealand ryegrass-clover based pastures. For a non-irrigated Waikato pasture Mudge et al. (2011) reported a range of 1984-2404 $\text{g C m}^{-2} \text{ y}^{-1}$ and Kirschbaum et al. (2015) reported a mean daily rate of 5.8 g C m^{-2} ($\sim 2113 \text{ g C m}^{-2} \text{ y}^{-1}$). At an irrigated site in the South Island of New Zealand, Graham et al. (2016) reported higher annual GPP of 2827 g C m^{-2} . Comparatively, annual GPP for many international pastures sites is much lower, likely because of shorter growing seasons. For example, Ma et al., (2015) reported mean annual GPP of 1230 g C m^{-2} for managed grasslands across Europe. At two of the study sites included in the Ma et al. (2015) synthesis the original authors compared intensive (higher fertiliser inputs and stocking rates) and extensively managed treatments. At the French site, Klumpp et al. (2011) reported higher GPP in all years under more intensive management (mean of 1770 g C m^{-2} compared to the 1544 g C m^{-2}). In Switzerland, Ammann et al. (2007) did not partition NEP into

GPP and ER but did measure higher NEP in all years under intensive management. These findings suggest increasing management intensity, which has occurred in New Zealand, increases productivity. Stocking rates on New Zealand dairy farms are about 3 times higher than those under intensive management at these European grasslands and combined with our year-round growing season likely explain the high annual GPP at our site and at New Zealand grasslands in general.

4.5.2 Evaporation

At an annual scale, site differences in E were within 2%; however, pre-treatment E was 1% higher at New Mix and post-treatment E was 2% lower at New Mix. Slope analysis showed this small difference was significant and E was 3% lower at New Mix. Visual interpretation of annual slope analysis showed the difference was at higher daily E rates suggesting that under warm conditions, when water was not limiting, the more diverse pasture was limiting water loss relative to ryegrass-clover pasture. This was supported by shoulder season E measurements (no water limitation) which showed a significant reduction in E at New Mix (5.8%) and again during warm wet conditions when a non-significant reduction in E of 4.3% was measured at New Mix. Others (e.g. Nobilly, 2015) have reported increased HWUE from more diverse pastures suggesting species used in diverse pastures can constrain water loss relative to ryegrass while not limiting production. Despite the shoulder season reduction in E at New Mix being relatively small (5.8%), such a trait could foreseeably conserve soil moisture reserves through spring for later use during summer when additional soil moisture would be valuable.

In contrast, under dry conditions E was higher at New Mix (8.5%) but this potentially large and important difference was not significant. Although E measurement comparisons between pasture swards are scarce, others have compared soil water extraction among a range of pasture mixes by measuring changes in soil moisture content through time (e.g. Brown et al., 2005; Moot et al., 2008; Nobilly, 2015). Generally, more diverse pastures have been found to extract more water during summer but differences over other seasons are typically small. This increased summer water extraction generally occurs from greater soil depths indicating increased rooting depth under more diverse pastures (Brown,

2004; Moot et al., 2008). We speculate that the non-significant increase in E under warm dry conditions was attributable to the deeper rooted species (chicory, plantain, lucerne) but this summer advantage was potentially limited at our site because of a very compact soil layer at about 1 m depth. Ryegrass pastures can generally extract moisture to at least 1 m depth (Moot et al., 2008; Woodward et al., 2001) and consequently the deeper rooting species in the diverse pasture may have had little advantage at our experimental site.

Annual E totals measured in the current study (719 – 739 mm) were lower (about 5%) than annual totals reported for the Old Rye site by Pronger et al. (2016) and we speculate this difference was linked to the combined effect of consecutive dry summers (2013 – 2015) combined with the new pastures not having established extensive root systems. Evaporation rates from grassland are strongly dependent on available water and energy (Krishnan et al., 2012) and therefore comparisons between studies are climate dependent. At a comparable non-irrigated ryegrass and clover pasture site in the Waikato region, Kirschbaum et al. (2015) reported mean E of 2.24 mm day⁻¹ (annually ~ 818 mm) while in a cooler New Zealand region with irrigated ryegrass and clover pasture Graham et al. (2016) reported annual E of 791 mm. Comparison to international pasture sites was problematic because of climatic differences. However, based on the relationship between the site water balance and the De Martonne-Gottman aridity index (following the approach of Ma et al. (2015)) three global grassland sites with somewhat similar climates were identified. Annual E totals were 547 mm at a site in Ireland (Byrne and Kiely, 2006), 539 at a site in Switzerland (Ammann et al., 2007), and 665 mm for a site in France (Klumpp et al., 2011). Overall, annual E totals from other New Zealand sites were similar to totals for New Rye and New Mix but international sites were lower. This difference was likely associated with the warmer climate in New Zealand where mean annual temperature were 3-4°C higher than the European sites and New Zealand's year-round growing conditions.

4.5.3 Water use efficiency

Under warm and dry conditions, we found EWUE was significantly lower at New Mix (6%) despite significantly higher GPP (4%). However, under warm and wet conditions EWUE was significantly higher at New Mix (12.9%) and during

shoulder season conditions there was a non-significant increase in EWUE at New Mix (7.5%) which occurred because of a significant reduction in E (5.8%). Site differences in HWUE (which did not include a pre-treatment period) also strongly suggested HWUE was generally higher at New Mix during warm and shoulder season conditions. However, during cool winter growing conditions both EWUE and HWUE were lower at New Mix. At an annual scale there were no significant differences in EWUE between sites and this was supported by very similar HWUE during the post-treatment period. These results suggest pasture diversity increases both EWUE and HWUE during warm wet conditions and potentially also during shoulder season conditions but these gains are offset by reduced EWUE and HWUE over winter. From an agricultural perspective the gains of over 5% during warm and shoulder seasons are large enough to be of practical importance and conservation of soil water during spring at New Mix likely conserves soil moisture for use during dry warm conditions – a period when we observed large (8.5%) but non-significant increases in E at New Mix.

Others have also reported higher HWUE as a result of increased pasture diversity. For example, Nobilly (2015) reported higher HWUE for a diverse mix of similar sown composition to our site (rye, clover, lucerne, chicory, plantain, timothy, and prairie grass) over two consecutive spring and summer periods under irrigation compared to simple mixes. However, the highest HWUE in this trial was for a ryegrass, clover, and lucerne mix and Nobilly (2015) consistently found mixes that contained lucerne had higher HWUE. Those that only contained additional herbs were not different from ryegrass and clover. Under non-irrigated conditions, Brown et al. (2005) also found lucerne had higher HWUE compared to monoculture chicory and red clover but suggested this finding may have been because lucerne was extracting water from below the depth of soil moisture measurements (2.3 m) – a problem we avoided by measuring E over the pasture rather than soil water extraction. Lucerne did not grow well in New Mix but we still measured increased HWUE during wet warm growing conditions. Given herbs (chicory and plantain) accounted for the main differences in species composition between New Rye and New Mix (Appendix B, Figure B1) it was likely that increased HWUE was due to their inclusion. Further research is required test this hypothesis.

Seasonal and annual comparisons of EWUE as affected by changes in pasture diversity were not found in the published literature. However, at an annual scale EWUE at both New Rye and New Mix was similar ($3.0 \text{ g C m}^{-2} (\text{kg H}_2\text{O})^{-1}$) and these annual values were similar to those reported for irrigated pastures in the South Island of New Zealand of $3.1 \text{ g C (kg H}_2\text{O})^{-1}$ by Graham et al. (2016). EWUE was lower for a non-irrigated ryegrass and clover pasture site in the Waikato region ($2.6 \text{ g C (kg H}_2\text{O})^{-1}$) based on reported GPP and *E* measurements from Kirschbaum et al. (2015). Data analysed by Kirschbaum et al. (2015) was previously presented by Wallace (2010) who showed that EWUE was very low during a severe 1 in 100-year drought that occurred in early 2008 and this drought likely explains the lower annual scale EWUE at this Waikato site. These values from New Zealand pastures are similar to reported values for global temperate grazed grasslands. For example, in a temperate French grassland we calculated EWUE averaged about $2.5 - 2.6 \text{ g C m}^{-2} (\text{kg H}_2\text{O})^{-1}$ over a six-year period based on data presented in Ma et al. (2015) from work done by Klumpp et al. (2011). Review of global grasslands under a range of climates showed very large variation in annual scale EWUE. Generally, EWUE was low in arid environments and high in temperate environments. For example, EWUE was $0.40 \text{ g C (kg H}_2\text{O})^{-1}$ for short sparse vegetation on sandy soils with low rainfall in China (Hu et al., 2008) and up to $3.79 \text{ g C (kg H}_2\text{O})^{-1}$ for a temperate mountain valley grassland site in Austria (Wohlfahrt et al., 2008). EWUE at our sites was toward the upper end of the global range.

The high EWUE measured at our site relative to many global grasslands was likely driven by our temperate climate where limitations on productivity (extremes in rainfall, air temperature and VPD) are moderated through the oceanic influence and grass growth therefore continues year-round. High VPD is often found to be the dominant limitation on EWUE because increasing the VPD results in a non-linear increase in transpiration relative to carbon uptake (Beer et al., 2009; Ponton et al., 2006; Scanlon and Albertson, 2004). This control of VPD likely drove the strong seasonal variation in EWUE. EWUE was high in winter when air temperature and VPD were low and EWUE was low in late summer when air temperature and VPD were high. In addition to the climatic advantages,

intensively grazed pastures typically receive high rates of N inputs which likely increase photosynthesis without increasing transpiration. Leaf nitrogen content enhances the CO₂ carboxylation rate (Hussain et al., 2011) with large proportions (50 – 80%) of plant N being allocated for the synthesis of photosynthetic protein (Evans, 1989). Consistent with this, Mills (2007) found HWUE was significantly higher for N fertilised cocksfoot pasture (38 kg DM ha⁻¹ mm⁻¹) compared to a non-fertilised cocksfoot control (17 kg DM ha⁻¹ mm⁻¹). Therefore, we can expect higher WUE in humid oceanic environments and where N inputs are increased either via the integration of legumes or the strategic use of N fertiliser.

4.5.4 Uncertainties

4.5.4.1 Asynchronous grazing

Grazing timing was dictated by farm managers on this commercial dairy farm and consequently grazing was not aligned between treatments. Intensive grazing events strongly reduced GPP (Appendix B, Figure B2) but had little effect on *E* (see Chapter 3) and therefore grazing reduced EWUE. Consequently, asynchronous grazing between sites increased differences in GPP and EWUE between treatments. The number and frequency of grazing events were the same between treatments (timing was just offset) and therefore differences caused by asynchronous grazing reduced as the analysis period increased in length. By pooling data from multiple periods where environmental conditions were similar over the pre- and post-treatment period we reduced the effect of grazing on GPP and EWUE. However, especially for the shorter pre-treatment period asynchronous grazing may have increased between site differences and therefore added to uncertainty when applying pre-treatment corrections to post-treatment data. For future paired site comparison of grazed pastures, we strongly recommend synchronising grazing events where practically possible.

4.5.4.2 Pre-treatment period

Despite many studies not including pre-treatment observations, Rutledge et al. (2017) demonstrated that pre-treatment site comparison was critical for determining post-treatment differences. For this study, the pre-treatment period was relatively short (7 months) and did not cover the full range of seasonal variation. Importantly the pre-treatment period did include dry and warm growing

conditions that were the main focus of this research. However, a longer pre-treatment period would have reduced the potential for asynchronous grazing to influence pre-treatment site differences. Future paired eddy covariance studies would benefit from longer pre-treatment comparison period because this period has a strong influence on post-treatment differences when it is used to account for any pre-treatment differences. We suggest that ideally the pre-treatment period should be of similar length to the post-treatment period.

4.5.4.3 Eddy covariance

Eddy covariance measurements include inherent uncertainties due to both systematic and random errors associated with measurement, data processing, and gap filling procedures. While error analysis is widely reported for NEP measured by eddy covariance (e.g. Campbell et al., 2016; Rutledge et al., 2017) few researchers have quantified errors for water vapour fluxes and EWUE measurements. Eichelmann et al. (2016) recently reported an annual gap filling error for evaporation measurements of ± 12 mm ($\sim 1.5\%$ of the annual flux) in a switchgrass ecosystem suggesting gap fillings errors are likely small but future work needs to focus on measurement and data processing errors. The largest known sources of systematic uncertainty are related to the apparent lack of energy balance closure and the site specific developed turbulence threshold chosen (Baldocchi, 2003). Energy balance closure was $> 84\%$ at both New Mix and New Rye over the study period and therefore at the upper end of the typical range reported for EC measurements (Wilson et al., 2002). The developed turbulence threshold was chosen following Reichstein et al. (2005) at both sites and the same artificial neural network (ANN) based gap filling routine was applied at both sites. Additionally, both EC systems were identical and the same data processing procedures were used at both sites. Consequently, any systematic errors affected both sites equally and were therefore unlikely to have any impact on conclusions. Finally, EC measurements also include random uncertainty that can be large at the half hourly timescale; however, as summation time increases the contribution of random uncertainty declines (Baldocchi, 2003). In this study, random uncertainty would have been negligible because data was integrated over multi-year timescales.

4.6 Conclusions

Measured GPP was higher at the New Mix compared to the New Rye treatment during both dry warm (4.0%) and wet warm (8.8%) conditions and these increases were supported by DM production measurements. Evaporation measurements were not significantly different and consequently both EWUE and HWUE were higher at New Mix during warm conditions. During shoulder season conditions no differences in production (GPP and DM) were found while E was significantly lower at New Mix (5.8%) resulting in higher EWUE. Hypothetically, higher EWUE during spring may conserve soil moisture for use later in the summer. Over cool winter conditions both GPP and DM production were lower at New Mix while E was not different resulting in lower cool season EWUE and HWUE at New Mix. At an annual timescale, there were no differences in GPP or DM production between treatments because the seasonal differences compensated. Seasonal production differences between swards suggested that increasing diversity was advantageous during warm months while traditional ryegrass and clover pastures were superior during cool months. Therefore, the strategic integration of both ryegrass-clover and more diverse pastures on different parts of a farm would likely be beneficial to ensure year-round productivity. A strong decline in diversity was observed in the third year (and consequently the final year was excluded from analysis) when the broadleaf herbs were out-competed by ryegrass and therefore future studies may benefit by excluding ryegrass. Alternatively, grazing should be managed to optimise the persistence of the broadleaf herbs. Finally, we observed strong reductions in EWUE post-grazing because GPP declined sharply while E was largely unchanged. Therefore, it is possible that changes in grazing management, in particular allowing greater pasture residuals to remain post-grazing, may increase pastoral EWUE. Further research is required to test this hypothesis.

4.7 References

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Chapter Five

5 Using the natural abundance of carbon isotopes as an indicator of paddock scale WUE

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The contributions of authors were (also see Appendix D):

Project conception was a collaboration led by Jack Pronger with contributions from all co-authors. Field sampling was done by Jack Pronger with help from Aaron Wall. Eddy-covariance measurements, filtering and gap filling were a collaboration between Suusanna Rutledge, Aaron Wall, (who both worked on an aligned project) and Jack Pronger with advice from Dave Campbell. Data analysis and writing of the manuscript was done by Jack Pronger. Louis Schipper was the primary reviewer of this chapter with Dave Campbell, Mike Clearwater, Susanna Rutledge, and Aaron Wall providing additional feedback to improve the manuscript.

5.1 Abstract

Pastoral agriculture is important for supplying global demand for animal products but pasture productivity is often limited by water availability. Increasing plant diversity has been shown to increase production, especially during warm and dry conditions, but the optimal mix of species varies spatially dependent on climate, soil type, and plant water requirements. Consequently, a fast and cost effective method to screen for productive drought tolerant species and mixes *in situ* at farm scale is needed. The use of carbon isotope discrimination ($\Delta^{13}\text{C}$) is attractive because the method does not modify the measurement environment, integrates over useful time scales, and is quick, simple, and cost effective. We tested the robustness of bulk leaf $\Delta^{13}\text{C}$ as a measure of water use efficiency (WUE) by comparing the seasonal progression of WUE calculated from bulk leaf $\Delta^{13}\text{C}$ ($\text{WUE}_{\Delta^{13}\text{C}}$) to ecosystem WUE (EWUE) measured at the paddock scale using eddy covariance (EC). Mixed species bulk leaf biomass samples were harvested pre-grazing, dried, sub-sampled, ground, and the ratio of ^{13}C to ^{12}C was measured. Correlation was strong between $\text{WUE}_{\Delta^{13}\text{C}}$ and EWUE ($r^2 > 0.79$, $p < 0.01$) at both study sites. Correlations were also strong between $\Delta^{13}\text{C}$ and soil moisture, air temperature, and vapour pressure deficit. $\Delta^{13}\text{C}$ declined as water stress and the vapour pressure deficit (VPD) increased, indicating an increase in intrinsic WUE (WUE_i), but $\text{WUE}_{\Delta^{13}\text{C}}$ and EWUE decreased because of the overriding control of increased VPD. The positive correlation between water stress and WUE_i highlighted the importance of simultaneously measuring both production and $\Delta^{13}\text{C}$ to avoid interpreting decreased fractionation (increased WUE_i) as an agriculturally desirable trait when, in some circumstances, increased WUE occurs at a cost to production. Considerable within-season differences in $\text{WUE}_{\Delta^{13}\text{C}}$ and production between co-existing pasture species indicated that manipulation of pasture species mixtures can lead to increased EWUE. Ultimately, in combination with traditional soil testing and production monitoring, I envisage farm advisors could use $\Delta^{13}\text{C}$ measurements to optimise species selection for site specific climate and soil conditions to maximise WUE and farm production and profit.

5.2 Introduction

Grazed pastures cover about one quarter of the global ice free land area (Steinfeld et al., 2006) and are important for supplying global demand for animal products. These grazed pastures often occupy landscapes where seasonal water shortages are common (O'Mara, 2012) and therefore maximising WUE (the ratio of carbon fixed to water used) is important (Martensson et al., 2017; White and Snow, 2012). A number of studies have reported a positive effect of increasing plant diversity on both harvest WUE and summer production (Goh and Bruce, 2005; Nobilly, 2015; Sanderson et al., 2006; Skinner, 2008) but selection of the optimal plant species and mixtures at any given location is challenging because of continuous variation in climate, topography, and soil type. Therefore, a rapid and cost effective method to identify pasture species and mixes with high WUE at farm scale across continuously varying landscapes is needed.

Carbon dioxide in the atmosphere is comprised of two stable carbon isotopes (^{12}C ~99% and ^{13}C ~1%) and plants discriminate against the heavier isotope during photosynthesis. The extent of the carbon isotope discrimination ($\Delta^{13}\text{C}$) can be used to make measurements of photosynthetic performance including WUE (Farquhar et al., 1982). The use of bulk leaf $\Delta^{13}\text{C}$ is attractive because it can provide a time-integrated measure of leaf scale gas exchange that would be difficult to obtain from direct physiological measurements (Farquhar et al., 1989), does not modify the measurement environment, and is a quick, simple, and cost effective.

Differences in $\Delta^{13}\text{C}$ are primarily dependent on the ratio of leaf internal (C_i) to ambient atmospheric (C_a) CO_2 concentration (Farquhar et al., 1982). In turn, the ratio of C_i/C_a is dependent on factors which regulate photosynthesis (A), for example light and nutrients, relative to stomatal conductance (g_s). Therefore, $\Delta^{13}\text{C}$ is a measure of leaf level intrinsic WUE ($\text{WUE}_i = A/g_s$). In general, as the ratio of C_i/C_a declines, the gradient driving CO_2 diffusion into the leaf through the stomata increases relative to the gradient driving diffusion of water vapour out of the leaf resulting in higher WUE_i (Farquhar et al., 1989). However, under circumstances where the leaf internal to atmospheric vapour pressure deficit (VPD) increases, for example during dry warm growing conditions, the gradient driving

diffusion of water out of the leaf increases. Therefore, when comparing WUE between plants using $\Delta^{13}\text{C}$ (WUE_i) it is important that all plants are exposed to equivalent VPD (Smedley et al., 1991) or alternatively the changes in VPD are accounted for using approaches outlined in Section 2.3 developed by Farquhar et al. (1982).

Assuming constant VPD, WUE_i increases under plant water stress because stomatal closure reduces transpiration more than CO_2 uptake. This differential effect on CO_2 uptake and water vapour loss largely occurs because CO_2 diffusion is subject to an additional internal leaf resistance whereas water vapour is only controlled by stomatal and leaf boundary layer resistance (Farquhar et al., 1989). Therefore, a reduction in $\Delta^{13}\text{C}$, indicative of increased WUE_i , can occur because of either a relative reduction in stomatal conductance, or a relative increase in photosynthesis. In an agricultural setting the mechanism increasing WUE is important because a decline in stomatal conductance will reduce production, whereas a relative increase in photosynthesis should increase production (Condon et al., 2002). Consequently, it is important to couple WUE measurements with measures of production or water flux to avoid inadvertently selecting for low production by solely relying on WUE ratios.

Strong correlations between $\Delta^{13}\text{C}$ and leaf level WUE have been demonstrated for many C3 crops including beans (*Phaseolus vulgaris*) (Ehleringer, 1990), peanuts (*Arachis hypogea*) (Wright et al., 1992), and cowpeas (*Vigna unguiculata*) (Ismail and Hall, 1992) in addition to those from the grass family including wheat (*Triticum aestivum*) (Condon et al., 1990), barley (*Hordeum vulgare*) (Anyia et al., 2007) and range grass (*Agropyron desertorum*) (Ehleringer et al., 1990). In a glasshouse experiment, Martensson et al. (2017) showed strong correlation between discrimination and WUE and shoot biomass production for the common pasture species, tall fescue (*Festuca arundinacea*) and cocksfoot (*Dactylis glomerata*). However, the method has not been widely used in productive pasture systems and comparisons to ecosystem scale measurements are scarce. Recently, Flanagan and Farquhar (2014) compared leaf and EWUE in a non-grazed native Canadian grassland using bulk leaf $\Delta^{13}\text{C}$ and eddy covariance methods. Leaf level isotope WUE ($\text{WUE}_{\Delta^{13}\text{C}}$) was found to be 2-3 times higher than EWUE and

this difference was largely attributed to isotope measurements not accounting for soil water evaporation which is included in EWUE measurements. In the wetter of the two years (1999) WUE was lower using both approaches and in the drier year (2000) WUE was higher using both approaches suggesting that, despite the 2 to 3-fold difference in absolute magnitude, the methods were correlated. However, while $WUE_{\Delta^{13}C}$ was measured at multiple points through each growing season Flanagan and Farquhar (2014) did not present seasonal correlations between the two methods. Due to the scarcity of comparison to ecosystem scale measurements in grassland there is still uncertainty regarding the strength of correlation between $WUE_{\Delta^{13}C}$ and EWUE.

Measurement of $\Delta^{13}C$ can be made on bulk leaf biomass or different fractions of the leaf biomass, for example cellulose or non-structural carbohydrates. The $\Delta^{13}C$ of a bulk leaf reflects fractionation during photosynthetic exchange over the entire lifespan of the leaf, including during leaf expansion (O'Leary, 1988). In contrast, measurement of $\Delta^{13}C$ on non-structural carbohydrates integrates WUE over the days prior to harvest (Bowling et al., 2008). Additionally, measuring $\Delta^{13}C$ of bulk leaf biomass may increase noise caused by fractionation steps post photosynthesis during the synthesis of secondary organic compounds such as oils, waxes, resins, tannins (Moreno-Gutierrez et al., 2012). However, Orchard et al. (2010) has shown that over 80% of the variation in $\Delta^{13}C$ among 44 different species was explained by variation in C_i/C_a suggesting post photosynthesis differences in fractionation among C3 plant species is not a significant problem. Ultimately, the plant component analysed should depend on the time period of interest, and from a pragmatic viewpoint, less complicated measurement approaches should be more desirable. In intensively grazed pasture systems plant biomass is removed regularly by grazing herbivores (typically every 3 to 6 weeks) and integration of WUE over the grazing cycle is advantageous and therefore bulk leaf biomass analysis is suitable.

The objective of this study was to examine the correlation between leaf level $WUE_{\Delta^{13}C}$ and EWUE determined by eddy covariance measurements in an intensively grazed pastoral situation. Seasonal variation in $\Delta^{13}C$ between a range of C3 pasture species was also measured to examine the extent of variation in

fractionation among common pasture species. It was hypothesised $\Delta^{13}\text{C}$ would vary between species because of differences in plant traits (rooting depth, leaf N content) and $\text{WUE}_{\Delta^{13}\text{C}}$ would be correlated with EWUE measured by eddy covariance. Assuming $\text{WUE}_{\Delta^{13}\text{C}}$ and EWUE were correlated, $\Delta^{13}\text{C}$ would represent a simple and cost effective method to screen pasture systems for water stress and WUE in multiple locations under variation in soil type, climate, and management. To compare approaches, we sampled pasture biomass within the integrated measurement area of two eddy covariance systems over a 12-month period just prior to grazing events thereby capturing the isotope signal over the pasture regrowth cycle for comparison to EWUE measurements made over aligned time periods.

5.3 Methods

The comparison of $\text{WUE}_{\Delta^{13}\text{C}}$ and EWUE was conducted at an intensively grazed commercial dairy farm in the Waikato region of New Zealand (37°46'07 S, 175°48'07 E) where the effect of increased pasture diversity on soil carbon storage (Rutledge et al., 2017) and water use and EWUE was also investigated (Chapter 4). Soils in the experimental paddocks were a complex of silt loams ranging from poorly to well drained that had formed on gently undulating volcanogenic alluvium that had been incrementally covered by up to 0.5 m of volcanic tephra. Climate was temperate with 30-year mean annual rainfall of 1249 mm and temperature of 13.3°C. The farm covered an area of 207 ha and was grazed year-round at a stocking rate of about 3.3 lactating dairy cows per hectare. The two sites used for the current research were New Mix (Fluxnet code NZ-Tr2) and New Rye (Fluxnet code NZ-Tr3). Both sites were direct drilled following glyphosate herbicide application in April 2013. At New Rye ryegrass (*Lolium perenne*) and white clover (*Trifolium repens*) pasture was sown. Species sown at New Mix included the broadleaf herbs chicory (*Cichorium intybus*) and plantain (*Plantago major*), the legumes lucerne (*Medicago sativa* also commonly known as alfalfa), and white clover, and multiple grass species including ryegrass, timothy (*Phleum pratense*), cockfoot (*Dactylis glomerata*), and prairie grass (*Bromus willdenowii*).

5.3.1 Eddy covariance measurement and gap filling

The eddy covariance system is described in Pronger et al. (2016) and Rutledge et al. (2017). Briefly, both New Mix and New Rye had identical instrumentation with 3D sonic anemometers (CSAT3, Campbell Scientific Inc., Logan, UT, USA (CSI)) and enclosed path gas analysers (LI-7200, LI-COR Inc., Lincoln, NE, USA) mounted at 1.55 m height. Data were collected at 20 Hz and stored on portable memory cards by CR3000 dataloggers (CSI). Ancillary measurements were made at both sites and stored as half hourly data on CR1000 dataloggers (CSI). These ancillary data included net radiation (NR01, Hukseflux Thermal Sensors, Delft, Netherlands), air temperature and relative humidity (HMP155, Vaisala Inc., Helsinki, Finland), soil heat flux (HFT3, Hukseflux), soil temperature at 50, 100, and 200 mm depth using thermistors (107 probes, CSI), VMC at 100 and 200 mm depth (CS616, CSI) and precipitation (tipping bucket rain gauge, TB5, Hydrological Services, NSW, Australia).

EddyPro software (Version 5.2, LICOR Inc.) was used to process high frequency data. Resultant half hourly fluxes were then filtered to remove low quality data using EddyPro quality control flags (Mauder and Foken, 2004), periods underdeveloped turbulent intensity (standard deviation of 30-min vertical wind velocity $< 0.11 \text{ m s}^{-1}$ (Acevedo et al., 2009)), periods of instrument malfunction, periods when cows were grazing more than 5% of the EC footprint, and periods when fluxes were sourced from behind the CSAT3. To calculate daily totals gaps were filled using an artificial neural network (ANN) described by Pronger et al. (2016) and Rutledge et al. (2017). The ANN was run in two monthly windows with day and night time periods filled separately. The modelled response of night time net ecosystem production (NEP) was then used to calculate day time ecosystem respiration (ER) which was added to daytime NEP to determine GPP.

EWUE was calculated by dividing daytime GPP by daytime E for the 3-week period prior to each pre-grazing biomass harvest for carbon isotope analysis. This was done because grazing intervals were 3 weeks or greater and therefore EWUE was compared to $\text{WUE}_{\Delta}^{13\text{C}}$ over the last 3 weeks of biomass growth. Daytime was defined as all half hour periods where the photosynthetic photon flux density (PPFD) was greater than $20 \mu\text{mol s}^{-1} \text{ m}^{-2}$.

5.3.2 Pasture biomass sampling and analysis

Both New Rye and New Mix were rotationally grazed year-round with grazing rotation lengths between about 20 days in late spring to in excess of 60 days through winter in line with standard practice for intensively managed pastures in New Zealand. We harvested bulk biomass at 10 random locations within the calculated footprint of the eddy covariance system (calculated following Kormann & Meixner, 2001) just prior to grazing at each site for selected grazing events throughout 2015. These random harvest locations were not from grazing exclusion cages because they needed to be representative of the paddock biomass for comparison to eddy covariance measurements. New random locations were generated for each pre-grazing harvest. The farm was managed as a commercial dairy farm and consequently grazing timing varied between treatments and we were not able to sample before all grazing events. At New Mix we harvested before eight out of 11 grazing events for the year and at New Rye we harvested before seven out of 11 grazing events for the year. These harvests were spread relatively evenly throughout 2015. At each random sampling location, a 0.2 m² quadrat was used and biomass was cut at a height such that harvested biomass consisted of sunlit leaves (typically about 80 mm).

In addition to the bulk species pre-grazing biomass sampling, individual species samples were collected from grazing exclusion cages at New Mix for the harvest that best aligned with each season through 2015. Sunlit leaves were selected and stalky or reproductive material was avoided. The purpose of collecting individual species samples was to compare WUE_{Δ}^{13C} between the dominant species (ryegrass, white clover, chicory, and plantain). Lucerne was also sampled where possible. Despite poor establishment at this site, lucerne is of particular interest to many farmers because it has been shown to be productive during warm and dry seasons (e.g. Nobilly, 2015).

On return to the lab samples were removed from chiller bins and dead material was removed from the samples prior to drying at 65°C for 7 days. Dried samples were then cut into small pieces (about 2 cm length) and well mixed to ensure homogenisation of the sample. The sample was then split continuously until

about 2 grams of dry plant material remained. Each sample was ground to a fine powder in a ball mill at the Waikato Stable Isotope Unit. Samples were analysed using a Europa Scientific 20/20 isotope analyser where samples were combusted, separated by gas chromatography, and then analysed by continuous-flow mass spectrometry (precision was $\pm 0.5\%$). Samples were referenced to pre-calibrated C_4 sucrose that had been cross referenced to the PeeDee Belemite standard. Carbon isotope composition ($\delta^{13}C$) with respect to the reference was calculated as:

$$\delta^{13}C = \left[\frac{\left[\frac{C^{13}}{C^{12}} \right]_{sample}}{\left[\frac{C^{13}}{C^{12}} \right]_{standard}} - 1 \right] \times 1000 \quad \text{Eqn. 1}$$

The carbon isotope discrimination factor with respect to source air ($\Delta^{13}C$) was calculated from $\delta^{13}C$ following Farquhar et al. (1982) as:

$$\Delta^{13}C = \frac{\delta^{13}C_a - \delta^{13}C_p}{1 + \delta^{13}C_p} \quad \text{Eqn. 2}$$

where C_p is the $\delta^{13}C$ of the plant material with respect to the standard (Pee Dee Bee belimite) and C_a is the $\delta^{13}C$ of the atmosphere plants are exposed to assuming a well-mixed atmospheric $\delta^{13}C$ value of -8.4% measured at Baring Head (NIWA, 2016). $\Delta^{13}C$ is a more useful descriptor than $\delta^{13}C$ because it is relative to the source and hence provides mechanistic insight into biological processes (Farquhar et al., 1989).

5.3.3 Calculating leaf level WUE from $\Delta^{13}C$

To compare leaf level $\Delta^{13}C$ in units directly comparable to EWUE measured by EC we calculated the ratio of leaf internal to external CO_2 concentration (C_i/C_a) from leaf level $\Delta^{13}C$ measurements using the linear model of Farquhar et al. (1982). The ratio of C_i/C_a was then combined with on-site measurements of atmospheric vapour pressure and CO_2 concentration and estimates of leaf saturation vapour pressure to calculate leaf level WUE ($WUE_{\Delta^{13}C}$) in units of $g\ C\ (kg\ H_2O)^{-1}$ for direct comparison to EC measured EWUE.

The ratio of $C_i:C_a$ was calculated as:

$$C_i:C_a = \frac{(\Delta - a)}{(b - a)} \quad \text{Eqn. 3}$$

where a is the fractionation during diffusion (4.4‰) and b is the enzymatic fractionation by Rubisco during carboxylation (27‰). Enzymatic fractionation is reduced to 27‰ when using the simple ‘linear’ Farquhar model to account for fractionation during respiration, photorespiration, and during diffusion of CO_2 from intercellular air spaces to the chloroplast. Leaf level WUE was calculated as:

$$WUE_{\Delta^{13}\text{C}} = \frac{A}{E} = \frac{(c_a - c_i)}{1.6(e_i - e_a)} = \frac{c_a(1 - c_i/c_a)}{e_i\left(1 - \frac{e_a}{e_i}\right)1.6} \quad \text{Eqn. 4}$$

where A is the molar rate of carbon assimilation, E is the molar rate of transpiration, c_a is the atmospheric CO_2 partial pressure calculated from onsite measurements of CO_2 concentration (LI-7200, LI-COR Inc., Lincoln, NE, USA) and atmospheric pressure (CS100 Campbell Scientific Ltd., Shepshed, UK), e_i is the leaf internal vapour pressure, e_a is the leaf external vapour pressure calculated from air temperature and relative humidity measurements at each site and 1.6 is the ratio of diffusivities of water vapour and CO_2 in air. Leaf internal vapour pressure is equal to the saturation vapour pressure for the leaf temperature (Osmond et al., 1980) and was calculated as:

$$e_i = \exp\left[52.57633 - \left(\frac{6790.49}{T}\right) - 5.02808 \log_e T\right] \quad \text{Eqn. 5}$$

where T is the leaf temperature which was estimated from surface temperature calculated from outgoing longwave radiation measurements (NR01, Hukseflux Thermal Sensors, Delft, Netherlands). All parameters used to calculate $WUE_{\Delta^{13}\text{C}}$ were daytime values ($\text{PPFD} > 20 \mu\text{mol s}^{-1} \text{m}^{-2}$) measured over the 3-week period prior to isotope biomass sampling.

5.3.4 Adjusting leaf level WUE to compare to EWUE

Leaf level measurements of WUE do not include soil water evaporation (E_s) and interception (E_i). Equation 6 is a modification of Equation 4 that allows for these additional sources of evaporation to be accounted for to allow direct comparison to EWUE measurements. Equation 6 also allows for the removal of leaf level carbon uptake subsequently lost through root respiration (Flanagan and Farquhar, 2014; Hubick and Farquhar, 1989).

$$WUE_{\Delta^{13}C} = \frac{A}{E} = \frac{(c_a - c_i)(1 - \phi_c)}{1.6(e_i - e_a)(1 + \phi_w)} \quad \text{Eqn. 6}$$

where ϕ_c is the fraction of carbon fixed during photosynthesis that is subsequently lost through root exudation and respiration and ϕ_w is the non-productive fraction of evaporative water use (primarily E_s and E_i). In this study partitioning of NEP into GPP and ER accounted for carbon lost through root respiration at the ecosystem scale and consequently it was not necessary to account for this when making comparisons between EWUE and leaf level $WUE_{\Delta^{13}C}$.

5.4 Results

5.4.1 Meteorology

Relative to 30-year normal rainfall (1249mm) 2015 was a dry year with total rainfall of 1078 mm. Low annual rainfall was largely a result of low summer rainfall and consequently soil volumetric moisture content (VMC) remained below permanent wilting point (about -1500 kPa) through much of summer and into early autumn (5th Jan to 15th March). During this dry period maximum daily vapour pressure deficits (VPD) often exceeded 2 kPa. Through much of autumn, winter, and spring air temperature and rainfall were very close to the 30-year normal (Figure 5.1).

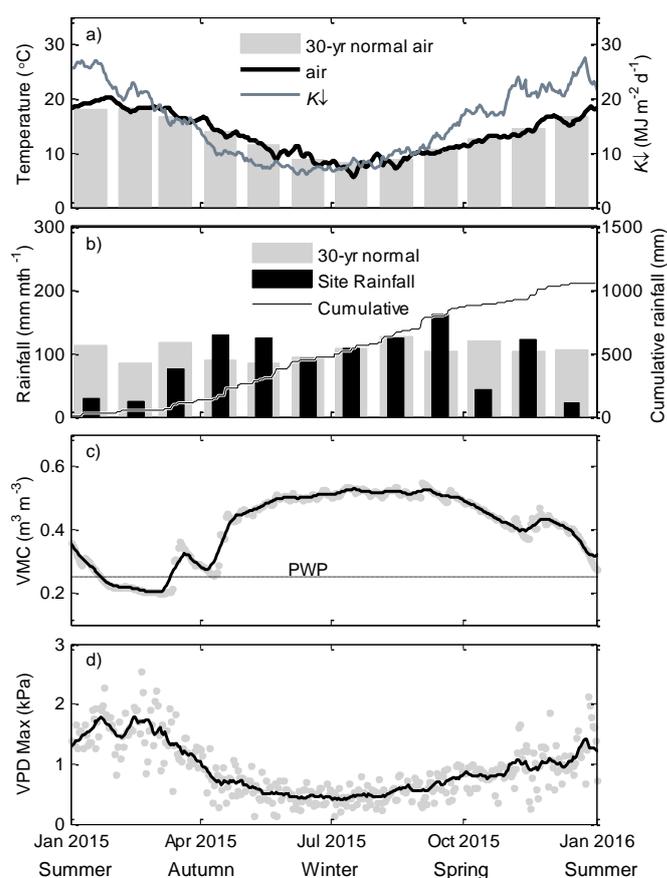


Figure 5.1. Variation in environmental conditions at New Mix (New Rye was similar) during 2015; (a) 15-day running mean of incoming shortwave radiation (K_{\downarrow}) and air temperature compared to the monthly normal air temperature (1981-2010); (b) monthly measured rainfall compared to monthly normal rainfall (1981-2010) and cumulative rainfall for 2015; (c) daily (grey dots) and 15-day running mean VMC at 10 cm depth (horizontal dashed line shows permanent wilting point); (d) daily (grey dots) and 15 day running mean of daily maximum vapour pressure deficit.

5.4.2 Seasonal variation in EWUE and isotopic discrimination

GPP varied through 2015 with mean daily rates ranging from about $2 \text{ g C m}^{-2} \text{ day}^{-1}$ during the dry late summer and early autumn period up to $8 \text{ g C m}^{-2} \text{ day}^{-1}$ during spring (Figure 5.2a). GPP was lower at New Rye compared to New Mix during the dry late summer and early autumn period and potentially also during late spring. In contrast, during winter and early spring GPP was higher at New Rye compared to New Mix. Some of this difference was associated with seasonal differences between treatments (see Chapter 4); however, the differences between sites need to be interpreted with caution because WUE was measured over offset

time periods due to asynchronous grazing. In contrast, differences in E between sites were small (Figure 5.2b) because differences in the timing of grazing had little effect on E (see Chapter 3). Evaporation rates were lowest in mid-winter (about 1 mm day^{-1}) and highest during summer (about 4 mm day^{-1} when averaged over a 3-week interval).

Seasonally, EWUE was lowest during warm dry growing conditions and highest during mid-winter when soil moisture was not limiting and the atmospheric VPD was low (Figure 5.2c). EWUE was marginally lower at New Rye compared to New Mix during the warm and dry late summer and early autumn period and higher during winter. These observations agree with the findings presented in Chapter 4 but must be interpreted with caution in this case because asynchronous grazing meant calculations were made over different growth periods when climatic differences could strongly effect both GPP and EWUE.

Intrinsic WUE (WUE_i) was highest during dry summer periods (low $\Delta^{13}\text{C}$) and lowest during mid-winter (high $\Delta^{13}\text{C}$) (Figure 2d). Therefore, EWUE and WUE_i were negatively correlated prior to accounting for differences in VPD (see Appendix C, Figure C1). The decrease in $\Delta^{13}\text{C}$ during warm and dry periods indicates plants were reducing stomatal conductance in response to soil drying and increased atmospheric VPD thereby increasing WUE_i . At New Rye, $\Delta^{13}\text{C}$ was lower relative to New Mix during the dry late summer and early autumn period. Diversity was still reasonably high at New Mix during this period and it was likely the deeper rooting herbs, chicory and plantain, were accessing deeper soil water and therefore the severity of water stress was relatively lower for chicory and plantain. During late autumn, WUE_i was lower at New Rye (higher $\Delta^{13}\text{C}$) relative to New Mix in agreement with species specific $\Delta^{13}\text{C}$ measurements (see Section 5.4.3) that showed WUE_i was low for ryegrass during winter.

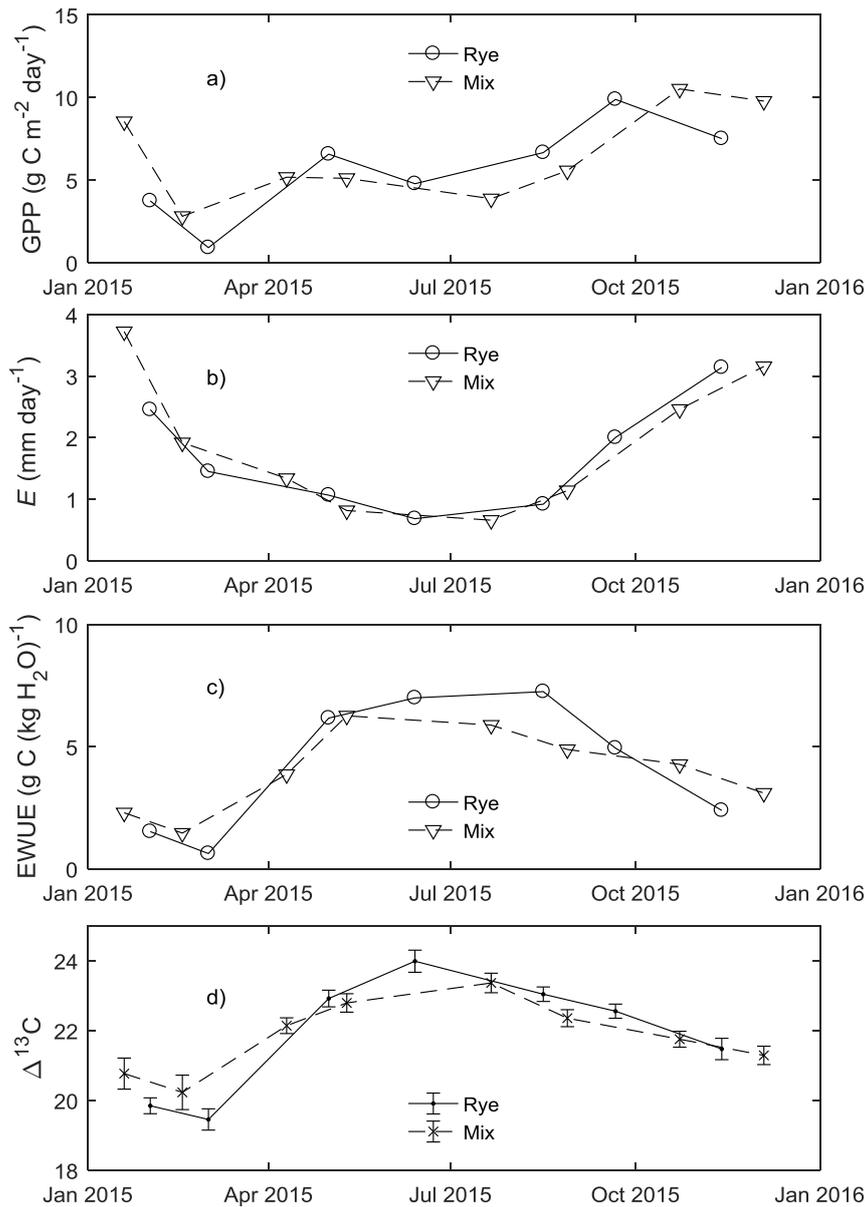


Figure 5.2. Mean GPP (panel a), E (panel b), and EWUE (panel c) measured by EC for 3-weeks prior to isotope sampling for New Rye (open circles) and New Mix (open triangle) and mean $\Delta^{13}\text{C}$ of harvested plant biomass grown over the same period (panel d) (Error bars show 95% CI). All data plotted at the mid-point of the period of interest (i.e. biomass harvested on 12 Feb 2015 was plotted at 2 Feb 2015 and EC data is mean for 22 Jan 2015 to 12 Feb 2015).

To directly compare $\Delta^{13}\text{C}$ to EWUE it was necessary to account for the seasonal changes in the atmospheric VPD on WUE_i using Equation 4 (Farquhar et al., 1982). After applying Equation 4 both EWUE and $\text{WUE}_{\Delta^{13}\text{C}}$ varied in unison (Figure 5.3a and 5.3b) and correlation between EWUE and $\text{WUE}_{\Delta^{13}\text{C}}$ was strong

with significant positive correlation coefficients of 0.88 at New Mix and 0.79 at New Rye (Figure 5.3c).

Despite the strong positive correlation between EWUE and $WUE_{\Delta}^{13}C$, the agreement between absolute values were poor (Figure 5.3c). For example, at New Mix mean EWUE during the annual grazing cycles examined was $3.77 \text{ g C m}^{-2} (\text{kg H}_2\text{O})^{-1}$ compared to a mean $WUE_{\Delta}^{13}C$ of $7.85 \text{ g C m}^{-2} (\text{kg H}_2\text{O})^{-1}$. Some of the discrepancy between $WUE_{\Delta}^{13}C$ and EWUE was because $WUE_{\Delta}^{13}C$ only accounts for transpiration whereas EWUE includes evaporation of soil water (E_S). While E_S was not measured separately at this site, Graham et al. (2016) modelled E_S from an intensively grazed ryegrass and clover pasture and reported contributions of 20 – 30% of total evaporation, similar to contributions used by Flanagan and Farquhar (2014). Figure 5.3 (panel d, e, and f) shows the annual comparison between EWUE and $WUE_{\Delta}^{13}C$ after adjusting Equation 4 to account for the likely contribution of E_S following Equation 6. After accounting for the probable contribution of E_S , agreement between the two methods improved; however, $WUE_{\Delta}^{13}C$ was still about twice the magnitude of EWUE throughout the year at New Mix and during the warmer months at New Rye.

Regression slopes between EWUE and $WUE_{\Delta}^{13}C$ were also different between New Rye and New Mix (Figure 5.3 panels c and f). At New Mix, the difference between EWUE and $WUE_{\Delta}^{13}C$ was higher during winter, resulting in a slope of 1.3 prior to adjustment for E_S and 1.0 post-adjustment. In contrast, at New Rye differences between EWUE and $WUE_{\Delta}^{13}C$ were larger during summer and spring and smaller during winter resulting in a slope of 0.5 prior to adjustment for E_S and 0.4 post-adjustment. This difference in slope between sites was possibly related to differences in diversity between sites (see Section 5.5.2 for discussion).

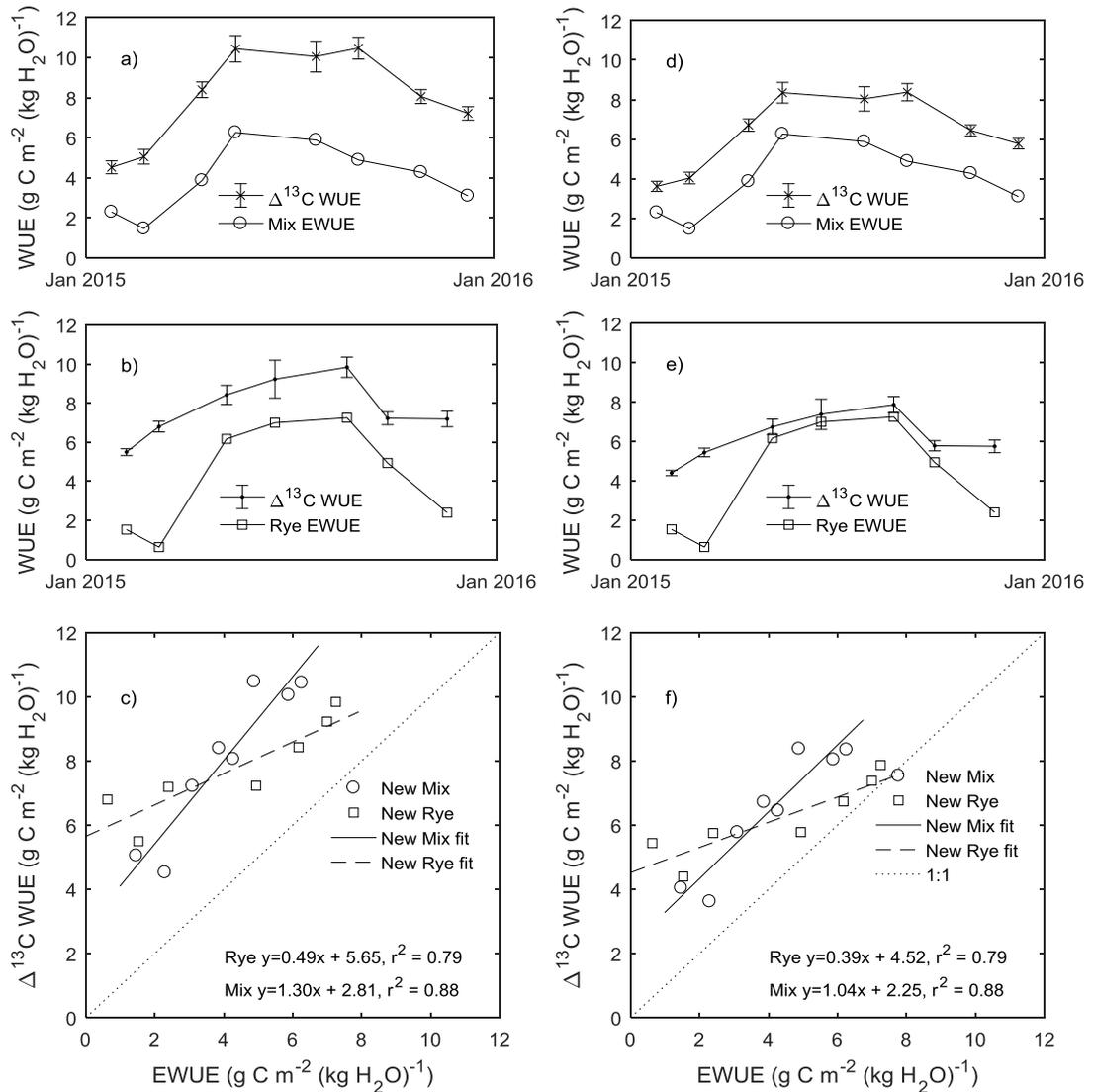


Figure 5.3. Annual comparison of EWUE and $\text{WUE}_{\Delta^{13}\text{C}}$ after applying Equation 4 at New Mix (panel a) and New Rye (panel b) and regression between EWUE and $\text{WUE}_{\Delta^{13}\text{C}}$ (panel c) and equivalent figures (d – f) after adjusting Equation 4 to account for the likely contribution of E_s following Flanagan and Farquhar (2014). Error bars show 95% CI.

5.4.3 Seasonal variation in $\text{WUE}_{\Delta^{13}\text{C}}$ between species

In addition to the comparison of EWUE to $\text{WUE}_{\Delta^{13}\text{C}}$ calculated from bulk biomass, the seasonal variation in $\Delta^{13}\text{C}$ between the dominant species was also measured to examine differences in $\text{WUE}_{\Delta^{13}\text{C}}$ between species (Figure 5.4a). During summer (Dec 2014 to Feb 2015), $\text{WUE}_{\Delta^{13}\text{C}}$ was higher for chicory relative to all other species except clover (Figure 5.4b). Relative production from chicory was also high (38.9%), compared to ryegrass (20.6%), plantain (19.6%), clover (8.5%), and lucerne (0.9%) and therefore the high summer $\text{WUE}_{\Delta^{13}\text{C}}$ measured for

chicory was not at the expense of production (Figure 5.4c). During autumn $WUE_{\Delta}^{13}C$ was again highest for chicory but this was not significantly different from ryegrass, clover, and lucerne. The high autumn $WUE_{\Delta}^{13}C$ did not come at the expense of production that was highest for chicory but not significantly different from ryegrass and plantain. In winter 2015, diversity was very low at New Mix with pasture composition dominated by ryegrass which had low relative $WUE_{\Delta}^{13}C$. In spring 2015 (and from this time onward) the contribution of chicory and plantain did not return as it had in previous springs suggesting persistence of chicory and plantain was limited to about two years under intensive grazing. Again, despite the high spring production from ryegrass, $WUE_{\Delta}^{13}C$ was relatively low while clover had high $WUE_{\Delta}^{13}C$. Combined, these results indicate chicory and clover use water more efficiently than ryegrass and further study is required to examine this potential.

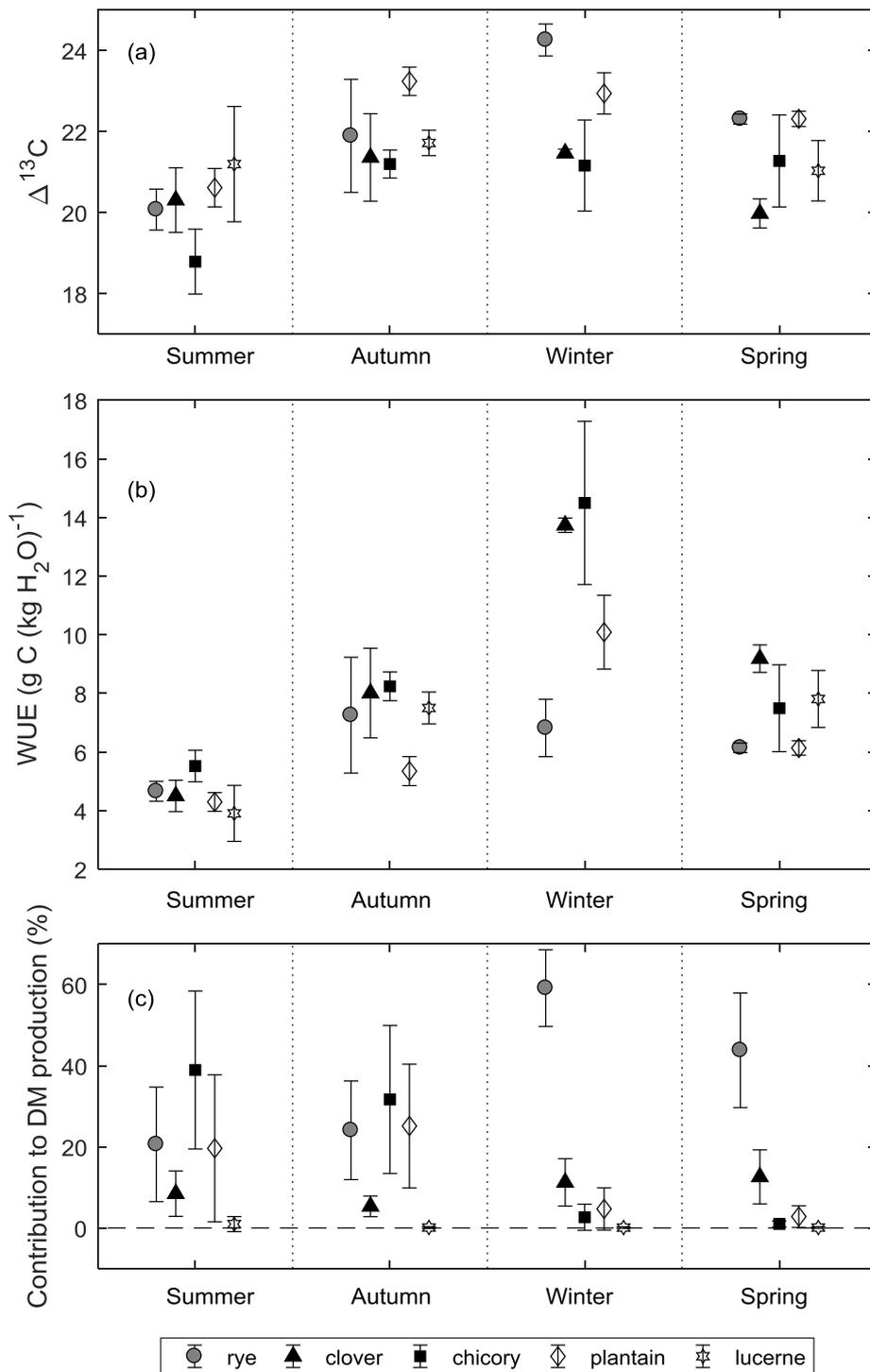


Figure 5.4. Seasonal variation in $\Delta^{13}\text{C}$ (panel a) and $\text{WUE}_{\Delta^{13}\text{C}}$ (panel b) for ryegrass, clover, chicory, plantain, and lucerne for all seasons of 2015 and the seasonal contribution to total DM yield (panel c) for each species over 2015. Error bars show 95% confidence intervals and non-overlapping confidence intervals show significant differences at $p < 0.05$.

5.4.4 Relationships between fractionation and soil moisture

Correlations between $\Delta^{13}\text{C}$ and soil moisture, air temperature, and VPD were strong at both sites with significant coefficients ranging from 0.68 to 0.96 (Figure 5.5). The strongest relationships were between $\Delta^{13}\text{C}$ and VPD (Figure 5.5c). Carbon isotope discrimination was low when soil moisture content was low and air temperatures and VPD were high. Discrimination decreased relatively linearly as soil moisture increased and air temperature and VPD declined. Examination of Figure 5.5 indicates $\Delta^{13}\text{C}$ was generally higher at New Mix when VMC was low and temperature and atmospheric VPD were high. These measurements were made during the dry late summer and early autumn of 2015, prior to the relative abundance of chicory and plantain declining sharply (winter 2015), and consequently plant water stress was potentially lower at New Mix because chicory and plantain were likely accessing deeper soil water.

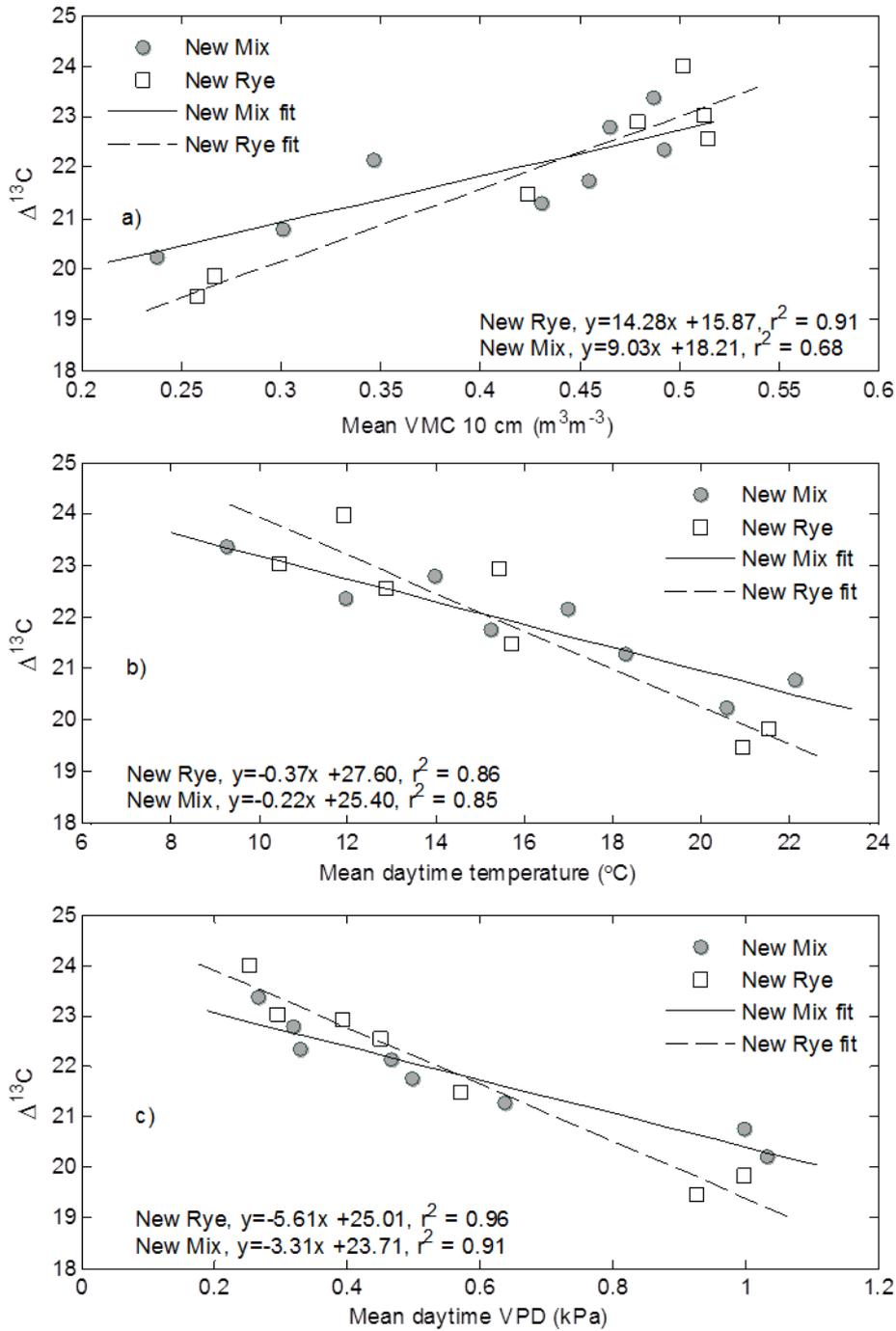


Figure 5.5. Correlation between $\Delta^{13}\text{C}$ and mean soil moisture at 10 cm depth (panel a), mean daytime air temperature (panel b), and mean daytime VPD (panel c).

5.4.5 Sensitivity of Farquhar equation to input variables

To determine if assumptions made during calculation of $\text{WUE}_{\Delta^{13}\text{C}}$ from $\Delta^{13}\text{C}$ measurements could explain the discrepancy between EWUE and $\text{WUE}_{\Delta^{13}\text{C}}$ the

sensitivity of the Farquhar et al. (1982) equations to the isotopic composition of the source atmosphere, CO₂ concentrations, and the VPD was examined (Figure 5.6). Decreases in atmospheric δ¹³C and CO₂ concentration or increases in VPD were required to close the discrepancy between EWUE and WUE_{Δ¹³C}. Atmospheric δ¹³C values from continuous measurements made at Baring Head in New Zealand were used, CO₂ concentration was measured onsite at 1.55 m, and the VPD was calculated from relative humidity and air temperature measured at 2 m above the pasture. It was more likely plants were experiencing more negative δ¹³C (respired CO₂ δ¹³C approaches -27 ‰) and lower atmospheric VPD at the leaf surface compared to what we measured at 2 m height, both of which increase the difference between WUE_{Δ¹³C} and EWUE. Surface CO₂ concentration may have been lower than what we measured when CO₂ uptake was strong during the daytime but the magnitude of this effect was likely relatively small (squares in Figure 5.6). Sensitivity analysis indicated the assumptions made with respect to inputs to equations 2, 3, and 4 are unlikely to explain the observed discrepancy between EWUE and WUE_{Δ¹³C}. Overall, while EWUE and WUE_{Δ¹³C} were highly correlated absolute values differed by about 40% (at New Mix) after accounting for *E_S* and further research is required to explain this discrepancy.

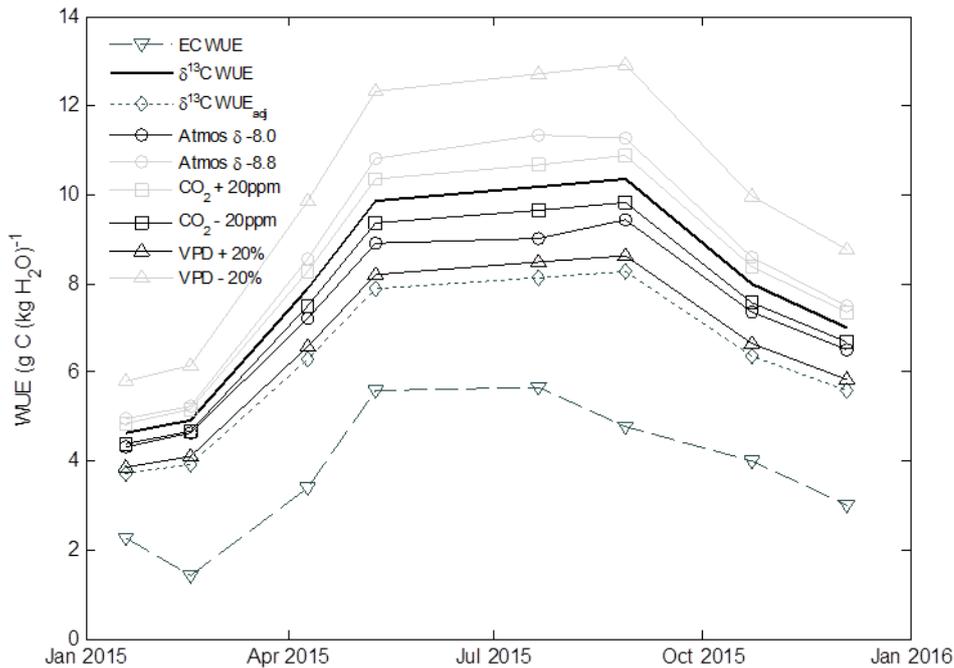


Figure 5.6. Sensitivity of WUE_{Δ}^{13C} (heavy black line) to variation in variables used to calculate WUE from bulk leaf Δ^{13C} including atmospheric δ^{13C} (circles), CO_2 concentration (squares), and VPD (triangles) using New Mix as an example. Solid fine black lines show variation that brought WUE_{Δ}^{13C} closer to measured EWUE and pale grey lines show variation that increased the discrepancy. Also shown is WUE_{Δ}^{13C} after adjusting for the likely contribution of E_s (diamonds with dotted line) and measured EWUE (triangles with dashed line).

5.5 Discussion

5.5.1 General relationship between WUE_{Δ}^{13C} and EWUE

The relationship between Δ^{13C} and leaf level WUE is well supported theoretically (Farquhar et al., 1982) and experimentally (e.g. Anyia et al., 2007; Condon et al., 1990; Ehleringer et al., 1990; Martensson et al., 2017); however, few researchers have compared bulk biomass leaf WUE_{Δ}^{13C} to ecosystem level measurements in grasslands. Strong correlation ($r^2 \geq 0.79$) was found between EWUE and WUE_{Δ}^{13C} at both New Rye and New Mix throughout 2015. However, absolute agreement between EWUE and WUE_{Δ}^{13C} was relatively poor. Using the more commonly applied ‘linear’ equation of Farquhar et al. (1982), WUE_{Δ}^{13C} was about twice as large as EWUE. Others have also observed large discrepancies between

WUE $_{\Delta}^{13}\text{C}$ and EWUE. For example, Flanagan and Farquhar (2014) calculated annual WUE $_{\Delta}^{13}\text{C}$ to be 5.11 g C (kg H $_2$ O) $^{-1}$ using the ‘linear’ equation compared to measured EWUE of 1.78 g C (kg H $_2$ O) $^{-1}$ for a native Canadian grassland. This difference occurred, at least partly, because WUE $_{\Delta}^{13}\text{C}$ only accounts for transpiration whereas EWUE includes E_S and E_T . Flanagan and Farquhar (2014) suggested that a conservative estimate of E_S at their site was about 20% (e.g. E_T 80% of total E) and by accounting for this they improved agreement but a large discrepancy still remained. E_S was not measured separately at our sites but recent modelling work at a ryegrass-clover pasture site in New Zealand estimated a contribution of 20 -30% (Graham et al., 2016). At our site after adjusting Equation 4 to account for the likely contribution of E_S (25% of total E) mean annual WUE $_{\Delta}^{13}\text{C}$ at New Mix declined from 7.85 g C m $^{-2}$ (kg H $_2$ O) $^{-1}$ to 6.28 g C m $^{-2}$ (kg H $_2$ O) $^{-1}$. This was still considerably higher than mean annual EWUE of 3.77 g C m $^{-2}$ (kg H $_2$ O) $^{-1}$ at New Mix (differences were similar at New Rye).

Some of the observed discrepancy between WUE $_{\Delta}^{13}\text{C}$ and EWUE may have been related to the use of the ‘linear’ model (Equation 4) as opposed to using the less frequently used but more complete ‘classic’ equation (Farquhar et al., 1982). Some have claimed the ‘linear’ model over estimates WUE (Seibt et al., 2008) while others have found very little difference between models (Hu et al., 2010) or that estimates using the ‘classic equation’ are still high compared to eddy covariance measurements (Flanagan and Farquhar, 2014). The ‘classic’ equation requires estimates of mesophyll conductance and photorespiration and Flanagan and Farquhar (2014) demonstrated that varying mesophyll conductance could improve agreement.

In addition to model selection, a number of assumptions were made regarding meteorological variables used to calculate WUE $_{\Delta}^{13}\text{C}$. Assumptions were similar to those made by others (e.g. Flanagan and Farquhar, 2014) and included that plant leaves were accessing a well-mixed atmosphere during the day and therefore ambient atmospheric $\delta^{13}\text{C}$ could be used in addition to mean day-time CO $_2$ concentration, atmospheric pressure, air temperature, and VPD measured at between 1.5 to 2 m above the canopy. Sensitivity analysis (Figure 5.6) showed Equation 4 was sensitive to VPD and atmospheric $\delta^{13}\text{C}$ in particular. It is possible

that during periods of low developed turbulence that ambient $\delta^{13}\text{C}$ could have been more enriched from respiratory processes or the VPD could have been lower at the leaf surface compared to the VPD measured at 2 m above the vegetation. Additionally, leaf boundary layer resistances may have resulted in lower VPD at the leaf surface compared to the VPD that was measured at 2 m above the canopy. However, more enriched ambient $\delta^{13}\text{C}$ and lower VPD would have increased discrepancy between EWUE and $\text{WUE}_{\Delta}^{13}\text{C}$ and therefore do not help to explain the observed differences between $\text{WUE}_{\Delta}^{13}\text{C}$ and EWUE.

A further consideration that affects absolute agreement between EWUE and $\text{WUE}_{\Delta}^{13}\text{C}$ is the time period that EWUE is calculated over. EWUE generally increases considerably between post-grazing, when leaf area is low and E_S is a larger proportion of total E , and pre-grazing when leaf area is high and total E is dominated by E_T . In this study, EWUE was calculated over the three weeks prior to sampling because $\Delta^{13}\text{C}$ was assumed to integrate over the life of the biomass (grazing rotations were typically three to four weeks). In contrast, if EWUE was calculated over the ten days prior to biomass sampling for isotope analysis absolute agreement improved but correlation between EWUE and $\text{WUE}_{\Delta}^{13}\text{C}$ decreased. For example, at New Mix EWUE averaged across all grazing events increased from $3.77 \text{ g C (kg H}_2\text{O)}^{-1}$ calculated over 21 days to $4.56 \text{ g C (kg H}_2\text{O)}^{-1}$ calculated over ten days pre-grazing. However, correlation strength decreased from 0.91 to 0.71 (see Figure C2).

Overall there was strong correlation between $\text{WUE}_{\Delta}^{13}\text{C}$ and EWUE. However, further research is required to reconcile the discrepancy between $\text{WUE}_{\Delta}^{13}\text{C}$ and EWUE.

5.5.2 Differences in the regression relationship between sites

The difference in regression slopes between sites was largely driven by site differences in late summer and early autumn and then again during winter. During late summer and early autumn, EWUE was higher at New Mix but $\text{WUE}_{\Delta}^{13}\text{C}$ was higher at New Rye resulting in strong differences in correlations at lower EWUE values between sites (Figure 5.3c and f). During this period soil VMC remained below PWP for an extended period resulting in senescence of a

large proportion of the ryegrass plants at both sites. However, because other species, such as chicory were still growing at New Mix, GPP was higher at New Mix while E was similar between sites resulting in higher relative EWUE at New Mix (Figures 5.2a, b, and c). In contrast, $WUE_{\Delta}^{13}C$ was higher at New Rye because isotope sampling was limited to the remaining live ryegrass plants that were likely experiencing higher water stress relative to remaining live plants at New Mix (and consequently increased $WUE_{\Delta}^{13}C$ at New Rye during summer was driven by reduced stomatal conductance and lower production). These factors may explain the differences in regression fits at lower EWUE values (Figure 5.3c and f). Differences at higher EWUE values (winter) are more difficult to explain. One hypothesis is that the rapid decline in diversity over winter 2015 at New Mix left more open gaps in the pasture resulting in lower ecosystem level GPP and a higher proportion of ecosystem E coming from E_S at New Mix. This would have decreased EWUE but had no effect on $WUE_{\Delta}^{13}C$ measurements that were dominated by ryegrass at this time of year and hence similar between sites. Together, these factors may explain the differences in regression slopes between sites (Figure 5.3c and f).

These differences in regression slopes also highlight some limitations that must be considered when using $WUE_{\Delta}^{13}C$ to make paddock scale inferences about EWUE. Firstly, $WUE_{\Delta}^{13}C$ does not account for soil water evaporation and consequently will likely over predict EWUE when pasture covers are low (e.g. winter at New Mix) or for uneven pastures with poor canopy closure. Secondly, $WUE_{\Delta}^{13}C$ can increase because of water stress during summer and therefore simultaneous measurements of production are critical to avoid erroneously interpreting high $WUE_{\Delta}^{13}C$ as agriculturally desirable when in some circumstances (dry conditions in particular) high $WUE_{\Delta}^{13}C$ may indicate increased plant water stress.

5.5.3 Differences in $\Delta^{13}C$ between species

Asynchronous grazing resulted in both EWUE and $WUE_{\Delta}^{13}C$ being calculated over different time periods at New Mix and New Rye limiting our ability to directly compare the effect of diversity on WUE because of differences in weather and consequently growing conditions between time periods. Therefore, to examine whether $\Delta^{13}C$ was different for the dominant pasture species when grown

over the same time period we measured differences in $\Delta^{13}\text{C}$ between co-existing species at New Mix toward the end of each season (Figure 5.4a). Differences in $\text{WUE}_{\Delta^{13}\text{C}}$ between species grown in the same environment could be expected because of differences in physiology associated with evolutionary development of these plants (e.g. differences in root depth and leaf N content) (Murphy and Bowman, 2009). For the seasonal comparison, differences in $\Delta^{13}\text{C}$ between species within each seasonal sampling could be directly linked to leaf level $\text{WUE}_{\Delta^{13}\text{C}}$ (the use of Equation 4 was not critical) assuming the VPD was equivalent for each species during the respective growth period (Smedley et al., 1991).

During warm dry summer conditions, relative $\text{WUE}_{\Delta^{13}\text{C}}$ and production was highest for chicory (Figure 5.4b and c) and this may have been because the deep tap root increased access to water. Increased access to deeper soil water during dry periods could lead to increased WUE because of increased evaporative cooling which maintains leaf temperatures closer to the optimum for photosynthesis (Osmond et al., 1980). During spring, $\text{WUE}_{\Delta^{13}\text{C}}$ was highest for white clover but white clover was not significantly different from chicory and lucerne. $\text{WUE}_{\Delta^{13}\text{C}}$ was lowest for ryegrass and plantain which were not significantly different from chicory. This suggests that during spring, when water limitations are rare, white clover and potentially lucerne (both legumes) had higher WUE. High leaf N content may increase WUE because N allows for increased rates of photosynthesis while not increasing transpiration (Hussain et al., 2011). Increasing EWUE during spring and summer are likely desirable in New Zealand pasture systems to conserve water for late summer and autumn dry periods. Consequently, including chicory and additional legumes into pasture mixes could be considered as a potential approach for increasing pastoral production and WUE at specific times of the year.

5.5.4 Dependence of $\Delta^{13}\text{C}$ on soil moisture

Correlations were strong between $\Delta^{13}\text{C}$ and soil moisture content, air temperature, and VPD (Figure 5.5). These relationships likely occur because as soil moisture declines, air temperatures increase (less evaporative cooling) resulting in higher atmospheric VPD. Plants respond to this increased water stress by reducing

stomatal conductance, thereby decreasing carbon isotope discrimination which is interpreted as an increase in WUE_i (Farquhar et al., 1989). Therefore, differences in $\Delta^{13}C$ between plant species during dry periods needs to be carefully interpreted and accompanied by simultaneous measurement of relative differences in production. Production measurements are necessary to avoid erroneously selecting for water stressed species with high WUE_i but low productivity (Condon et al., 2002). This complication arises because increased WUE_i can occur because of relative decreases in stomatal conductance (not always desirable) or relative increases in carboxylation (desirable in an agricultural context).

5.5.5 Limitations and uncertainties

Relatively large differences in $\Delta^{13}C$ between species (Figure 5.4a and b) show that fractionation can be significantly different between co-existing C3 pasture species. However, there is potential for post carboxylation fractionation during the synthesis of species specific organic compounds (oils, waxes, resins, tannins) to influence bulk leaf $\Delta^{13}C$ post-photosynthesis (Moreno-Gutierrez et al., 2012). Despite this complication, Orchard et al. (2010) showed that 80% of the variation in $\Delta^{13}C$ between different C3 plant species was a consequence of variation in the ratio of C_i to C_a and therefore variation in WUE . This suggests post carboxylation fractionation differences among C3 plant species is likely small and potentially unimportant from a pragmatic viewpoint.

Additionally, to make inferences about WUE based on carbon isotope discrimination stomatal conductance and mesophyll conductance must be strongly positively correlated (Flexas et al., 2012). There is still uncertainty about the strength of this correlation but evidence suggests a positive correlation does occur (Barbour et al., 2010; Evans, 1999). Also, mesophyll conductance is typically greater in higher flux species such as grasses and herbs (see Flexas et al., 2012) and therefore less likely to result in further fractionation.

5.5.5.1 Experimental design and measurement techniques

This study was designed to examine the correlation between $EWUE$ and leaf scale $WUE_{\Delta^{13}C}$ to determine the suitability of leaf scale $\Delta^{13}C$ measurements for comparing paddock and farm scale $EWUE$ between pasture swards.

Asynchronous grazing between sites resulted in offset timing of pre-grazing isotope sampling. Consequently, both metrics of WUE ($WUE_{\Delta^{13}C}$ and EWUE) were calculated for offset time periods when weather conditions were potentially different. This offset meant it was not reasonable to draw direct conclusions about differences between the New Mix and New Rye sites based on comparison of the bulk isotope data at this site because WUE is strongly controlled by changes in meteorology and soil water content (Yang et al., 2010). The future use of $\Delta^{13}C$ to compare $WUE_{\Delta^{13}C}$ between pasture swards should use a synchronised grazing approach, or alternatively grazing exclusion cages, to avoid this complication.

Ecosystem scale estimates of NEP and evaporation gathered by EC measurements also include uncertainty as a result of measurement and data processing errors and gap filling procedures. In general, the largest known sources of uncertainty are related to low developed turbulence and biases associated with gap filling techniques (Campbell et al., 2015). These uncertainties are larger at night when low turbulence results in more gaps and therefore the effect on daytime EWUE used in the current study are minimised. Additionally, ecosystem scale estimates of daytime GPP are partitioned from NEP using a modelling approach (see Section 5.3.1). This may result in some bias in daytime GPP estimates (Oikawa et al., 2017). However, there is currently not a widely accepted alternative routine for partitioning GPP from NEP.

5.6 Conclusions

Strong correlation between bulk leaf $WUE_{\Delta^{13}C}$ and EWUE at both individual sites suggest that, in combination with pasture production monitoring, $\Delta^{13}C$ measurements are suitable for comparing WUE between pasture species and mixes across continuously varying agricultural landscapes. However, future use of the approach to compare sites should use a synchronised grazing approach or grazing exclusion cages. Additionally, large within season differences in $\Delta^{13}C$ between co-existing species suggest WUE of pastoral systems can be improved by manipulating species composition. Differences in $\Delta^{13}C$ were also strongly correlated with soil water content and ensuing increases in the atmospheric VPD and therefore $\Delta^{13}C$ can identify differences in water stress between adjacent plant

species. This dual function highlights the importance of simultaneously measuring both production and $\Delta^{13}\text{C}$ to avoid interpreting decreased $\Delta^{13}\text{C}$ (increased WUE_i) as an agriculturally desirable trait during periods of potential water stress. Despite strong correlation between $\text{WUE}_{\Delta^{13}\text{C}}$ and EWUE, agreement of absolute values was poor and further study is required to reconcile this apparent scaling issue. Given limitations are recognised and understood and production is monitored simultaneously, $\Delta^{13}\text{C}$ provides a powerful tool for quick, simple, and cost effective measurement of WUE and water stress. Therefore, $\Delta^{13}\text{C}$ measurement can likely be used to optimise species selection for site specific climate and soil combinations at paddock and farm scale.

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Chapter Six

6 Summary and Conclusions

Conflict over freshwater is increasing globally, largely as a result of population growth and climate induced changes in supply (Kundzewicz and Gerten, 2015). Simultaneously, agriculture is under pressure to provide food for an expanding global population. Given restrictions in freshwater supply, increasing reliance solely on irrigation is not sustainable, and consequently increasing water use efficiency (WUE) is critical for the agricultural sector to maintain and increase productivity (White and Snow, 2012). Increasing pastoral diversity has often been shown to increase production during periods of soil water scarcity (e.g. Nobilly et al., 2013; Ruz-Jerez et al., 1991; Skinner, 2008). The positive effect of increasing diversity is typically linked to complementarity in plant traits such as rooting depth and facilitation among species (Sanderson et al., 2006) or the inclusion of plants with divergent life histories that use water more efficiently (Moreno-Gutierrez et al., 2012) as opposed to the number of species per se (Pembleton et al., 2015).

Intensively grazed pastures cover about one third of New Zealand (Ministry for the Environment, 2009) and efficient production and profitability is tightly linked to year-round pasture growth (Pembleton et al., 2015). However, the increasing incidence of extended dry periods (Clark et al., 2011) threaten profitability and consequently more farmers are interested in exploring the potential of pastoral diversity to improve dry season production (Pembleton et al., 2015). Prior to this thesis, little work had been done in New Zealand, or globally, comparing ecosystem scale measurements of WUE between simple and more diverse pastures. This lack of research limits farmer uptake because of uncertainty about the benefits and trade-offs in WUE and production. Broadly, my objective was to contrast seasonal evaporation, WUE, and production strategies of a simple binary ryegrass-clover and a more diverse pasture at the ecosystem scale. To achieve this objective, I used a novel paired site eddy covariance (EC) approach and this was the main focus of Chapter 4. However, through the literature review process it became apparent that baseline evaporation measurements from traditional ryegrass-clover pasture were scarce and therefore this gap was addressed first in

Chapter 3. Finally, Chapter 5 examined the potential to use natural carbon isotope discrimination during photosynthesis as a cost effective method to examine WUE. Such an approach avoids the technical expertise needed, and cost associated with using the EC method and may enable the selection of site specific pasture mixes at paddock to farm scale to optimise WUE and maximise pasture production. With these objectives in mind, the specific questions were (more detail in Section 1.2):

Chapter 3

What is the annual flux of water from traditional ryegrass-clover pastures and how does the flux vary spatially and temporally as a result of grazing and the seasonal progression of controls?

Chapter 4

Is increased summer production from more diverse pastures a consequence of increased access to water through deeper roots or increased WUE and what are the differences in seasonal plant growth and production characteristics?

Chapter 5

Can natural carbon isotope discrimination ($\Delta^{13}\text{C}$) during photosynthesis be used to compare WUE between pasture swards at the paddock scale and consequently allow quick and cost effective monitoring of WUE at paddock and farm scale?

The following section (6.1) summarises findings for each of the questions outlined above prior to overall conclusions in section 6.2. The final section (6.3) then briefly outlines some pertinent remaining questions.

6.1 Summary

6.1.1 Chapter 3 Summary – Evaporation from ryegrass-clover pastures

What is the annual flux of water from traditional ryegrass-clover pastures and how does the flux vary spatially and temporally as a result grazing and the seasonal progression of controls?

The focus of this thesis was on the effect of pasture diversity on evaporation (E) and EWUE; however, during review of literature it became apparent that E

measurements from traditional ryegrass-clover pastures were scarce despite identification of this gap over 20 years ago (Kelliher et al., 1993). Ecosystem scale measurements of E from intensively managed pasture systems are important for informing water resource decision making and validation of hydrologic models and remote sensing methods. Therefore, the objective was to quantify spatial and temporal variation in E from ryegrass-clover pastures and examine the effect of grazing on E using three eddy covariance towers located on a commercial dairy farm. The three systems measured continuously and simultaneously over ryegrass and clover pasture for about one year to examine spatial variation and one system measured continuously for three consecutive years to examine temporal variability.

Spatial variation in E was low across the three sites at daily, seasonal, and annual scales. Over the first year, annual E totals were 770 mm at Site 1 and 783 mm at Site 2 and over the spring and summer period when all three sites were measured simultaneously E totals were 520 mm at Site 1, 505 mm at Site 2, and 504 mm at Site 3. At a daily scale, E was strongly correlated between sites with r^2 values of 0.89 – 0.95 and RMSE ranging from 0.25 mm to 0.39 mm day⁻¹. Inter-annual variation was also low with annual E totals at Site 1 of 770 mm in 2012, 757 mm in 2013, and 776 in 2014. The largest differences between years occurred in 2013 when the late summer and autumn period was drier than usual and consequently soil moisture content constrained E . The low spatial and temporal variability across these ryegrass-clover pasture sites likely occurred because during most of each year E was strongly controlled by net radiation (R_n). The decoupling coefficient (Ω) (Jarvis and McNaughton, 1986) was about 0.8 during most of each year supporting the conclusion that evaporation was strongly radiation driven at these sites. However, during late summer and autumn dry periods, Ω declined sharply and the Bowen ratio spiked when soil VMC declined below about 30% indicating a switch in dominant control from R_n to available water.

The effect of rapid reductions in leaf area on E following intensive grazing events was also examined. The replicated EC experimental design, with non-synchronised grazing, was ideally suited for this purpose. Some studies have suggested grazing induced reductions in leaf area have little effect on E from

grazed grasslands (Shuttleworth et al., 1989; Stewart and Verma, 1992; Wang et al., 2012) while others have found a grazing resulted in a reduction in E (e.g. Bremer et al., 2001; Day and Detling, 1994; Frank, 2003). To determine whether E declined following intensive grazing events the relationship between two sites before and after one site was grazed was compared using linear regression. On average, across the year, leaf area was $3.4 \text{ m}^2 \text{ m}^{-2}$ pre-grazing and about $1.5 \text{ m}^2 \text{ m}^{-2}$ post-grazing. After all autumn and winter grazing's there was no observable reduction in E post-grazing but following some spring and summer grazing events a small but significant reduction in evaporation was measured. The reduction in E was estimated to be about 0.2 mm day^{-1} based on changes in slope of the relationship between the grazed and non-grazed site amounting to a reduction of about 5%. The nil to small reduction in E following grazing likely occurred because evaporation of soil water was able to compensate for the reduction in transpiration in this typically moist growing environment. During extended dry periods biomass growth, and hence reduction in leaf area following grazing, was small likely minimising the reductions in E post grazing.

Measured evaporative fluxes were also compared to FAO-PM reference crop evaporation (E_o). Measuring E at field scale is technically difficult (Fisher et al., 2005) and consequently models are often used to predict E for water balance, Earth system, and primary production models (Seaquist et al., 2003). While multiple models have been developed, the FAO recommend the sole use of the FAO-PM approach because it works well in a wide range of environments and it is generally desirable to use consistent methodology (Howell and Evett, 2004). Agreement between E_o and measured E was strong at a daily ($r^2 = 0.91$, $p < 0.01$) and monthly ($r^2 = 0.98$, $p < 0.01$) scale when soil moisture was not limiting. Under non-water limiting conditions over the 3-year period the crop coefficient (K_c) was calculated to be 0.99 and consequently a K_c value of 1 seemed appropriate. However, during periods of soil moisture limitation, E_o was higher than measured E . Under moisture limitation, Allen et al. (1998) recommend applying a soil moisture stress factor (K_s) derived from a soil water balance model. Applying K_s improved model performance at an annual scale but because of a lag in response daily and monthly performance was poor at the beginning and end of dry periods. A simple three bin VMC correction factor (K_{VMC}) was also trialled

which improved performance at a daily and monthly scale. However, this approach would require comparison at other pasture sites to determine appropriate correction factors below wilting point and between wilting point and the lower limit of readily available soil moisture content.

This was one of the first studies to use a replicated eddy covariance approach to examine spatial variation in E from intensively grazed pastures. Findings demonstrated that spatial and temporal variation in E was low, E was largely insensitive to large changes in leaf area, and the performance of the FAO-PM model was good in the absence of soil moisture limitation. Strong correlation was observed between R_n and E ($r^2 > 0.80$). The strong dependence of E on radiation largely explained the low spatial and temporal variation, relative insensitivity to leaf area, and the good performance of the FAO-PM model when soil moisture was not restrictive. During periods of soil moisture limitation, poor performance of FAO-PM was not overcome using the soil moisture stress factor (K_s). These findings imply that FAO-PM is only suitable for modelling E from ryegrass-clover pastures when water is not limiting and further study is required to determine a suitable method for varying surface resistances based on soil water balance modelling.

6.1.2 Chapter 4 Summary – Pasture diversity and EWUE

Is increased summer production from more diverse pastures a consequence of increased access to water through deeper roots or increased WUE and what are the differences in seasonal plant growth and production characteristics?

Globally, grazed pastures cover a larger land area than any other land use (Steinfeld et al., 2006) and often occupy areas where seasonal water shortages commonly limit productivity (O'Mara, 2012). Increasing pasture diversity has been demonstrated to increase relative production during warm wet and warm dry conditions (Goh and Bruce, 2005; Nobilly, 2015) but whether this is because of increased access to water or increased WUE is contentious (e.g. Brown, 2004). Prior to this research, comparison of measured E and EWUE between simple ryegrass-clover and more diverse pastures were absent. The objective of this chapter was to contrast E and EWUE between a ryegrass-clover pasture and a

more diverse pasture mix that included multiple grasses, legumes, and herbs. It was hypothesised that diversity would increase dry season production through increased access to soil water that may also improve EWUE by reducing plant water stress and consequently increasing productivity. This hypothesis was tested using a novel paired eddy covariance approach with a pre-treatment calibration period. The exchange of water and carbon was measured at paddock scale along with driver variables and above ground DM production.

Evaporation, GPP, EWUE, DM production, and HWUE was compared between a high diversity treatment (New Mix) and a simple ryegrass-clover treatment (New Rye) during a two-year period. Seasonal growing conditions were divided into warm, shoulder and cool based on soil temperatures that corresponded with sensible seasonal divisions. Warm season data was further divided into dry, intermediate, and wet conditions based on soil moisture. Treatment differences were examined using a paired site regression method that accounted for pre-treatment differences.

During warm and dry conditions GPP was significantly higher at New Mix and this was strongly supported by DM production measurements. Despite also measuring higher HWUE, a large (8.8%), but non-significant, increase in E resulted in a significant decrease in EWUE at New Mix. Under warm and wet conditions, both GPP and EWUE were significantly higher at New Mix while E was similar. This was supported by higher HWUE at New Mix. These results indicated that under both dry and wet warm conditions diversity had a positive effect on production. During warm wet growing conditions, EWUE was also higher; however, during dry conditions increased productivity likely occurred because of increased access to water.

During shoulder season conditions, E was significantly lower at New Mix (5.8%) while GPP and DM production were similar. This resulted in a non-significant increase in EWUE at New Mix (no difference in HWUE). The reduction in E indicated EWUE was higher at New Mix and this potentially could conserve soil moisture during spring for later use during dry summer conditions. During cool winter growing conditions, both production and WUE metrics were higher at New

Rye. At an annual scale, there were no large and significant differences between swards (E was significantly lower but only by 3% which was largely driven by the reduction during spring). These results suggested that gains in production and WUE during warm conditions at New Mix were offset by gains at New Rye during cooler conditions.

Annual EWUE ($3.0 \text{ g C m}^{-2} (\text{kg H}_2\text{O})^{-1}$) was similar to reported values for other New Zealand grasslands (e.g. Graham et al., 2016) but higher than for many international grasslands (e.g. Ma et al., 2015) where annual total GPP is often much lower largely due to the absence of year-round pasture production. Seasonal variation in EWUE was high ranging from about $2 \text{ g C m}^{-2} (\text{kg H}_2\text{O})^{-1}$ during dry summer periods up to about $8 \text{ g C m}^{-2} (\text{kg H}_2\text{O})^{-1}$ during cool winter growing conditions. Strong seasonal variation in EWUE has been observed by many and is typically linked to the strong influence of increases in the atmospheric vapour pressure deficit (VPD) that increases transpiration relative to CO_2 uptake (Ponton et al., 2006). In agreement with the relative increases in GPP and DM production at New Mix during warm conditions, many others have also found increases in warm season DM production from more diverse pastures (e.g. Goh and Bruce, 2005; Skinner and Dell, 2016).

This was one of the first studies to compare EWUE between pasture swards of contrasting diversity at an ecosystem scale by measuring evaporation and both GPP and harvestable DM production. Results showed increased pasture diversity had small, but important positive effects on production and EWUE during warm growing conditions but these benefits were offset during cool season growing conditions when ryegrass dominated pastures were superior. Given ryegrass was a dominant species at both sites it is likely larger increases in summer production and EWUE can be achieved by optimising species mixtures and a rapid and cost effective measurement technique is required to compare WUE between co-existing pasture species.

6.1.3 Chapter 5 Summary – Carbon isotope discrimination and EWUE

Can natural carbon isotope discrimination ($\Delta^{13}\text{C}$) during photosynthesis be used to compare WUE between pasture swards at the paddock scale and consequently allow quick and cost effective monitoring of WUE at paddock and farm scale?

Eddy covariance is a powerful ecosystem scale measurement tool that enables examination of water vapour and carbon fluxes at half hourly to multi-year time scales and can be coupled to multiple driver variables. However, the technology is expensive and requires a high level of technical expertise constraining its widespread use. The objective of this chapter was to examine the correlation between carbon isotope discrimination ($\Delta^{13}\text{C}$) during photosynthesis and EWUE as measured by EC. Bulk leaf $\Delta^{13}\text{C}$ should provide a time integrated measure of leaf scale WUE because both $\Delta^{13}\text{C}$ and WUE are dependent on the ratio of leaf internal to external CO_2 concentration (Farquhar et al., 1982). Assuming this leaf scale measure of WUE was positively correlated with EWUE, isotopic discrimination should represent a fast and cost effective method that could be used to select plant species and mixes to optimise WUE at farm or paddock scale. To examine correlation between these two methods bulk leaf biomass were collected within the integrated measurement area of the New Rye and New Mix EC systems prior to each grazing event over a 12-month period (2015). The harvested biomass had grown since the previous grazing event and therefore should integrate WUE over the grazing cycle.

A strong correlation was found between EWUE and $\text{WUE}_{\Delta^{13}\text{C}}$ ($r^2 \geq 0.79$, $p < 0.01$) at both New Rye and New Mix. During warm and dry conditions EWUE declined to about $1 \text{ g C m}^{-2} (\text{kg H}_2\text{O})^{-1}$ and $\Delta^{13}\text{C}$ decreased to about 19.5%. During mid-winter EWUE exceeded $7 \text{ g C m}^{-2} (\text{kg H}_2\text{O})^{-1}$ and $\Delta^{13}\text{C}$ increased approaching 24%. A relative reduction in $\Delta^{13}\text{C}$ is usually interpreted as an increase in WUE_i but this is only valid if plants are exposed to equivalent atmospheric VPD (Smedley et al., 1991). The atmospheric VPD varied seasonally and was at a maximum during warm and dry growing conditions when EWUE and $\Delta^{13}\text{C}$ was lowest. Once this variation in atmospheric VPD was accounted for, using the approach of Farquhar et al. (1982), EWUE and $\text{WUE}_{\Delta^{13}\text{C}}$ were strongly positively correlated.

Despite the strong positive correlation, EWUE was much lower than WUE_{Δ}^{13C} . For example, during winter EWUE was about $5 \text{ g C m}^{-2} (\text{kg H}_2\text{O})^{-1}$ while isotopic WUE exceeded $10 \text{ g C m}^{-2} (\text{kg H}_2\text{O})^{-1}$. Some of this difference occurred because the isotopic method did not include non-productive water loss which was included at the ecosystem scale. Assuming a likely contribution of 25% from E_s , based on estimates made by Graham et al. (2016), agreement between EWUE and WUE_{Δ}^{13C} improved but a large discrepancy remained unexplained. An unrealistic contribution from E_s was required to achieve good absolute agreement between approaches (about 60%). A number of other assumptions were made to calculate WUE_{Δ}^{13C} from Δ^{13C} (Chapter 5, Section 5.4.5) but sensitivity analysis indicated these assumptions were unlikely to resolve the discrepancy. A further potential factor that may explain some of the discrepancy was the time period used to calculate EWUE because EWUE increased as pasture biomass increased. Consequently, if EWUE was calculated over 10 days prior to isotope sampling rather than 21 days (approximate age of biomass) EWUE increased thereby decreasing the discrepancy between approaches. It is likely a number of factors contributed to the discrepancy between methods and further study is required to find a resolution.

Important differences in WUE_{Δ}^{13C} were also found among co-existing species grown in the more diverse sward. For example, during summer fractionation was significantly lower for chicory relative to ryegrass, plantain, and lucerne. Simultaneous measurement of DM production showed relative production was high for chicory during summer and consequently increased WUE was not occurring at a cost to production. These findings suggested increased WUE_{Δ}^{13C} during summer for chicory was likely due to increased carboxylation rates as opposed to decreased stomatal conductance. Differences in WUE_{Δ}^{13C} between species support the hypothesis that bulk Δ^{13C} measurements are suitable for identifying relative increases in WUE between pasture swards.

Correlation between EWUE and WUE_{Δ}^{13C} was strong despite poor absolute agreement. Additionally, important differences in WUE_{Δ}^{13C} were found between co-existing pasture species. Strong correlations were also observed between Δ^{13C}

and soil moisture content, air temperature, and the atmospheric VPD. Consequently, bulk leaf isotopic analysis is likely a cost effective method to compare water stress and WUE between adjacent pasture species. Ultimately, in combination with traditional production monitoring, it is envisaged that farm advisors could use $\Delta^{13}\text{C}$ measurements to optimise species selection for site specific climate and soil combinations to maximise WUE and farm production and profit.

6.2 Overall Conclusions

Given the current and future pressures on agriculture to increase production despite limited water resources, increasing WUE of pasture systems is an important priority. Prior to this thesis, long-term measurements of E from intensively grazed ryegrass-clover pastures were scarce. Chapter 3 showed both spatial and temporal variation in E were low (<3%), largely because E was strongly controlled by available energy. Additionally, grazing events caused, at most, minor reduction in E and it was likely soil water evaporation compensated for reductions in transpiration. Others have shown grazing events reduce GPP post-grazing (e.g. Hunt et al., 2016) and therefore EWUE should decline sharply following grazing given they do not effect E . This implies that maintaining high post-grazing pasture residuals will likely increase EWUE and further research is needed to examine this in more detail. Two of the sites were subsequently sown in a new ryegrass-clover and a new diverse pasture. Research reported in Chapter 4 showed that increasing diversity increased productivity during warm dry periods, increased productivity and WUE during warm and wet periods, and increased WUE during shoulder season growing conditions. These advantages were relatively small (~5%) and largely offset by gains in productivity and WUE during cool season growing conditions at the ryegrass-clover site. Therefore, warm season gains came at a cost to cool season production and consequently the integration of both systems at farm scale is likely advantageous to maintain year-round productivity. However, identifying optimal placement of species and mixes spatially over a farm and region is complicated by continuous variation in climate and soils. Chapter 5 showed a strong correlation between EWUE and $\text{WUE}_{\Delta^{13}\text{C}}$ and relatively large measured differences in $\Delta^{13}\text{C}$ among co-existing species suggesting there were important differences in WUE and water stress between

species. Therefore, I propose that $\Delta^{13}\text{C}$ could be developed for use by farm advisors, in conjunction with production monitoring, to optimise species selection at farm scale and thereby maximise pasture production to help feed a growing global population.

6.3 Future work

A number of questions remain regarding the effect of practical management interventions on EWUE, uncertainty in interpretation of bulk leaf $\Delta^{13}\text{C}$ between species, and consequently site specific plant species selection to optimise WUE. The following section briefly outlines some pertinent questions, their importance, and suggests how they may be addressed.

6.3.1 Management interventions to improve pastoral EWUE

Under what conditions do reductions in leaf area through grazing decrease evaporation and can improved understanding be used to modify grazing management practices to increase pastoral EWUE?

Ecosystem scale evaporation measurement from this study (Pronger et al., 2016) and another recent study from New Zealand (Graham et al., 2016) suggest E is relatively insensitive to large changes in leaf area following intensive grazing events. However, other recent work has suggested leaf area can strongly reduce E in managed grasslands (Gebler et al., 2015; Van Housen, 2015). This lack of consensus is also apparent in past literature with some finding grazing reduces E (e.g. Bremer et al., 2001; Day and Detling, 1994; Frank, 2003) and others finding no effect of grazing on E (Shuttleworth et al., 1989; Stewart and Verma, 1992; Wang et al., 2012). At the site used for this research, large reduction in leaf area had little effect on E but caused strong reductions in GPP (see Appendix B) and consequently EWUE declined post-grazing. Given the lack of consensus in the literature and the implications for EWUE, more research would seem justified to determine under what conditions leaf area effects E and whether there is potential to increase EWUE through grazing management. For example, in an irrigated system it may be advantageous to withhold irrigation the week prior to grazing to reduce surface soil moisture and consequently limit the ability of soil water evaporation to compensate for reductions in transpiration.

Is there potential to increase EWUE by optimising leaf N content using fertiliser inputs or increasing legume content of pastures?

Leaf nitrogen (N) content strongly influences the CO₂ carboxylation rate (Hussain et al., 2011) and consequently has potential to increase photosynthetic carbon uptake without increasing transpiration thereby increasing EWUE. Leaf N content could be manipulated by increasing the legume content of pastures or alternatively by timing fertiliser application to optimise summer leaf N content. This question could be addressed at paddock scale using a replicated split site EC approach using either increased abundance of summer active legumes or increased summer N fertiliser. It would seem wise to couple such work to study of the effects of increased leaf N content on production, N leaching, and nitrous oxide emissions. Alternatively, a cost effective approach would be to use leaf $\Delta^{13}\text{C}$ discrimination to measure differences in WUE which would allow the comparison of multiple plant species at a range of N application rates. This question could be addressed by comparing plant production and resource use of multiple plant species under a range of N fertiliser application rates across New Zealand.

Which plant species have high WUE at high temperatures with and without soil moisture stress?

Differences in life history and consequently leaf and root traits result in variation in WUE between pastoral plant species (Moreno-Gutierrez et al., 2012). Many studies have found increased relative production as a result of increased diversity during both dry (Skinner, 2008) and wet (Skinner and Dell, 2016) summer periods and this increased productivity is often attributed to complementarity in traits as opposed to purely a diversity effect in an ecological sense (Pembleton et al., 2015). There is an opportunity for further applied research that uses $\Delta^{13}\text{C}$ and dry matter production measurements to compare the performance of a range of commonly used pasture grasses, legumes, and herbs in both monoculture and mixes across a range of sites that represent large proportions of New Zealand's pastoral landscape. I would envisage this to be a multi-year project that captured a range of summer and autumn conditions and also examined timing of productivity relative to ryegrass-clover in addition to pasture persistence.

6.3.2 Improving understanding of uncertainties when using $\Delta^{13}\text{C}$ WUE

How problematic is post carboxylation fractionation when comparing WUE between different pasture species using carbon isotopic discrimination?

The use of bulk leaf $\Delta^{13}\text{C}$ analysis is advantageous for comparison of WUE in grazed pastures because bulk leaf material integrates over the grazing cycle (leaf age) and sample collection and preparation is simple. However, potential differences in post carboxylation fractionation during the synthesis of oils, waxes, and tannins between pasture species may confound results increasing uncertainty and consequently complicating interpretation (Moreno-Gutierrez et al., 2012). Leaf sugars are generally accepted to represent more direct products of photosynthesis and consequently reduce uncertainty when comparing multiple plant species (Moreno-Gutierrez et al., 2012). Indeed, Scartazza et al. (2014) found stronger agreement between isotope discrimination from leaf sugars and EWUE compared to isotope WUE calculated from bulk leaf material or respired CO_2 . However, these comparisons of methods are difficult because leaf sugars record a day or two prior to harvest whereas bulk leaf samples integrate over the age of the leaf. Therefore, this question may be best addressed using a plant growth chamber where a consistent growing environment can be maintained for multiple plant species over multiple weeks. New leaves could be harvested at regular intervals and split – half used for bulk leaf analysis and half snap frozen for sugar extraction. Ideally this research would be combined with simultaneous gas exchange measurement of CO_2 and H_2O to confirm the relationship between $\Delta^{13}\text{C}$ and leaf level WUE.

Does translocation of carbohydrates stored in roots and below grazing height biomass influence the measured $\Delta^{13}\text{C}$ signal over a grazing cycle?

Plants translocate carbohydrates between roots and shoots (Campbell and Reece, 2005) which could contaminate isotopic signals with carbohydrates fixed during previous grazing cycles. The potential for contamination from carbohydrates stored in root systems could be determined using a paired growth chambers approach or field based with rainout shelters. A replicated treatment could be exposed to water stress over a prolonged period while a replicated control was not stressed. After a growth cycle (3 – 4 weeks) above ground biomass could be

harvested from both treatments. Over the following re-growth cycle, moisture stress would not be applied to either treatment and both would be exposed to equivalent environmental conditions. After the re-growth cycle, $\Delta^{13}\text{C}$ should be equivalent between treatments if translocated carbohydrates were not influencing the $\Delta^{13}\text{C}$ signal.

6.4 References

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Appendix A: Supplemental material for Chapter 3

Low spatial and inter-annual variability in evaporation from a year-round intensively grazed temperate pasture system in New Zealand

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Calculating reduction in E following vegetation senescence and grazing

To estimate the reduction in E attributable to vegetation senescence and grazing we used the change in slope of the relationship between the paired sites before and after vegetation senescence or grazing. The following paragraph describes the procedure using the autumn herbicide application as an example (Figure A1).

Firstly, we calculated the difference in slope of the relationship between LE from site 1 and 2 for pre- and post-vegetation senescence periods (Table A1). We then calculated the mean 30 minute LE from the vegetated site for April 2013 (Table A2). For each half hour period we multiplied mean April LE from the vegetated site by the difference in slope between sites 1 and 2 and added the difference in intercept values (effectively the vertical distance between the best fit lines in Figure A1). This gave us the mean difference in LE between sites which we attributed to vegetation senescence for each half hour (Table A2). We then converted LE from $W\ m^{-2}$ to $mm\ day^{-1}$ to give the daily reduction in E (mm) attributable to vegetation senescence. Confidence intervals were calculated using the differences between the 95% upper and lower bounds for the slope pre- and post-vegetation senescence. Table A3 gives slope statistics for the seasonal grazing events and the herbicide event shown in Figure 6 of the main manuscript.

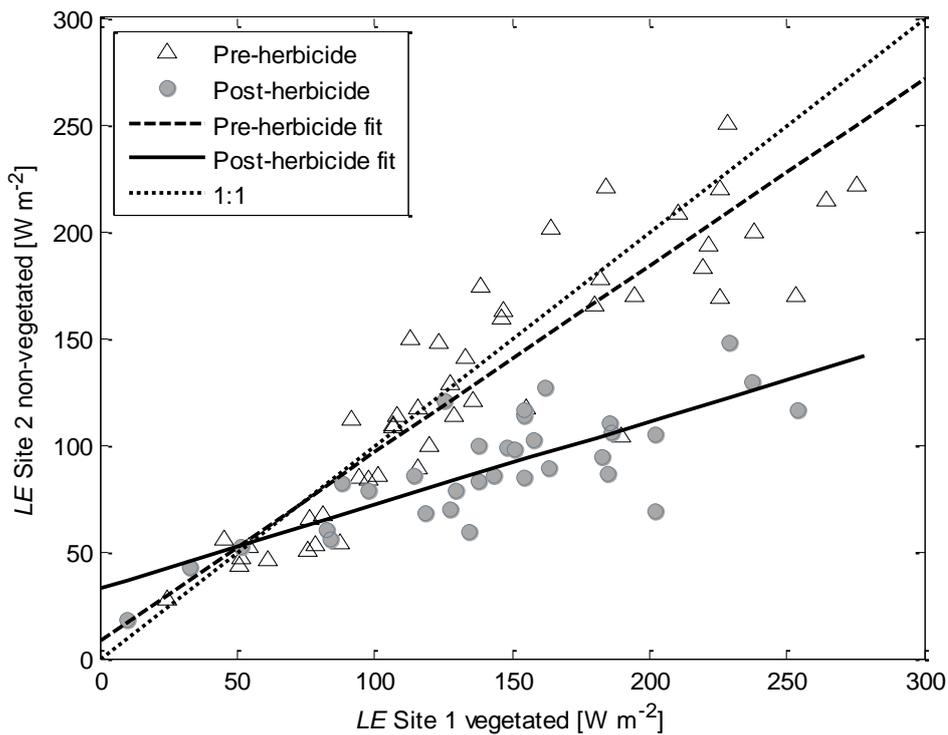


Figure A1. Relationship between LE from the vegetated and non-vegetated sites pre (open triangles) and post herbicide application (grey circles) with lines of best fit.

Table A1. Slope and intercept values for the relationship between site 1 and 2 pre and post herbicide application and the difference in slope and intercept values.

Pre herbicide application			
	Estimate	95% CI lower	95% CI upper
Intercept	8.50	-11.99	28.99
Slope	0.88	0.74	1.01
Post herbicide application			
	Estimate	95% CI lower	95% CI upper
Intercept	32.59	15.53	49.65
Slope	0.39	0.28	0.50
Difference in intercept	24.09	27.52	20.66
Difference in slope	-0.48	-0.46	-0.50

Table A2. Differences in *LE* and *E* attributable to vegetation senescence and 95% confidence interval bounds for the difference

Time of day	Mean April 30 min <i>LE</i> at vegetated site (W m-2)	Mean slope difference for 30 min period (W m-2)	95% lower bound slope difference (W m-2)	95% upper bound slope difference (W m-2)
09:30:00 a.m.	62.42	-6.07	-1.37	-10.78
10:00:00 a.m.	87.86	-18.37	-13.14	-23.59
10:30:00 a.m.	94.61	-21.63	-16.26	-27.00
11:00:00 a.m.	125.40	-36.51	-30.51	-42.51
11:30:00 a.m.	125.96	-36.78	-30.77	-42.79
12:00:00 p.m.	155.12	-50.87	-44.27	-57.48
12:30:00 p.m.	168.12	-57.16	-50.28	-64.03
01:00:00 p.m.	178.37	-62.11	-55.03	-69.19
01:30:00 p.m.	182.92	-64.31	-57.13	-71.48
02:00:00 p.m.	171.51	-58.80	-51.85	-65.74
02:30:00 p.m.	172.77	-59.40	-52.43	-66.37
03:00:00 p.m.	161.31	-53.87	-47.13	-60.60
03:30:00 p.m.	153.53	-50.10	-43.53	-56.68
04:00:00 p.m.	124.95	-36.29	-30.31	-42.28
04:30:00 p.m.	105.50	-26.89	-21.30	-32.48
05:00:00 p.m.	83.39	-16.21	-11.07	-21.35
05:30:00 p.m.	53.40	-1.72	2.81	-6.24
Difference in daily <i>E</i> (mm)		-0.48	-0.41	-0.56

Table A3. Slopes and intercept statistics to support Figure 3.6

	Slope	Adj. r ²	p value	95% CI lower	95% CI upper	p value (slopes difference)	Intercept	p value	95% CI lower	95% CI upper
Winter										
Pre-graze	0.90	0.83	0.00	0.75	1.05	0.83	5.06	0.55	-12.15	22.26
Post-graze	0.87	0.69	0.00	0.63	1.11		7.28	0.37	-9.15	23.70
Spring										
Pre-graze	0.87	0.72	0.00	0.74	1.01	0.04	24.30	0.09	-3.72	52.32
Post-graze	1.11	0.81	0.00	0.97	1.25		-7.04	0.57	-31.96	17.89
Summer										
Pre-graze	0.86	0.92	0.00	0.80	0.91	< 0.01	23.67	0.02	4.70	42.63
Post-graze	1.11	0.91	0.00	1.00	1.22		-25.43	0.10	-55.52	4.66
Autumn										
Pre-graze	1.04	0.88	0.00	0.88	1.20	0.35	-5.54	0.67	-31.70	20.62
Post-graze	0.95	0.87	0.00	0.83	1.07		7.02	0.42	-10.53	24.57
Spray out period										
Pre-spray	0.88	0.79	0.00	0.74	1.01	< 0.01	8.50	0.41	-11.99	28.99
Post-spray	0.39	0.61	0.00	0.28	0.50		32.59	0.00	15.53	49.65

Post-hoc leaf area measurement to show grazing reduces LAI

We measured leaf area from grazing exclusion cages between July 2015 and May 2016 to quantify the likely effect grazing events had on LAI. In sequence with the grazing rotation 5 randomly positioned quadrats (0.2 m²) were cut to 4 cm height (which is representative of typical grazing residual height) and then the residual material was cut to ground level. The paired quadrat samples (below and above grazing height) were returned to the lab and well mixed to homogenise and then subsampled. Leaf area was measured on the subsample using the LiCOR LI3100C. Using this approach we were able to infer leaf area prior to and post grazing. Figure A2 shows that throughout the year grazing events likely resulted in a significant reduction in leaf area. Pasture management and grazing rotation lengths did not change between the early period when the grazing comparison analysis was done and the later period when the LAI measurements were made.

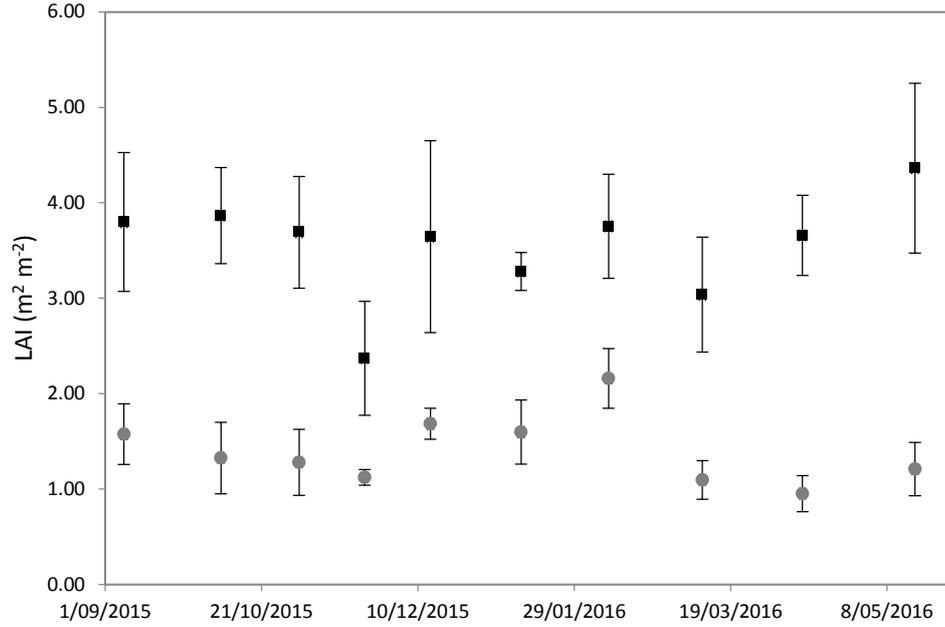


Figure A2. Leaf area pre-cutting (black squares) and post cutting to grazing height (grey circles) showing a significant reduction in LAI throughout the year for all grazing events. Error bars are 95% confidence intervals.

Calculating the soil moisture stress factor

The soil moisture stress factor (K_s) was determined as:

$$K_s = \frac{TAW - D_r}{TAW - RAW} = \frac{TAW - D_r}{(1-p)TAW} \quad (1)$$

where total available water (TAW, mm) is the difference between soil water content at field capacity (FC, $m^3 m^{-3}$) and permanent wilting point (PWP, $m^3 m^{-3}$), RAW (mm) is the amount of TAW that can be removed from the root zone depth (Z_r , 1 m was assumed based on mid-range rooting depth for ryegrass pasture systems (Hayman and Stocker, 1983; Parfitt et al., 1985)) before moisture stress occurs, p is the fraction value of TAW that can be extracted before moisture stress occurs (0.6 assumed based on Allen et al. (1998)), and D_r is daily root zone depletion calculated using a daily water balance computation as follows:

$$D_{r, i} = D_{r(i-1)} - P_i + E_{0,i} \quad (2)$$

where $D_{r,i}$ is root zone depletion at the end of day i (mm), $D_{r,i-1}$ is water content in the root zone at the end of the previous day ($i - 1$) (mm), P_i is precipitation on day

i (mm), and $E_{o,i}$ is evaporation on day i (mm). To initiate the water balance for the root zone the initial depletion ($D_{r,i-1} \equiv D_{r0}$) was estimated from the soil water content by:

$$D_{r,i-1} = 1000(\theta_{FC} - \theta_{i-1}) * Z_r \quad (3)$$

where θ_{i-1} is the average soil water content for the effective root zone. See Allen et al. (1998) for full details on calculating adjusted FAO-56 E_o .

References

- Allen, R.G., Pereira, L.S., Raes, D. and Smith, M., 1998. FAO Irrigation and Drainage Paper No. 56: Crop Evapotranspiration, Food and Agriculture Organisation, Rome, Italy.
- Hayman, J.M. and Stocker, R.V., 1983. Soil water extraction patterns under pasture and lucerne on two soil types in Canterbury. Proceedings of the Agronomy Society of New Zealand, 12: 61 - 63.
- Parfitt, R.L., Joe, E.N. and Cook, F.J., 1985. Water use and pasture growth on Judgeford silt loam. New Zealand Journal of Agricultural Research, 28: 387 - 392.

Appendix B: Supplemental material for Chapter 4

Changes in species composition through time post-treatment

The contribution of the dominant plant species (ryegrass, clover, chicory, and plantain) for both treatments (organised by season) is shown in Figure B1. In addition to these dominant species the New Mix included prairie grass, timothy, cocksfoot, and lucerne but these other species contributed very little to the sward and are included in the ‘other’ category along with weeds and dead material. Lucerne did not establish well at New Mix and over the three-year period contributed less than 1% to sward composition. The contribution of the broadleaf herbs, chicory and plantain, declined through time at New Mix as ryegrass became more dominant. In winter, spring, and summer there was a fairly linear increase in ryegrass dominance through time between years and by the final year ryegrass contributed between 38% (summer) and 59% (winter) of the sward. In summer and autumn (typically the driest months) the broad leaf herbs, chicory and plantain, were more dominant.

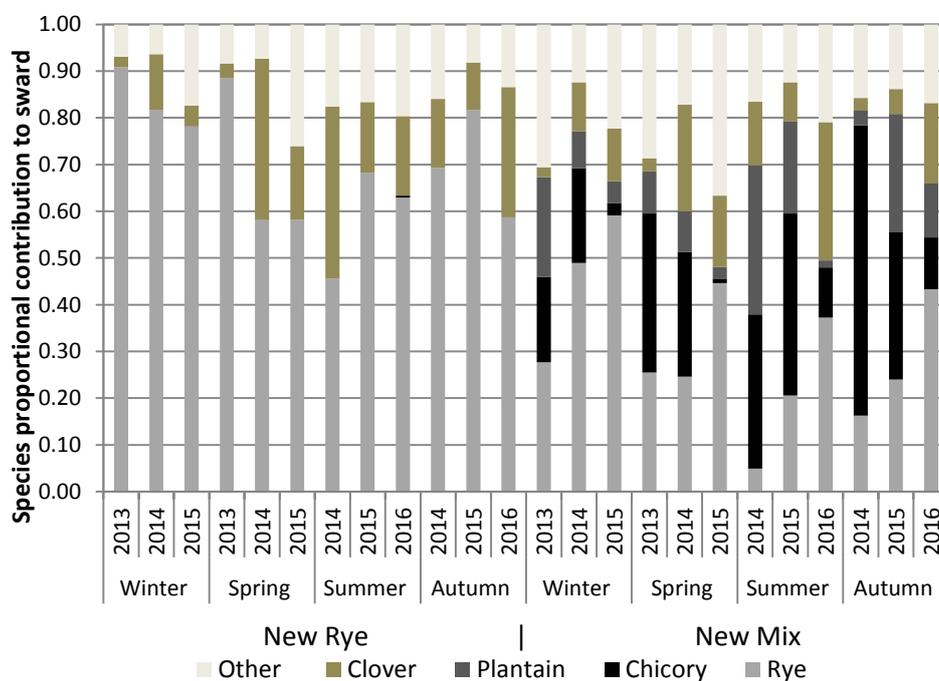


Figure B1. Seasonal and inter-annual changes in sward composition for the dominant pasture species through time at New Rye (left side) and New Mix (right side). Weeds, dead material, and sub-dominant species are all in the ‘other’ category.

Grazing induced reductions in GPP

Figure B2 shows daily GPP from New Rye (panel a) and New Mix (panel b) over the spring pre-treatment period when both sites were in ryegrass. The vertical dashed lines show the occurrence of grazing events which were not synchronised between treatments for practical farm management purposes. These grazing events caused strong declines in GPP and given grazing events caused, at most, small reduction in evaporation (see Chapter 3) grazing events reduce EWUE.

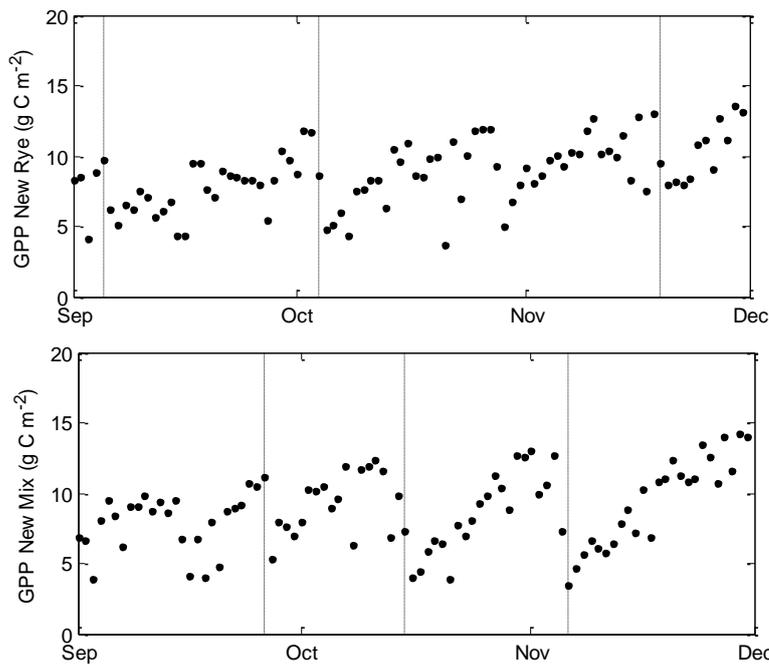


Figure B2. Daily GPP at New Rye (top panel) and New Mix (middle panel) over spring 2012 prior to imposing treatments when both sites were still in old ryegrass and clover pasture. The vertical dashed lines show the timing of grazing events that were not synchronised between sites.

Appendix C: Supplemental material for Chapter 5

Correlation between EWUE and $\Delta^{13}\text{C}$ prior to accounting for seasonal changes in VPD

Strong correlation was found between EWUE and $\Delta^{13}\text{C}$ (Figure C1, $r^2 > 0.90$) prior to accounting for the changes in the atmospheric vapour pressure deficit (VPD) through 2015. This strong relationship likely occurred because both EWUE and intrinsic WUE (WUEi) were responding to strong seasonal variation in VPD. WUE is strongly dependent on the VPD because increases in VPD increase transpiration disproportionately compared to CO_2 uptake.

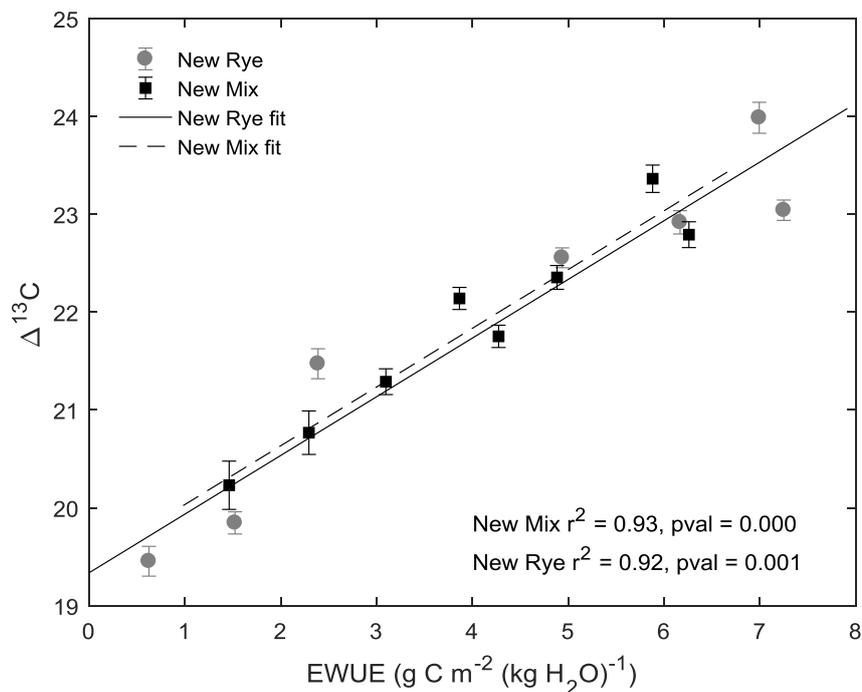


Figure C1. Correlation between EWUE and isotopic discrimination of bulk plant biomass ($\Delta^{13}\text{C}$) with respect to source atmosphere at New Rye (grey circles, solid line) and New Mix (black squares, dash line). Error bars show SEM for $\Delta^{13}\text{C}$ measurements, errors were not able to be calculated for EWUE ($n=1$).

Examining effect of time period EWUE was calculated

EWUE generally increases between post-grazing, when leaf area is low and E_S is a larger proportion of total E , and pre-grazing when leaf area is high and total E is dominated by E_T . Consequently, the discrepancy between EWUE and $WUE_{\Delta^{13}C}$ decreases if the time period that EWUE is calculated over shorter and closer to the grazing event when leaf area is higher. In Figure 5.3 a 21-day period was used because bulk leaf isotope measurements are typically considered to integrate WUE over the leaf lifespan (Bowling et al., 2008) which in an intensively grazed New Zealand pasture is 3 weeks or greater. Figure C2 is equivalent to Figure 5.3 but EWUE was calculated as the average for 10 days prior to isotope sampling as opposed to 21 days shown in Figure 5.3. $WUE_{\Delta^{13}C}$ is also different than Figure 5.3 because input data used to calculate $WUE_{\Delta^{13}C}$ from $\Delta^{13}C$ was also averaged over a 10-day period. Using a 10-day period absolute agreement between $WUE_{\Delta^{13}C}$ and EWUE improved but correlation strength decreased. For example, at New Mix EWUE averaged across all grazing events increased from 3.77 g C (kg H₂O)⁻¹ calculated over 21 days to 4.56 g C (kg H₂O)⁻¹ calculated over ten days pre-grazing. However, correlation strength decreased from 0.91 to 0.71 (Figure C2).

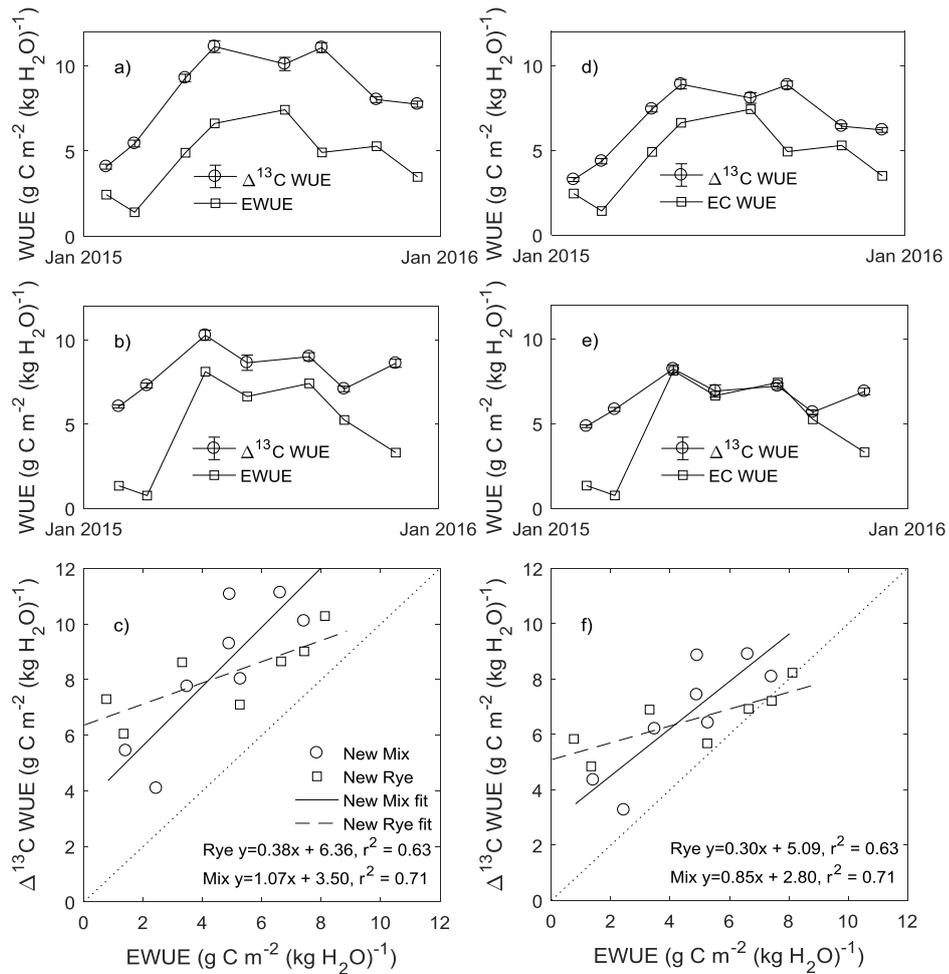


Figure C2. Annual comparison of EWUE and WUE_{Δ¹³C} after applying equation 4 at New Mix (panel a) and New Rye (panel b) and regression between EWUE and WUE_{Δ¹³C} (panel c) and equivalent figures (d – f) after increasing evaporative component of WUE_{Δ¹³C} by 25% to account for likely contribution of soil water evaporation and consequently more directly comparable to EWUE measurements. Error bars show 95% CI.

Raw $\delta^{13}\text{C}$ data

Table C1. Pre-grazing bulk biomass $\delta^{13}\text{C}$ data showing the ten replicates at each sampling time for both New Rye (Tcon, left column) and New Mix (Tmix, right column) through 2015 (continued over page)

Sample date	Treatment	Random Quadrat Number	Sample Identification	$\delta^{13}\text{C}$	Sample date	Treatment	Random Quadrat Number	Sample Identification	$\delta^{13}\text{C}$
11/02/2015	Tcon	21	1_11	-27.64	29/01/2015	Tmix	11	1_1	-29.74
11/02/2015	Tcon	22	1_12	-27.87	29/01/2015	Tmix	12	1_2	-29.13
11/02/2015	Tcon	23	1_13	-27.47	29/01/2015	Tmix	13	1_3	-29.10
11/02/2015	Tcon	24	1_14	-28.20	29/01/2015	Tmix	14	1_4	-28.70
11/02/2015	Tcon	25	1_15	-27.31	29/01/2015	Tmix	15	1_5	-27.69
11/02/2015	Tcon	26	1_16	-28.31	29/01/2015	Tmix	16	1_6	-27.75
11/02/2015	Tcon	27	1_17	-27.45	29/01/2015	Tmix	17	1_7	-28.55
11/02/2015	Tcon	28	1_18	-27.80	29/01/2015	Tmix	18	1_8	-28.64
11/02/2015	Tcon	29	1_19	-27.43	29/01/2015	Tmix	19	1_9	-27.80
11/02/2015	Tcon	30	1_20	-27.48	29/01/2015	Tmix	20	1_10	-28.64
12/03/2015	Tcon	21	1_51	-26.87	27/02/2015	Tmix	11	1_41	-28.17
12/03/2015	Tcon	22	1_52	-26.44	27/02/2015	Tmix	12	1_42	-29.80
12/03/2015	Tcon	23	1_53	-27.07	27/02/2015	Tmix	13	1_43	-27.60
12/03/2015	Tcon	24	1_54	-27.23	27/02/2015	Tmix	14	1_44	-27.46
12/03/2015	Tcon	25	1_55	-27.31	27/02/2015	Tmix	15	1_45	-27.31
12/03/2015	Tcon	26	1_56	-28.10	27/02/2015	Tmix	16	1_46	-27.59
12/03/2015	Tcon	27	1_57	-27.68	27/02/2015	Tmix	17	1_47	-28.29
12/03/2015	Tcon	28	1_58	-27.49	27/02/2015	Tmix	18	1_48	-28.68
12/03/2015	Tcon	29	1_59	-27.53	27/02/2015	Tmix	19	1_49	-27.70
12/03/2015	Tcon	30	1_60	-27.51	27/02/2015	Tmix	20	1_50	-28.03
11/05/2015	Tcon	21	1_91	-31.06	20/04/2015	Tmix	11	1_81	-29.99
11/05/2015	Tcon	22	1_92	-31.01	20/04/2015	Tmix	12	1_82	-30.22
11/05/2015	Tcon	23	1_93	-30.66	20/04/2015	Tmix	13	1_83	-29.63
11/05/2015	Tcon	24	1_94	-30.02	20/04/2015	Tmix	14	1_84	-29.57
11/05/2015	Tcon	25	1_95	-30.33	20/04/2015	Tmix	15	1_85	-29.65
11/05/2015	Tcon	26	1_96	-30.49	20/04/2015	Tmix	16	1_86	-29.71
11/05/2015	Tcon	27	1_97	-31.06	20/04/2015	Tmix	17	1_87	-30.64
11/05/2015	Tcon	28	1_98	-30.46	20/04/2015	Tmix	18	1_88	-29.63
11/05/2015	Tcon	29	1_99	-30.77	20/04/2015	Tmix	19	1_89	-29.97
11/05/2015	Tcon	30	1_100	-30.30	20/04/2015	Tmix	20	1_90	-29.77
23/06/2015	Tcon	21	2_11	-31.05	20/05/2015	Tmix	11	2_1	-31.12
23/06/2015	Tcon	22	2_12	-31.90	20/05/2015	Tmix	12	2_2	-30.20
23/06/2015	Tcon	23	2_13	-31.44	20/05/2015	Tmix	13	2_3	-29.97
23/06/2015	Tcon	24	2_14	-32.35	20/05/2015	Tmix	14	2_4	-30.32
23/06/2015	Tcon	25	2_15	-30.88	20/05/2015	Tmix	15	2_5	-30.37
23/06/2015	Tcon	26	2_16	-31.87	20/05/2015	Tmix	16	2_6	-30.34
23/06/2015	Tcon	27	2_17	-31.29	20/05/2015	Tmix	17	2_7	-30.06
23/06/2015	Tcon	28	2_18	-31.85	20/05/2015	Tmix	18	2_8	-30.84
23/06/2015	Tcon	29	2_19	-32.14	20/05/2015	Tmix	19	2_9	-30.87
23/06/2015	Tcon	30	2_20	-31.50	20/05/2015	Tmix	20	2_10	-30.86
26/08/2015	Tcon	21	2_31	-30.86	31/07/2015	Tmix	11	2_21	-30.63
26/08/2015	Tcon	22	2_32	-31.03	31/07/2015	Tmix	12	2_22	-31.41
26/08/2015	Tcon	23	2_33	-30.40	31/07/2015	Tmix	13	2_23	-30.99
26/08/2015	Tcon	24	2_34	-31.02	31/07/2015	Tmix	14	2_24	-31.08
26/08/2015	Tcon	25	2_35	-30.94	31/07/2015	Tmix	15	2_25	-30.46
26/08/2015	Tcon	26	2_36	-30.15	31/07/2015	Tmix	16	2_26	-30.93
26/08/2015	Tcon	27	2_37	-30.48	31/07/2015	Tmix	17	2_27	-31.10
26/08/2015	Tcon	28	2_38	-31.00	31/07/2015	Tmix	18	2_28	-30.86
26/08/2015	Tcon	29	2_39	-30.89	31/07/2015	Tmix	19	2_29	-30.92
26/08/2015	Tcon	30	2_40	-30.55	31/07/2015	Tmix	20	2_30	-31.98

Sample date	Treatment	Random Quadrat Number	Sample Identification	$\delta^{13}\text{C}$	Sample date	Treatment	Random Quadrat Number	Sample Identification	$\delta^{13}\text{C}$
1/10/2015	Tcon	21	2_51	-30.78	7/09/2015	Tmix	11	2_41	-29.73
1/10/2015	Tcon	22	2_52	-30.45	7/09/2015	Tmix	12	2_42	-30.15
1/10/2015	Tcon	23	2_53	-30.16	7/09/2015	Tmix	13	2_43	-30.05
1/10/2015	Tcon	24	2_54	-30.29	7/09/2015	Tmix	14	2_44	-30.43
1/10/2015	Tcon	25	2_55	-29.59	7/09/2015	Tmix	15	2_45	-30.36
1/10/2015	Tcon	26	2_56	-30.37	7/09/2015	Tmix	16	2_46	-29.77
1/10/2015	Tcon	27	2_57	-30.33	7/09/2015	Tmix	17	2_47	-30.52
1/10/2015	Tcon	28	2_58	-30.22	7/09/2015	Tmix	18	2_48	-30.41
1/10/2015	Tcon	29	2_59	-30.11	7/09/2015	Tmix	19	2_49	-29.98
1/10/2015	Tcon	30	2_60	-30.42	7/09/2015	Tmix	20	2_50	-29.41
23/11/2015	Tcon	21	2_71	-29.39	2/11/2015	Tmix	11	2_61	-29.56
23/11/2015	Tcon	22	2_72	-29.20	2/11/2015	Tmix	12	2_62	-29.54
23/11/2015	Tcon	23	2_73	-28.98	2/11/2015	Tmix	13	2_63	-29.62
23/11/2015	Tcon	24	2_74	-29.70	2/11/2015	Tmix	14	2_64	-29.99
23/11/2015	Tcon	25	2_75	-29.59	2/11/2015	Tmix	15	2_65	-29.71
23/11/2015	Tcon	26	2_76	-29.04	2/11/2015	Tmix	16	2_66	-29.36
23/11/2015	Tcon	27	2_77	-28.52	2/11/2015	Tmix	17	2_67	-29.40
23/11/2015	Tcon	28	2_78	-28.78	2/11/2015	Tmix	18	2_68	-29.87
23/11/2015	Tcon	29	2_79	-29.17	2/11/2015	Tmix	19	2_69	-29.24
23/11/2015	Tcon	30	2_80	-30.07	2/11/2015	Tmix	20	2_70	-28.79
					14/12/2015	Tmix	11	2_81	-29.20
					14/12/2015	Tmix	12	2_82	-29.11
					14/12/2015	Tmix	13	2_83	-28.28
					14/12/2015	Tmix	14	2_84	-29.71
					14/12/2015	Tmix	15	2_85	-29.28
					14/12/2015	Tmix	16	2_86	-29.08
					14/12/2015	Tmix	17	2_87	-29.33
					14/12/2015	Tmix	18	2_88	-28.86
					14/12/2015	Tmix	19	2_89	-29.21
					14/12/2015	Tmix	20	2_90	-28.64

Table C2 Raw species $\delta^{13}\text{C}$ data for summer, autumn, winter, and spring 2015

Date	Season	Site	Species	Rep	Sample Number	$\delta^{13}\text{C}$
17/02/2015	Summer	TMix	Rye	1	131	-27.81
17/02/2015	Summer	TMix	Rye	2	132	-28.50
17/02/2015	Summer	TMix	Rye	3	133	-27.72
17/02/2015	Summer	TMix	Clover	1	134	-28.80
17/02/2015	Summer	TMix	Clover	2	135	-27.49
17/02/2015	Summer	TMix	Clover	3	136	-28.41
17/02/2015	Summer	TMix	Chicory	1	137	-26.17
17/02/2015	Summer	TMix	Chicory	2	138	-27.51
17/02/2015	Summer	TMix	Chicory	3	139	-26.67
17/02/2015	Summer	TMix	Plantain	1	140	-28.43
17/02/2015	Summer	TMix	Plantain	2	141	-28.18
17/02/2015	Summer	TMix	Plantain	3	142	-28.96
17/02/2015	Summer	TMix	Lucerne	1	143	-30.45
17/02/2015	Summer	TMix	Lucerne	2	144	-28.48
17/02/2015	Summer	TMix	Lucerne	3	145	-28.30
20/04/2015	Autumn	TMix	Rye	1	146	-30.37
20/04/2015	Autumn	TMix	Rye	2	147	-30.45
20/04/2015	Autumn	TMix	Rye	3	148	-28.39
20/04/2015	Autumn	TMix	Clover	1	149	-30.22
20/04/2015	Autumn	TMix	Clover	2	150	-29.03
20/04/2015	Autumn	TMix	Clover	3	151	-28.45
20/04/2015	Autumn	TMix	Chicory	1	152	-28.93
20/04/2015	Autumn	TMix	Chicory	2	153	-29.41
20/04/2015	Autumn	TMix	Chicory	3	154	-28.90
20/04/2015	Autumn	TMix	Plantain	1	155	-31.08
20/04/2015	Autumn	TMix	Plantain	2	156	-31.27
20/04/2015	Autumn	TMix	Plantain	3	157	-30.69
20/04/2015	Autumn	TMix	Lucerne	1	158	-29.76
20/04/2015	Autumn	TMix	Lucerne	2	159	-
20/04/2015	Autumn	TMix	Lucerne	3	160	-29.39
31/07/2015	Winter	TMix	Rye	1	161	-31.62
31/07/2015	Winter	TMix	Rye	2	162	-32.27
31/07/2015	Winter	TMix	Rye	3	163	-32.04
31/07/2015	Winter	TMix	Clover	1	164	-29.37
31/07/2015	Winter	TMix	Clover	2	165	-29.40
31/07/2015	Winter	TMix	Clover	3	166	-29.24
31/07/2015	Winter	TMix	Chicory	1	167	-28.66
31/07/2015	Winter	TMix	Chicory	2	168	-28.35
31/07/2015	Winter	TMix	Chicory	3	169	-30.12
31/07/2015	Winter	TMix	Plantain	1	170	-30.24
31/07/2015	Winter	TMix	Plantain	2	171	-30.96
31/07/2015	Winter	TMix	Plantain	3	172	-30.99
31/07/2015	Winter	TMix	Lucerne	1	173	-
31/07/2015	Winter	TMix	Lucerne	2	174	-
31/07/2015	Winter	TMix	Lucerne	3	175	-

Date	Season	Site	Species	Rep	Sample Number	$\delta^{13}\text{C}$
2/11/2015	Spring	TMix	Rye	1	176	-30.01
2/11/2015	Spring	TMix	Rye	2	177	-30.21
2/11/2015	Spring	TMix	Rye	3	178	-30.17
2/11/2015	Spring	TMix	Clover	1	179	-27.87
2/11/2015	Spring	TMix	Clover	2	180	-28.24
2/11/2015	Spring	TMix	Clover	3	181	-27.64
2/11/2015	Spring	TMix	Chicory	1	182	-29.21
2/11/2015	Spring	TMix	Chicory	2	183	-29.37
2/11/2015	Spring	TMix	Chicory	3	184	-28.87
2/11/2015	Spring	TMix	Plantain	1	185	-30.11
2/11/2015	Spring	TMix	Plantain	2	186	-30.31
2/11/2015	Spring	TMix	Plantain	3	187	-29.99
2/11/2015	Spring	TMix	Lucerne	1	188	-28.48
2/11/2015	Spring	TMix	Lucerne	2	189	-28.65
2/11/2015	Spring	TMix	Lucerne	3	190	-29.64

Appendix D: Co-Authorship Forms



Co-Authorship Form

Postgraduate Studies Office
Student and Academic Services Division
Wahanga Raranga Mātauranga Aorangi
The University of Waikato
Private Bag 3105
Hamilton 3240, New Zealand
Phone +64 7 838 4439
Website: <http://www.waikato.ac.nz/sasd/postgraduate/>

This form is to accompany the submission of any PhD that contains research reported in published or unpublished co-authored work. **Please include one copy of this form for each co-authored work.** Completed forms should be included in your appendices for all the copies of your thesis submitted for examination and library deposit (including digital deposit).

Please indicate the chapter/section/pages of this thesis that are extracted from a co-authored work and give the title and publication details or details of submission of the co-authored work.

Chapter 3 has been published in the Journal Agriculture, Ecosystems, and Environment. The title of this paper is Low spatial and inter-annual variability in evaporation from a year-round intensively grazed temperate pasture system.

Nature of contribution by PhD candidate: Collaboration on project conception; data collection, filtering, and gap filling. Conducted majority of data analysis and interpretation and writing of manuscript/chapter

Extent of contribution by PhD candidate (%): 85%

CO-AUTHORS

Name	Nature of Contribution
David Campbell	Collaboration on project conception, data analysis, and interpretation; provided substantial comments on manuscript versions
Michael Clearwater	Collaboration on data analyses and provided substantial comments on manuscript versions
Susanna Rutledge	Collaboration on data collection, filtering and gap filling; provided comments on data analyses and manuscript versions
Aaron Wall	Collaboration on data collection, filtering and gap filling; provided comments on data analyses and manuscript versions
Louis Schipper	Collaboration on project conception, data analysis, and interpretation; provided substantial comments on manuscript versions

Certification by Co-Authors

The undersigned hereby certify that:

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

Name	Signature	Date
David Campbell		31/03/2017
Michael Clearwater		20/4/2017
Susanna Rutledge		6/4/2017
Aaron Wall		31/03/17
Louis Schipper		31/3/17

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Please indicate the chapter/section/pages of this thesis that are extracted from a co-authored work and give the title and publication details or details of submission of the co-authored work.

As it stands in the submitted thesis, Chapter 4 is being prepared for submission to a suitable journal. The title of the chapter is currently 'Contrasting water use and water use efficiency in simple and diverse pasture mixes grazed by dairy cows'

Nature of contribution by PhD candidate: Collaboration on project conception; data collection, filtering, and gap filling. Conducted majority of data analysis and interpretation and writing of manuscript/chapter

Extent of contribution by PhD candidate (%): 80%

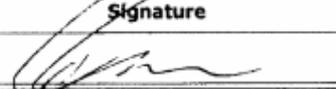
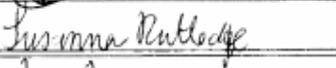
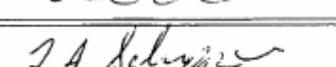
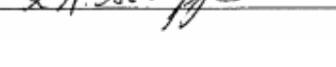
CO-AUTHORS

Name	Nature of Contribution
David Campbell	Collaboration on project conception, data analysis, and interpretation; provided substantial comments on manuscript versions
Susanna Rutledge	Collaboration on project conception, data collection, filtering and gap filling (evaporation and NEP); provided comments on data analyses and manuscript versions
Aaron Wall	Collaboration on data collection, filtering and gap filling; provided comments on data analyses and manuscript versions
Paul Mudge	Collaboration on project conception, provided substantial comments on manuscript versions
Michael Clearwater	Collaboration on data analyses and provided substantial comments on manuscript versions
Louis Schipper	Collaboration on project conception, data analysis, and interpretation; provided substantial comments on manuscript versions

Certification by Co-Authors

The undersigned hereby certify that:

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

Name	Signature	Date
David Campbell		31/03/2017
Susanna Rutledge		6/4/2017
Aaron Wall		31/3/17
Paul Mudge		20/04/2017
Michael Clearwater		20/4/2017
Louis Schipper		31/3/17



THE UNIVERSITY OF
WAIKATO
Te Whare Raukawa o Waikato

Co-Authorship Form

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Please indicate the chapter/section/pages of this thesis that are extracted from a co-authored work and give the title and publication details or details of submission of the co-authored work.

As it stands in the submitted thesis, Chapter 5 is being prepared for submission to a suitable journal. The title of the chapter is currently 'Natural carbon isotope discrimination as a tool to optimise pasture species selection at farm scale'

Nature of contribution by PhD candidate	Project conception; data collection; data analysis and interpretation; writing of manuscript/chapter
Extent of contribution by PhD candidate (%)	90%

CO-AUTHORS

Name	Nature of Contribution
David Campbell	Collaboration on project conception, data analysis, and interpretation; provided comments on manuscript versions
Michael Clearwater	Collaboration on project conception, data analyses and provided comments on manuscript versions
Paul Mudge	Collaboration on project conception, provided comments on data analysis and manuscript versions
Susanna Rutledge	Collaboration on eddy covariance data collection, filtering and gap filling (evaporation and NEP); provided comments on data analyses and manuscript versions
Aaron Wall	Collaboration on data collection, filtering and gap filling; provided comments on data analyses and manuscript versions
Louis Schipper	Collaboration on project conception, data analysis, and interpretation; provided substantial comments on manuscript versions

Certification by Co-Authors

The undersigned hereby certify that:

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

Name	Signature	Date
David Campbell		31/3/2017
Michael Clearwater		20/4/2017
Paul Mudge		20/04/2017
Susanna Rutledge		6/4/2017
Aaron Wall		31/03/17
Louis Schipper		31/3/17