NUTRIENT LEACHING UNDER INTENSIVE SHEEP GRAZING

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ABSTRACT

The use of some alternative forages may help sheep farmers to reduce nitrogen (N) leaching while increasing production. This thesis explores the effects of four forages (perennial ryegrass/white clover: RGWC; Italian ryegrass/white clover: IRWC; plantain/white clover: PWC; and a winter brassica) on sheep performance, urinary N excretion and N loss in drainage over two and a half years (Year 1: July to December 2019; Year 2: January to December 2020; Year 3: January to December 2021).

This study was conducted on an artificially drained, fine textured Tokomaru silt loam soil at Massey University's Keeble farm, near Palmerston North, Manawatu, New Zealand. The study design included four self-contained farmlets (each approximately 3.3 ha): three farmlets had 0.8 ha (24% of their grazing area) sown to include one of three alternative forages (IRWC or PWC or brassica), and the remaining 2.5 ha was sown in a perennial ryegrass/white clover sward. The entire area (100% of grazing area) of the fourth farmlet was sown in RGWC. Approximately 0.4 ha of each farmlet was located in a paddock where a series of 20 drainage plots (each 40 m by 20 m) were established to measure N leaching. Each of the alternative forages, and the RGWC, were sown on five of the drainage plots i.e., five replicates (combined area of 0.4 ha), which composed about one-half of the area of each alternative forage on each farmlet. The amount of N leached through a mole-pipe network on each drainage plot was also measured. Breeding ewe productivity including liveweight, condition score and lambing performance, as well as N excretion was also measured. In addition, forage growth and DM production were monitored along with chemical and botanical composition.

The inclusion of alternative forages into the RGWC system did not affect animal performance. This was due, in part, to animal management. The N leached under various forages was, therefore, able to be compared without the confounding effects of differences in animal

performance. The daily urinary N excretion per animal by sheep grazing PWC or brassica was lower (18 to 70%) than the daily urinary N excretion by sheep grazing RGWC or IRWC. It is likely that the diuretic effect of plantain and a lower N concentration in the brassica caused lower N concentrations in urine. Nitrate (NO_3^-) leaching losses under RGWC, IRWC and PWC were very small in Years 1 and 2 (ranging from 0.4 to 0.8 kg N/ha). The poor persistence of IRWC and PWC at this site and the need to re-establish these forages on the plots resulted in greater NO_3^- leaching under these forages in Year 3, negating some of the advantages associated with these forages in Years 1 and 2. In contrast, NO_3^- leaching losses were greater under brassica forages (ranging from 0.4 to 6.4 kg N/ha) than under RGWC (ranging from 0.5 to 1.5 kg N/ha). Although sheep grazing brassica forages excreted less urinary N (on an individual animal basis), leaching losses under the brassica treatments were higher. In addition to the effect of cultivation, this increased leaching was likely because brassica plots were grazed for a more extended period during winter than other forages, and there was no crop (forage) cover until the spring resowing; therefore, the urinary N accumulated during winter grazing was displaced by subsequent drainage. With the assumption that the cropped area occupies a relatively small portion of the farm, grazing brassica is likely to result in a relatively small increase in whole farm NO_3^- leaching.

Overall, NO_3^- leaching losses under sheep grazing forages were lower (ranging from 0.5 to 9.5 kg N/ha) than those reported under dairy cattle grazing forages, which suggests that sheep production may offer an alternative land use option for dairying areas where it is difficult to achieve the large reductions in NO_3^- leaching required to meet water quality objectives.

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COMMON ABBREVIATIONS

The abbreviations are defined at the first use and then without definition throughout this dissertation. Abbreviations are re-defined in each chapter.

ADF	Acid detergent fibre
BCS	Body condition scores
Ca	Calcium
CEC	Cation exchange capacity
CEF	Creatinine excretion factor
CP	Crude protein
CT	Condensed tannins
d	Day
DAP	Diammonium phosphate
DM	Dry matter
DMI	Dry matter intake
FEC	Faecal worm egg counts
GLM	General linear model
HPLC	High-performance liquid chromatography
ICP-OES	Inductively Coupled Plasma-Optical Emission Spectroscopy
IRWC	Italian ryegrass and white clover mix
K	Potassium
LSD	Least significance difference
LW	Live weight
LWG	Liveweight gain
ME	Metabolisable energy
me	Milliequivalents
Mg	Magnesium

mg	Milligrams
N	Nitrogen
Na	Sodium
NDF	Neutral detergent fibre
NIRS	Near infra-red spectrophotometer
NIWA	National Institute of Water and Atmospheric Research
OMD	Organic matter digestibility
P	Phosphorus
PD	Purine derivatives
PWC	Plantain and white clover mix
RGWC	Perennial ryegrass and white clover mix
RPM	Rising plate meter
SAS	Statistical Analysis System
SSS	Soluble sugars and starch
TN	Total nitrogen
WC	White clover
WSC	Water-soluble carbohydrates

CHAPTER 1: GENERAL INTRODUCTION

The New Zealand sheep industry produces 454, 000 tons of meat and 137, 000 tons of wool annually (Beef + Lamb New Zealand Economic Service 2022a). As the majority is exported, this produce contributes over 15% of the country's pastoral export earnings (Beef + Lamb New Zealand Economic Service 2022a). Over time, sheep farming systems in New Zealand have intensified as a result of increased fertiliser use, improved 'per head' animal performance and/or higher stocking rates (Morris & Kenyon 2014). However, intensification may result in a greater loss of nutrients to the environment (Field et al. 1985; Ruz-Jerez et al. 1995). In recent years, there has been an increased concern about the potential contamination of waterways with nutrients, in particular the nitrogen (N) leached from pastoral farm systems (Ruz-Jerez et al. 1995; Silva et al. 1999; Houlbrooke et al. 2004; Ministry for the Environment 2020).

Urine patches that are deposited by livestock during grazing are the main sources of leached N (Haynes & Williams 1993). Leached N, primarily nitrate (NO_3^-) enters the surface and ground water via water movement through the soil profile. At higher concentrations (> 11.3 mg N/L), NO_3^- can be hazardous to human health and aquatic life (Di & Cameron 2002). An even greater challenge to freshwater in New Zealand is that even relatively small concentration of NO_3^- can promote undesirable biological growth which, following eutrophication, results in a deterioration in water quality (Howarth 1988; Smith & Schindler 2009). In New Zealand, NO_3^- leaching from dairy cattle systems is relatively well characterised, and regional councils have begun to place limits on NO_3^- leaching rates from dairy farms (Ministry for the Environment 2020). A number of mitigation technologies have been developed to reduce NO_3^- leaching under dairy systems (Houlbrooke 2005; Anastasiadis et al. 2012; Cameron et al. 2013; Christensen et al. 2019). However, knowledge of the effect of present-day sheep grazing systems on nutrient leaching and mitigation strategies are currently limited in New Zealand.

Furthermore, due to differences in the management of sheep and dairy cows, many of the mitigation strategies that can be applied to dairy farms may not be feasible for sheep farms, for example housing, stand-off pads and duration-controlled grazing (Anastasiadis et al. 2012).

There is increasing interest in the use of alternative forages in pastoral agriculture systems in New Zealand. Some of these forages can be used to reduce NO_3^- leaching, i.e., improve environmental outcomes (e.g., plantain; *Plantago lanceolata* L. and Italian ryegrass; *Lolium multiflorum* Lam.), while others (e.g., winter crops) are used to increase animal performance in periods of low pasture availability (Anastasiadis et al. 2012; Morris & Kenyon 2014). Therefore, the use of alternative forages could be beneficial in sheep grazing systems as they may result in both increased production and/or a reduction in leaching levels.

Previous studies have shown that plantain on dairy farms can help to mitigate NO_3^- leaching losses (Box et al. 2016; Bryant et al. 2019). This is because plantain reduces urine N concentration and the urinary N excretion rate per patch, as a result of increased urine volume (Box et al. 2016; Bryant et al. 2019) and a decrease in ammonia production (Navarrete et al. 2016). In addition, the secondary metabolites produced by plantain may inhibit nitrification in the soil (Dietz et al. 2013; De Klein et al. 2019). Italian ryegrass, a rapidly growing pasture species, has been shown to exhibit greater N uptake during the winter months and, therefore, reduce NO_3^- leaching compared to perennial ryegrass (*Lolium perenne* L.)/white clover (*Trifolium repens*) swards when grazed by dairy cows (Woods et al. 2018). Winter crops such as brassicas e.g., turnips (*Brassica rapa* ssp. *rapa*), swedes (*Brassica napus* ssp. *napobrassica*), and kale (*Brassica oleracea ssp. acephala*) produce large amounts of forage (e.g., 8-15 t DM/ha) and are grazed with high stocking rates during winter when the grass growth rate is slow (Sprague et al. 2014; Anon 2019). Following grazing, the land is left bare until conditions are favourable for re-sowing in spring. This process of grazing winter brassica species may

result in large amounts of N being returned to the soil during winter, which can subsequently lead to high levels of NO_3^- leaching (Monaghan et al. 2013). In addition, greater NO_3^- leaching is often observed following cultivation (Monaghan et al. 2013).

In New Zealand, no long-term studies have been conducted on N leaching under current intensive sheep production systems, particularly when alternative forages are grazed. The aim of this study was to evaluate N leaching losses under a range of forage types (perennial ryegrass/white clover: RGWC; Italian ryegrass/white clover: IRWC; plantain/white clover: PWC; a winter brassica) and their effect on sheep performance to identify the potential for forages to reduce N leaching while maintaining or improving sheep performance.

The research objectives of this study were to:

i. Determine forage production and sheep performance following the incorporation of different forages into a sheep grazing system.

ii. Determine the potential of these forages to reduce urinary N excretion to the soil.

iii. Provide quantitative information on N leaching when sheep graze different forages.

To achieve these objectives, four self-contained contiguous farmlets or "treatments" (each approximately 3.32 ha) were established in the Manawatu region of New Zealand, on a Pallic soil with a mole-pipe drainage network. An area of 0.81 ha (24% of the grazing area) of the three farmlets was sown in one of three alternative forages (IRWC or PWC or brassica), and the remaining 2.51 ha (76% of grazing area) were sown in a perennial ryegrass/white clover. The entire area (100% of grazing area) of the fourth farmlet, which acted as the control, was sown in RGWC. In one paddock, a series of 20 drainage plots (each 40 m by 20 m) were established to measure N leaching losses. Each of the alternative forages, and the RGWC, were

sown on five of the drainage plots i.e., five replicates (combined area of 0.4 ha), which comprised about one-half of the total area in the alternative forage species within each farmlet. This trial design facilitated the measurement of sheep performance, forage intake and N excretion per sheep and losses of N through drainage.

1.1 Thesis Structure

This thesis consists of seven chapters including the general introduction (Chapter 1), a review of literature (Chapter 2) and a general methodology (Chapter 3). Chapters 4 to 6 present and discuss the data collected from the field research which was conducted over a period of two and a half years. Each of these chapters has been written in the form of a journal paper, comprising abstract, introduction, methods, results, discussion, and conclusion sections. Chapter 4 focuses on forage production and sheep performance, Chapter 5 focuses on sheep N intake and excretion in urine, and Chapter 6 details N losses in drainage. A broad discussion and conclusions are presented in Chapter 7. A part of the literature review is published (see Chapter 2; Maheswaran et al. (2022b)). In addition, data collected on nitrate leaching under sheep grazing systems in 2020 (Year 2) has been published and is included in Appendix 1 (Maheswaran et al. 2021).

CHAPTER 2: LITERATURE REVIEW

This review examines the present status of New Zealand sheep farming (2.1), forages used in sheep grazing systems (2.2), effects of sheep grazing systems on water quality (2.3), and the possible mitigation strategies (2.4). A summary identifying gaps in our knowledge concludes the review (2.5). Section 2.3 has been published in a scientific journal (Maheswaran et al. 2022b).

2.1 Present status of New Zealand sheep farming

The sheep meat and wool industries have contributed significantly to the development of the New Zealand economy (Cranston et al. 2017), currently producing 454, 000 tons of sheep meat and 130, 000 tons of wool annually (Beef + Lamb New Zealand Economic Service 2022a) with the majority being exported, contributing over 15% of export earnings obtained from pastoral products (Beef + Lamb New Zealand Economic Service 2022a). New Zealand is the largest exporter of lamb meat in the world, with the majority being exported to North Asia, European Union and Great Britain, North America and the Middle East (Beef + Lamb New Zealand Economic Service 2021). The contribution of wool and meat to the total income on sheep farms has changed relatively over the past years and now is primarily driven by meat production compared to wool (Table 2.1; Cranston et al. (2017)).

New Zealand's sheep farms are classified into eight classes based on a combination of farming system, topography and climatic condition (Cranston et al. 2017). Sheep performance varies widely among the farm classes and a brief comparison of sheep production data collected from a subset of farms within each class between the years of 1990/1991 and 2021/2022 are shown

Table 2.1 A comparison of sheep production data collected from a subset of farms within each class between the years of 1990/1991 and 2021/2022.

	Year	Farm type								
		Class 1	Class 2	Class 3	Class 4	Class 5	Class 6	Class 7	Class 8	WA
Area (ha)	1990/1991	10389	1604	607	376	215	381	183	251	514
	2021/2022	8096	1500	845	459	280	520	256	355	698
Stock units/ha	1990/1991	0.9	3.6	8.1	10.7	12.0	8.4	12.9	7.1	6.6
	2021/2022	1.6	4.5	8.1	9.3	10.1	8.3	10.9	8.7	6.5
Stock numbers at open	1990/1991	9112	4978	3673	2817	1785	2961	2430	1716	2804
	2021/2022	12759	6702	6828	4283	2827	4314	2796	3094	4499
Ewe lambing %	1990/1991	84.1	94.6	91.9	100.6	98.3	103.2	120.2	113.7	102.8
	2021/2022	107.1	128.3	129.9	130.5	134.5	137.6	134.0	133.3	130.8
Shorn wool sold (kg/sheep)	1990/1991	4.12	4.00	4.27	4.81	4.66	4.24	5.02	4.48	4.52
	2021/2022	4.52	4.11	4.49	4.72	4.30	4.38	4.3	2.25	4.41
% of gross income from wool sales	1990/1991	70.6	40.4	31.8	27.6	21.7	32.2	32.2	11.5	29.9
	2021/2022	30.2	6.0	3.7	2.6	1.4	3.2	3.6	0.5	4.3
% of gross income from sheep sales	1990/1991	13.1	33.4	25.1	23.4	23.9	41.5	53.7	21.9	31.8
	2021/2022	43.2	70.3	62.3	50.5	32.4	58.8	73.1	12.5	51.2

Source: Beef + Lamb New Zealand (1992); (Beef + Lamb New Zealand Economic Service 2022a, 2022b)
Class 1: S.I high country; Class 2: S.I hill country; Class 3: N.I hard hill country; Class 4: N.I hill country Class 5: N.I intensive finishing farms; Class 6: S.I finishing breeding farms; Class 7: S.I intensive finishing farms; Class 8: S.I mixed finishing farms; S.I = South Island, N.I = North Island; WA: Weighted average of all classes
* Weighted average of all classes: These data represent the average of each of eight farm classes weighted by the proportion of farms to total farms.

in Table 2.1 (Beef + Lamb New Zealand 1992, 2021). Farm area has increased in most of the farm types (except the class 1 and 2) and total stock units per farm increased in all classes; however, stock units/ha only increased in four farm types (class 1, 2, 3 and 8; Table 2.1).

The total number of sheep in New Zealand decreased from 57.9 million in 1990/1991 to 26 .0 million in 2020/2021 (Beef + Lamb New Zealand Economic Service 2022a). However, there was a significant improvement in the lambing percentage of ewes and the meat production (kg) per ewe (Tables 2.1 and 2.2). The weighted average ewe lambing percentage of all classes increased by 28.7% in the last three decades (Table 2.1). Lamb weight (kg/head) and lamb production efficiency (kg/ewe) increased by 37 and 118%, respectively, from 1990/1991 to 2020/2021 (Table 2.2). Morris & Kenyon (2014) indicated that even though the total number of sheep has declined, total production in terms of carcass production across the industries has been maintained due to the increase in individual sheep performance. Over the past decades, as stock units and sheep performance have increased N fertiliser application rates have risen (Table 2.3).

Table 2.2 Comparison of sheep production in New Zealand between 1990/1991 and 2020/2021.

Sheep productivity	Unit	1990/1991	2020/2021	change
Lambing performance	lambs/100 ewe	102	130	+28%
Lamb weight	kg/head	13.9	19.1	+37%
Lamb production	kg/ewe	9.8	21.3	+118%
Wool production	kg/head	5.3	5.0	-6%

Source: Beef + Lamb New Zealand Economic Service (2022a)

Table 2.3 Comparison of fertiliser application between 1990/1991 and 2019/2020.

Fertilizer usage	Unit	1990/1991	2019/2020
Pasture fertilizer application	kg/ha[1]	217	163.2
Pasture nitrogen fertilizer application	kg/ha[2]	1.1	17.0
Pasture phosphorus fertilizer application	kg/ha[2]	9	11.4

[1]Total applied fertiliser includes other nutrients and inactive components to improve application and utilization; [2]Effective nutrient; Source: (Beef + Lamb New Zealand 1992; Beef + Lamb New Zealand Economic Service 2022a).

2.2 Grazed forage systems

Intensive sheep farming in New Zealand has traditionally been based on perennial ryegrass (*Lolium perenne* L.) and white clover (*Trifolium repens*) (RGWC) swards (Kemp et al. 1999). However, dry matter production and nutritive value of this pasture can be limited during the summer and autumn period because of moisture and temperature stress (Matthews et al. 1999b). Alternative pasture species such as chicory, plantain, lucerne and red clover have been used, particularly under dryland systems (Fraser & Rowarth 1996; Kemp et al. 2010; Cranston 2017), and a range of short-term crops (brassicas) have been used to fill feed gaps in winter and summer (Valentine & Kemp 2007). Due to the present national concern on water quality, finding alternative species that yield adequately, are of high quality, and reduce N losses into the environment would be of benefit to the New Zealand sheep industry. This section of the review covers literature related to perennial ryegrass, white clover, Italian ryegrass (*Lolium multiflorum* Lam.), plantain (*Plantago lanceolata* L.) and brassicas such as turnips (*Brassica rapa* ssp. rapa), swedes (*Brassica napus* ssp. napobrassica) and kale (*Brassica oleracea* L.) in relation to use in sheep farming systems in New Zealand.

2.2.1 Perennial ryegrass

Perennial ryegrass is the most widely sown temperate grass in New Zealand (Charlton & Stewart 1999). Perennial ryegrass is easy to establish and manage and grows well in a wide range of fertile soils (Charlton & Stewart 1999). It can rapidly regrow quality forage after heavy grazing (Charlton & Stewart 1999); however, when the soil moisture is inadequate, it performs poorly in hot and dry seasons (Charlton & Stewart 1999; Easton & StJohn 2001). The annual dry matter (DM) production of ryegrass in New Zealand varies between 10 and 25 t DM/ha (Easton & StJohn 2001). The wide variation is due to the differences across regions in temperature, grazing management, soil moisture, and soil fertility (Kemp et al. 1999).

Table 2.4 Crude protein (CP), neutral detergent fibre (NDF), acid detergent fibre (ADF), water-soluble carbohydrate (WSC) and metabolisable energy (ME) content of perennial ryegrass.

Author	Herbage quality (g/kg DM)				ME
	CP	NDF	ADF	WSC	(MJ/kg DM)
New Zealand					
Summer					
Harrington et al. (2006)	232		281		
Sun et al. (2012)	217	446	246	67	9.7
Autumn					
Harris et al. (1998)	205	527	284	80	10.2
Barrell et al. (2000)	225	483	229	81	11.8
Burke et al. (2000)	155	487	255	91	11.0
Winter					
Sun et al. (2012)	150	536	277	106	9.4
Spring					
Hutton et al. (2011)					
P140	150	347	196		10.8
L66	80	500	269		9.6
Sun et al. (2012)	150	536	277	106	9.4
Other Countries					
Summer					
Fulkerson et al. (2007)	221	515	313	57	9.9
Raeside et al. (2014)	125	544			10.6
Dineen et al. (2021)	177	354	211		
Autumn					
Moorby et al. (2004)	187	510	303	117	
Fulkerson et al. (2007)	240	497	266	53	10.0
Dineen et al. (2021)	191	355	236		
Winter					
Fulkerson et al. (2007)	243	489	232	78	11.4
Raeside et al. (2014)	240	364			12.5
Spring					
Fulkerson et al. (2007)	263	552	259	63	11.1
Raeside et al. (2014)	155	402			11.7
Dineen et al. (2021)	214	325	205		

P140- ewe pregnancy 140 days, L-66 = 66 days after lambing

Nutrient composition

Across several studies conducted in New Zealand, crude protein (CP), neutral detergent fibre (NDF) and acid detergent fibre (ADF) contents of perennial ryegrass ranged between 8.0 and

23.2%, 34.7 and 53.6%, 19.6 and 28.4%, respectively, and metabolisable energy (ME) content varied between 9.4 and 11.8 MJ/ kg DM (Table 2.4).

The nutritional value of the perennial ryegrass differs depending on the management practices, season and stage of maturity (Burke et al. 2002). A study by Raeside et al. (2014), conducted in western Victoria, Australia, observed lowest percentage of CP and highest percentage NDF during summer (Table 2.4). Hodgson & Brookes (1999) stated that CP, WSC, and digestible organic matter digestibility (DOMD) values of perennial ryegrass are at their lowest and NDF is its maximum during summer. Ryegrass provides sufficient minerals for livestock during ad-libitum feeding; however, the Mg and Na are at marginal concentrations (Grace 1983).

2.2.2 White clover

White clover is a legume commonly sown in a mixed pasture sward with grass, herbs or other legume species. In a mix, it improves the sward quality, forage intake and utilization rates of animals and provides N by N fixation (Caradus et al. 1996). White clover is a summer active legume with optimal growing temperature ranging between 18 and 30°C (Mitchell 1956); however, productivity can be limited during severe dry summer conditions (Hoglund & Brock 1987; Brock et al. 1989). It can grow well in moderate to highly fertile soils; and therefore, can be successfully established in a range of soil types throughout New Zealand (Stewart et al. 2014). Compared to other species, white clover is low yielding with yield of 1 to 7 t DM/ha depending on the pasture management, rainfall and soil fertility (Brock 1973).

Nutrient composition

Across a number of studies conducted in New Zealand, the average CP, WSC, and ME contents of white clover were higher than the average of perennial ryegrass (Tables 2.4 and 2.5). Ulyatt (1981) stated that the white clover has a higher level of digestible protein and lower level of structural carbohydrates compared to perennial ryegrass. Fulkerson et al. (2007) reported

greater value for CP, WSC and ME contents in white clover during winter compared to other seasons in Australia (Table 2.5). White clover is rich in readily fermentable carbohydrates and minerals such as calcium, phosphorus, magnesium, copper, zinc and cobalt; however, it is low in lipids, sugars, cellulose, lignin, sodium and selenium (Caradus et al. 1996).

Table 2.5 Crude protein (CP), neutral detergent fibre (NDF), acid detergent fibre (ADF), water-soluble carbohydrate (WSC) and metabolisable energy (ME) content of white clover.

Author	Herbage quality (g/kg DM)				ME
	CP	NDF	ADF	WSC	(MJ/kg DM)
New Zealand					
Summer					
Lindsay et al. (2007)	276	263			11.7
Harrington et al. (2006)	270		228		
Autumn					
Harris et al. (1998)	246	361	237	94	10.7
Barrell et al. (2000)	298	266	201	102	12.2
Other Countries					
Summer					
Fulkerson et al. (2007)	242	276	210	41	10
Winter					
Fulkerson et al. (2007)	298	291	193	90	10.5
Spring					
Fulkerson et al. (2007)	281	339	233	75	9.3

2.2.3 Perennial ryegrass and white clover sward

Perennial ryegrass with a component of white clover (RGWC) are the main forage species sown in New Zealand (Kemp et al. 1999). The seasonal growth patterns of the RGWC somewhat complement each other (Valentine & Kemp 2007). The growth rate of ryegrass in winter is 20 kg/ha/day greater than white clover while the growth rate of ryegrass can reach 100 kg/ha/day in spring (Figure 2.1). Moisture stress and growth (reproductive) stage affect the growth of ryegrass in summer (Valentine & Kemp 2007) while white clover grows over summer and early autumn when temperatures and day length are highest leading to the highest

levels of N fixation (Carran 1978). Most hill country pastures contain 5% legume or less (Moot et al. 2007), whereas it can be 10% or more in lowland pastures (Brock & Hay 2001).

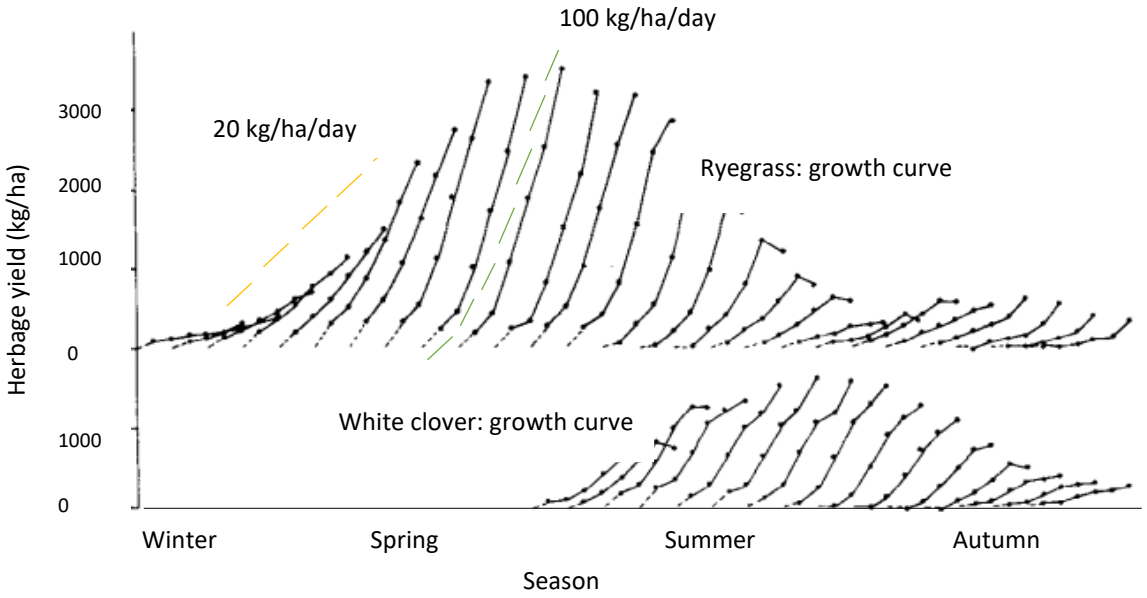

Figure 2.1 Seasonal production of perennial ryegrass and white clover at Palmerston North. Slopes for 20 and 100 kg/ha/day of dry matter production are indicated by the dashed lines (Brougham 1959; Valentine & Kemp 2007).

Nutrient composition

Previous studies conducted in New Zealand showed a wide variation in the nutrient content of RGWC swards across seasons (Table 2.6). The CP content was highest in winter and lowest in summer (Table 2.6; Somasiri (2014)). The average CP content of RGWC sward was 19.3% which was higher than the average of perennial ryegrass (Tables 2.4 and 2.6). The average ME content (10.3 MJ/kg DM) of RGWC sward was closer to the ME (10.4 MJ/kg DM) content of perennial ryegrass over various New Zealand studies (Tables 2.4 and 2.6).

Table 2.6 Crude protein (CP), neutral detergent fibre (NDF), acid detergent fibre (ADF), organic matter digestibility (OMD) and metabolisable energy (ME) content of perennial ryegrass/white clover sward.

Author	Herbage quality (g/kg DM)				ME (MJ/kg DM)	Clover % in mix
	CP	NDF	ADF	OMD		
New Zealand						
Summer						
Kenyon et al. (2010)	90.0	618		644	8.90	
Hutton et al. (2011)	92.0	502		644	9.50	0.2
Somasiri (2014)						
2011	187	463	236	694	10.1	11.5
2012	115	537	283	645	9.50	4.0
Cranston (2014)	210	400	254	740	10.6	18.0
Navarrete (2015)	205		12.1			
Al-Marashdeh et al. (2021)						
2017/2018	214	425	248		11.7	
2018/2019	223	411	239		12.0	
Navarrete et al. (2022)	98.4	477			9.8	
Autumn						
Golding et al. (2011)	200	480		640	9.0	
Somasiri (2014)						
2011	198	485	239	677	9.90	7.5
2012	293	395	193	749	10.8	1.0
Navarrete (2015)			184			
Box et al. (2017)	234	419	276	899	11.1	25.7
Al-Marashdeh et al. (2021)						
2017/2018	260	429	242		12.0	
2018/2019	246	422	239		12.2	
Navarrete et al. (2022)	205	467			10.0	
Winter						
Sinhadipathige et al. (2012)	228	396				5.0
Somasiri (2014)						
2011	263	432	233		10.2	4.5
2012	320	394	212		10.4	2.5
Spring						
Kenyon et al. (2010)	131	544			9.2.0	
Hutton et al. (2011)	123	362		720	10.6	4.9
Somasiri (2014)						
Early spring						
2011	247	430	219	711	10.4	5.0
2012	263	418	215	713	10.4	4.5
Late spring						
2011	155	482	262	683	10.1	7.0
2012	167	472	236	701	10.3	6.0
Navarrete (2015)		350	204		10.7	
Box et al. (2017)	186	460	265	903	11.7	7.28
Al-Marashdeh et al. (2021)						
2017/2018	182	416	233			12.4
2018/2019	226	407	223			12.5
Navarrete et al. (2022)	211	456				10.0

2.2.4 Italian ryegrass

Italian ryegrass has larger leaves and tillers, with less prolific tillering than perennial ryegrass (Woods 2017). Italian ryegrass typically lasts 12-18 months in drier areas, and up to three years under mild summer conditions (DairyNZ 2022a). Endophyte may be present in recently introduced Italian ryegrass cultivars and potentially has greater persistence, depending upon the insect pests present (DairyNZ 2022a). Italian ryegrass shows relatively high winter growth rates and high nutritive value compared with perennial ryegrass (Charlton & Stewart 1999). However, its growth in summer and autumn tends to be lower than that of the perennial ryegrass (Figure 2.2; Kemp et al. (1999)). Autumn sowed Italian ryegrass can be grazed two to three times before spring due to its fast winter growth (Ryan-Salter 2011). It grows rapidly and is suitable for light grazing 4-6 weeks after sowing under good growing conditions (Stewart et al. 2014). Yields of Italian ryegrass are quite variable, and annual DM production of Italian ryegrass ranges between 12.5 and 17 t DM/ha (New Zealand Plant Breeding and Research Association 2021). The DM production is influenced by seeding dates, rainfall, temperature and fertility (Bagg 2014). Ryan-Salter & Black (2012) observed 41% higher DM annual yield in Italian ryegrass when mixed with red clover compared to a monoculture of Italian ryegrass.

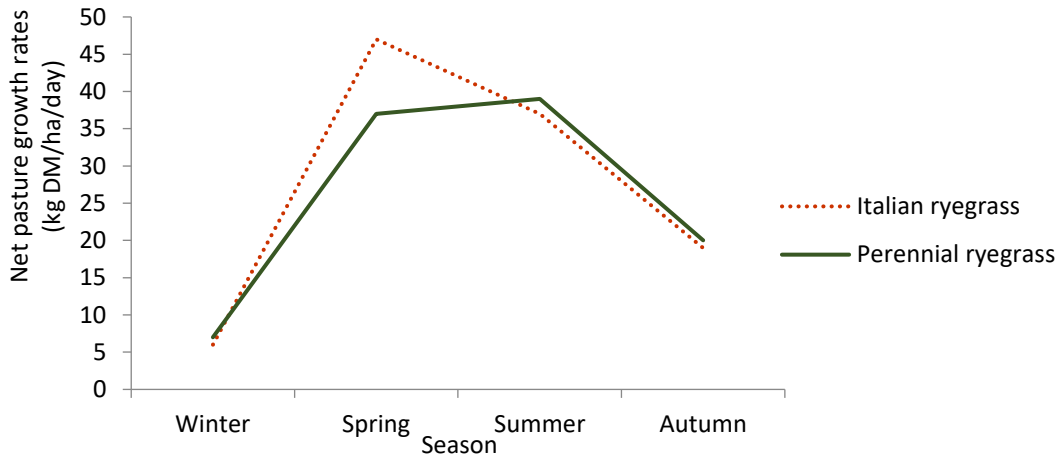

Figure 2.2 Seasonal production of perennial ryegrass and Italian ryegrass (adapted from Stevens (2016)).

Table 2.7 Crude protein (CP), neutral detergent fibre (NDF), acid detergent fibre (ADF), water-soluble carbohydrate (WSC) and metabolisable energy (ME) content of Italian ryegrass.

Author	Herbage quality (g/kg DM)				ME (MJ/kg DM)
	CP	NDF	ADF	WSC	
New Zealand					
Summer					
Ryan-Salter & Black (2012)					
Early Summer	55.0				11.3
Late Summer	96.0				9.5
Autumn					
Ryan-Salter & Black (2012)					
Early autumn	143.0				11.7
Late autumn	287				12.7
Winter					
De Ruiter et al. (2007)					
Early Winter	182	226	142	115	13.3
Mid-Winter	196	383	206	183	12.6
Late Winter	199	462	233	194	12.2
Ryan-Salter & Black (2012)	243				12.7
Spring					
Ulyatt et al. (1974)					
Early spring				153	
Mid spring				190	
Late spring				178	
Other Countries					
Summer					
Hopkins (2003)	214	502	292	65.0	
Autumn					
Thompson et al. (1992)	196	507	356		
Winter					
Thompson et al. (1992)	202	446	335		
Hopkins (2003)	197	510	263	150	

Nutrient composition

The CP percentage of Italian ryegrass ranges between 5.5 and 24% (Table 2.7). Ryan-Salter & Black (2012) stated that the CP content of the Italian ryegrass varies significantly at different times of the year and is at its maximum in May and July (28.7 and 24.3% respectively), and reduced considerably during December and February (5.5 and 9.6% respectively). Hopkins (2003) found a WSC content of 6.5% in a warm climate and 15.0% in cold climate. The variation in the WSC content depends on the season, with higher WSC in a cool-season due to the accumulation of plant metabolites (carbohydrate and fructose) in cool condition (Kobayashi

et al. 2008). The mean ME content of Italian ryegrass was 12.4 MJ/kg DM (Table 2.7), higher than the ME content of pure ryegrass or ryegrass white clover mixed swards (Tables 2.4 and 2.6).

2.2.5 Plantain

Plantain is a perennial herb that is grown as a monoculture or as a diverse pasture mixed sward with or without clover (Lee et al. 2015). It can grow quickly under a wide range of soil types (Stewart 1996). Due to this hardiness, it is widely used in dryland environments to improve dry matter production in summer (Stewart 1996). Moorhead & Piggot (2009) did not find any significant difference in the dry matter yield between plantain-based sward which included plantain, red and white clover and ryegrass during winter and spring. Under New Zealand grazing management, the annual DM yield of plantain ranges between 10 and 18 t DM/ha (Powell et al. 2007; Cranston 2014; Somasiri 2014; Lee et al. 2015; Navarrete 2015) and in a mixed sward with white and red clover, the yield was 11.7 t DM/ha (Rumball et al. 1997). Pure sward of plantain can be grazed over at least two to four years (Powell et al. 2007). Plantain contains biologically active secondary plant compounds including aucubin, iridoid, glucosides and mucilage at levels up to 3% of DM (Stewart 1996). The effect of secondary compounds on rumen microbial activity is explained in Section 2.4.

Nutrient composition

The CP percentage of plantain ranges between 9.1 and 30% (Table 2.8) but is typically lower than perennial ryegrass, white clover, RGWC and Italian ryegrass (Tables 2.4 to 2.7).

Table 2.8 Crude protein (CP), neutral detergent fibre (NDF), acid detergent fibre (ADF), water-soluble carbohydrate (WSC) and metabolisable energy (ME) content of plantain.

Author	Herbage quality (g/kg DM)				ME (MJ/kg DM)
	CP	NDF	ADF	WSC	
New Zealand					
Summer					
Cranston (2014)	220	276	198		11.6
Cave et al. (2014)	180	330			
Lee et al. (2015)	220	345			
Pain et al. (2015)	177	340	236	102	10.7
Navarrete (2015)	208	252	181	128	11.4
Navarrete et al. (2022)	166	379		81.7	9.7
Autumn					
Harrington et al. (2006)	283		256		
Cranston (2014)	210	247	159		11.8
Lee et al. (2015)	240	235			
Navarrete (2015)	264	198	152		12.0
Box et al. (2017)	223	305	228	103	11.0
Cranston et al. (2018)					
Autumn					
Farm 1	208	295			11.1
Farm 2	207	327			10.7
Farm 3	172	312			10.9
Navarrete et al. (2022)	208	329		64	10.3
Spring					
Cranston (2014)					
Early spring	238	194	135		12.1
Late spring	247	193	132		12.2
Pain et al. (2015)	91	415	270	135	10.0
Navarrete (2015)	195	285	214	112	11.4
Box et al. (2016)	195	340	247	60.7	11.8
Navarrete et al. (2022)	180	267		111	10.3
Other countries					
Summer					
Raeside et al. (2014)	138	420			9.8
Winter					
Raeside et al. (2014)	158	267			12.3
Spring					
Raeside et al. (2014)					
Early spring	117	229			11.8
Late spring	160	299			11.1

The CP content of the plantain varies with season, being greater in warmer conditions and lower in cooler conditions (Sanderson et al. 2003). Lee et al. (2015) mentioned that the CP content of plantain declined with increasing grazing interval due to the extended leaf height maturity when left un-grazed for longer intervals (Lee et al. 2015). Plantain has lower levels of NDF and ADF (average percentage) and higher levels of WSC (average percentage) than perennial ryegrass (Tables 2.4 and 2.8). Compared to perennial ryegrass (10.4 MJ/kg DM) improved level of ME content (11.4 MJ/kg DM) content was reported in the previous New Zealand studies (Tables 2.4 and 2.8).

2.2.6 Brassicas

Brassicas are used as annual crops to fill periods of feed deficits, particularly in winter and summer (Valentine & Kemp 2007). Brassicas are highly productive and may be ready to graze from 45 - 250 days from sowing, depending on the species and variety (Valentine & Kemp 2007). Leaves and bulb of the brassicas are highly digestible and a rich source of soluble carbohydrate (starch and soluble sugars; De Ruiter et al. (2007)). Bulbs of brassica contain a higher proportion of readily fermentable CHO than grass, while they have similar CP content (Barry 2013). Forage brassicas such as turnips, swedes and kale are high in ME (12.0-13.8 MJ ME/kg DM) and low in DM% and fibre (Litherland & Lambert 2007). These species have adequate CP content for optimum live weight gain (Litherland & Lambert 2007). The important trace minerals like copper, cobalt and selenium are low in brassicas. Also, they are low in iodine and can cause thyroid and reproductive problems (De Ruiter et al. 2007).

Turnips

Bulb turnips are one of the main brassicas' crops used for supplementation of poor pasture growth in winter due to their high potential for growth and yield during winter (Moot et al. 2007). Turnips are quicker maturing than swedes, and cultivar like "Green Globe" take

approximately 110 days to mature (White & Hodgson 1999). Turnips are a more favoured winter feed than swedes in areas where a cool, short growing season limits the use of slower maturing crops (Moot et al. 2007). The DM production of the brassicas is influenced by thermal time and interception of radiation (Moot et al. 2007). Annual DM yield of bulb turnips is highly variable (6 to 15 t DM/ha), with earlier sown crops producing greater yields (Moot et al. 2007).

Swedes

Swedes are normally used as a winter feed crop to fill the winter feed deficit (De Ruiter et al. 2007). Swedes produce a higher yield over a longer period (5 months) of the growing season than turnips (White & Hodgson 1999) because of their longer vegetative growth period. They perform well in cooler, moist environments as they have a low tolerance to drought; however, they cannot grow well in waterlogged areas (DairyNZ 2022d). Yields of 12-16 t DM/ha can be obtained under good growing conditions, although this varies with soil type, fertility, disease severity, and available moisture (DairyNZ 2022d). The keeping quality of swedes is greater than turnips and they can be grazed from early to late winter (White & Hodgson 1999). However, adverse weather and disease damage during winter can cause yield declines (DairyNZ 2022d).

Kale

Kale is normally used as a second brassica crop, especially after swedes, because of its tolerance to club root and dry rot (DairyNZ 2022c). It has a deep root system, and therefore has good drought tolerance (DairyNZ 2022c). Kestrel, a short to medium kale type, produces high-quality stems and can carry higher leaf percentages through the winter (PGG Wrightson Seeds 2017). Combining high leaf percentages with soft quality stems, Kestrel can offer a high ME feed that is suitable for chasing live weight targets in priority stock classes (PGG Wrightson Seeds 2017). The potential DM yield of short to medium cultivars is around 12 to

15 t DM/ha under good growing conditions (DairyNZ 2022c); however, yields are variable depending on the soil type, fertility and available moisture.

Nutrient composition of turnips, swedes and kale

Nutritional compositions of turnips, swedes and kale are given in Tables 2.9 to 2.11. Across a number of studies conducted in New Zealand, the mean CP and ME contents of turnips and swede leaf were higher than the bulb (Tables 2.9 and 2.10). Pelletier et al. (1976) stated that the CP content of brassica is greater in leaves than bulbs, but bulbs accumulated more nitrate than leaves. The nutritional content of brassicas varies with environmental conditions and degree of maturity of the plant at the time of harvest (Winkler 2017). The average WSC content of swede bulb is almost double that of the WSC of both swede leaf and turnip leaf (Tables 2.9 and 2.10).

Table 2.9 Crude protein (CP), neutral detergent fibre (NDF), acid detergent fibre (ADF), water-soluble carbohydrate (WSC) and metabolisable energy (ME) content of turnips.

Author		Herbage quality (g/kg DM)				ME (MJ/kg DM)
		CP	NDF	ADF	WSC	
New Zealand						
Winter						
Barry & Drew (1978)	Leaf	199				
	Bulb	172				
De Ruiter et al. (2007)	Bulb	127	237	183	579	11.1
	Whole plant	158	177	278	270	12.3
Westwood & Mulcock (2012)	Whole plant	143	232	189	263	11.4
Dalley et al. (2017)	Leaf	218	262	221	197	
	Bulb	134	266	199	380	
	Whole plant	126	417	328	233	
Other Countries						
Winter						
Koch et al. (2002)	Leaf	109	269	246		
Keim et al. (2020)	Whole plant	174	179			

Table 2.10 Crude protein (CP), neutral detergent fibre (NDF), acid detergent fibre (ADF), water-soluble carbohydrate (WSC) and metabolisable energy (ME) content of swedes.

Author		Herbage quality (g/kg DM)				ME (MJ/kg DM)
		CP	NDF	ADF	WSC	
New Zealand						
Winter						
Drew et al. (1974)	Whole plant	179				
Barry & Drew (1978)	Leaf	163				
	Bulb	172				
De Ruiter et al. (2007)	Whole plant	119	213	175	481	11.6
Sun et al. (2012)	Whole plant	162	176	121	301	14.1
Westwood & Mulcock (2012)	Whole plant	145	160	150	473	13.7
Dalley et al. (2017)	Leaf	245	171	169	191	
	Bulb	113	154	145	473	
Thant et al. (2020)	Whole plant	149	218	194	275	10.2
Other countries						
Winter						
Keogh et al. (2009)	Whole plant	114	265	253		
Winkler (2017)						
Major plus	Leaf	242	227	160		
	Bulb	143	412	323		
Am.Purple Top	Leaf	256	237	183		
	Bulb	156	421	329		
Dominion	Leaf	252	229	168		
	Bulb	156	421	329		
Keim et al. (2020)	Whole plant	132	300			

Table 2.11 Crude protein (CP), neutral detergent fibre (NDF), acid detergent fibre (ADF), water-soluble carbohydrate (WSC) and metabolisable energy (ME) content of kale.

Author	Herbage quality (g/kg DM)				ME (MJ/kg DM)
	CP	NDF	ADF	WSC	
New Zealand					
Winter					
De Ruiter et al. (2007)	131	271	226	387	11.5
Westwood & Mulcock (2012)	138	246	213	280	11.7
Dalley et al. (2017)	135	342	252	270	
Thant et al. (2020)	93	353	279	242	9.13
DairyNZ (2022c)					12 or more
Other countries					
Winter					
Keim et al. (2020)	110	300			

2.2.7 Animal performance on different forages

Ewe weight gain and BCS change during late pregnancy and lactation.

Research conducted in Canterbury, New Zealand, found that ewes grazed on plantain during late pregnancy and lactation had increased live weight gain compared to ewes grazed on perennial ryegrass (Table 2.12; Judson et al. (2009)). One of the possibilities for the above could be the ability of plantain to supply more metabolisable protein than the perennial ryegrass (Judson et al. 2009). In contrast, research conducted at Palmerston North, New Zealand (Kenyon et al. 2010) and Victoria, Australia (Raeside et al. 2014) found that mixed swards of plantain (Raeside et al. 2014) or plantain/perennial ryegrass (Kenyon et al. 2010; Raeside et al. 2014) did not improve ewe performance relative to RGWC swards (Table 2.12).

Lamb birth weight

Raeside et al. (2014) did not find any significant differences in the birth weight of the lambs of ewes grazed on perennial ryegrass or plantain at the same level of birth type (twins or singles) (Table 2.13).

Lamb growth to weaning and weaning weight

Judson et al. (2009) reported that the lambs grazed on plantain displayed improved live weight gain to weaning and live weight at weaning compared to lambs grazed on perennial ryegrass (Table 2.13). However, Kenyon et al. (2010) did not find any significant differences in lambs' growth to weaning between the lambs grazed on RGWC and those grazed on plantain/perennial ryegrass mixed swards (Table 2.13). Similarly, Raeside et al. (2014) did not find any significant differences in weaning weight of the lambs grazed on perennial ryegrass or plantain from the same birth type (twins or singles) (Table 2.13).

Lamb post-weaning growth and carcass weight

Lambs fed with RGWC had greater live weight gain from weaning to slaughter (Table 2.13; Eerens & Ryan (2000)) than lambs fed perennial ryegrass. Pure swards of perennial ryegrass may not be enough for all livestock to obtain their potential growth (Barry 1981; Fraser & Rowarth 1996). This is due to the higher feeding value of the white clover than that of ryegrass (Ulyatt 1981). Harris et al. (1997) reported that the higher level of white clover in a mixture of RGWC sward enhances the digestibility and intake of protein and energy. Farmers can produce nearly 25% more dry matter, 40% more carcass weight and 25% more wool from RGWC sward than from pastures with ryegrass alone receiving 270 kg N/ha/year (Eerens & Ryan 2000).

Lamb grazing on plantain did not improve lamb performance relative to perennial ryegrass (Fraser & Rowarth 1996) and RGWC sward (Appleton 2011) (Table 2.13). In contrast, Somasiri (2014) reported that the plantain and clover sward can support greater lamb live weight gains and carcass weight than RGWC sward (Table 2.13). Similarly, Deaker (1994) observed greater carcass weight in the lambs fed with plantain than the perennial ryegrass (Table 2.13). This variation could be due to the differences in the cultivar used. Fraser &

Rowarth (1996) used Lancelot which is not as good as Tonic. Other studies (Deaker 1994; Somasiri 2014) used Tonic.

The plants with greater nutrient composition would likely have resulted in higher feeding values (Waghorn et al. 2007). The greater energy content of the plantain than that of the perennial ryegrass (Tables 2.4 and 2.6) would result in higher growth rate in lambs in plantain/white clover mix compared to RGWC sward (Table 2.13). Further, the fibre content of perennial ryegrass was higher than that for plantain, which can limit feed intake (Waghorn et al. 2007) and can affect lamb growth negatively (Somasiri 2014). Further, Somasiri (2014) recommended using plantain/white clover swards to improve lamb carcass weights and value over RGWC swards.

Studies comparing the performance of young sheep grazing brassica have shown that sheep grazing turnips, or a mixture of swedes and Italian ryegrass, gained more weight than sheep grazing swedes alone (Table 2.14).

Table 2.12 Effect of grazing ewe on perennial ryegrass (RG), ryegrass/white clover (RGWC), Italian ryegrass (IR), plantain (P) and ryegrass/plantain (RG/P) during late pregnancy and lactation on ewe live weight change to weaning and ewe body conditioning score (BCS). Means within rows with different superscripts are significantly different at P<0.05 level.

Author	Ewe live weight change from late pregnancy to weaning (kg)				Ewe BCS change from late pregnancy to weaning	
	RG	RGWC	P	RG/P	RGWC	RG/P
Judson et al. (2009)						
Year 1	-7.5[a]		6.6[b]			
Year 2	-2.3[a]		7.2[b]			
Kenyon et al. (2010)		-9.3		-11.8	-0.1	-0.3
Raeside et al. (2014)						
Single		0.73		1.68		
Twin		-7.26		-7.88		
Cranston et al. (2016)						
L59		70.6		70.7	2.7	2.7
L 96		75.9		75.5	2.9	2.9

L59: 59 days after the midpoint of lambing; L96: 96 days after the midpoint of lambing.

Table 2.13 Effect of grazing ewe on perennial ryegrass (RG), ryegrass/white clover (RGWC), Italian ryegrass (IR), plantain (P) and plantain/white clover (PWC) on their lamb birth weight, lamb live weight gain to weaning, lamb survival to weaning % and lamb weight at weaning. Means within rows with different superscripts are significantly different at P<0.05 level.

Author	Lamb birth weight (kg)		Lamb growth to weaning (g/day)				Lamb growth (g/day) between L59 and L96		Lamb weight at weaning (kg)		
	RGWC	P	RG	RGWC	P	PWC	RGWC	PWC	RG	RGWC	P
Eerens & Ryan (2000)			247.0^{b}	276.0^{a}					21.6^{b}	23.9^{a}	
Judson et al. (2009)											
Year 1			296.0^{b}		376.0^{a}				33.9^{b}		41.1^{a}
Year 2			309.0^{b}		346.0^{a}				30.7^{b}		33.9^{a}
Kenyon et al. (2010)				242.0		249.2					
Raeside et al. (2014)											
Single	5.3	5.6							35.6		29.9
Twin	3.9	4.6							25.7		28.0
Cranston et al. (2016)							318	327			

L59: 59 days after the midpoint of lambing; L96: 96 days after the midpoint of lambing.

Table 2.14 Effect of grazing sheep on turnips, swedes and swedes/Italian ryegrass mixture on daily weight gain of young sheep.

Author		Turnips	Swedes	Swedes/Italian ryegrass (20%)
		Weight gain (g/day)	Weight gain (g/day)	Weight gain (g/day)
Winter				
Drew et al. (1974)	Wether hoggets	111	101	
Barry & Drew (1978)	Wether hoggets	116	101	
Stevens et al. (1994)	Young sheep		109	132

2.3 Sheep grazing system effects on nitrate leaching

Publication

Maheswaran S, Cranston LM, Millner JP, Horne DJ, Hanly JA, Kenyon PR, Kemp PD 2022. Effects of sheep grazing systems on water quality with a focus on nitrate leaching. Agriculture 12: 758. https://doi.org/10.3390/agriculture12060758.

2.3.1 Abstract

This article reviews the literature on nitrate (NO_3^-) leaching under sheep grazing systems and focuses on identifying future research needs. Urinary nitrogen (N) is an important source of the NO_3^- leached from pastoral agriculture. Urinary N excretion can be measured or simulated using models and has been well characterised for dairy systems. It is difficult to continuously monitor the urinary N excretion of sheep under field conditions, consequently, measurements of N excretion in sheep urine are limited. Urination events by sheep vary greatly in volume (0.5 L to 6.9 L), concentration (3 to 13.7 g N/L) and frequency (8 to 23 events/day): this variation results in a corresponding variation in N loading rates in urine patches. The amount of NO_3^- leached under pastures grazed by sheep has typically varied between 1 and 50 kg N/ha/year, but rates as high as 300 kg N/ha/year have been reported. The quantity of NO_3^- leached under sheep depends on; season, climate, quantity and timing of drainage and the interaction between forage production and stocking rate, fertiliser applied, N fixation by legumes, forage type and grazing management. Most studies examining NO_3^- leaching under sheep grazing systems are more than 20 years old, so there is little recent information on NO_3^- leaching under modern, pasture-based sheep production systems. Further research is required to; quantify NO_3^- leaching levels under current sheep farming practices, understand the impacts of this leaching on water quality and, to help identify effective strategies to reduce the transfer of N from grazed paddocks to receiving water bodies. This additional information will help

provide information for decision support tools, including models, and management practices, to help sheep farmers minimise their impact on the aquatic environment.

Keywords: climate; nitrogen; pasture system; season; soil, urine

2.3.2 Introduction

Nitrogen (N) losses in drainage and runoff from agricultural soils, particularly from intensively grazed pasture, can be a significant cause of water quality deterioration in many parts of the world (Ruz-Jerez et al. 1995; Cuttle et al. 1996; Silva et al. 1999; Di & Cameron 2002; Houlbrooke et al. 2004). Nitrate (NO_3^-) is the most common form of N in drainage water (Haynes & Williams 1993; Monaghan et al. 2002). At higher concentrations (> 11.3 mg N/L), NO_3^- can be hazardous to human health and aquatic life (Di & Cameron 2002): more problematically, a relatively small concentration of NO_3^- can promote undesirable biological growth, which following eutrophication, results in a deterioration in water quality (Di & Cameron 2002). Nitrate has a negative charge and is repelled by cation exchange sites on soil surface (Garwood & Ryden 1986); therefore, it is easily leached when water drains through soil (Di & Cameron 2002). In comparison, ammonium (NH_4^+) does not generally move far within the soil profile because it is attracted to cation exchange sites (Haynes & Williams 1993; Monaghan et al. 2002).

Nitrogen cycling in a grazing system is influenced by the grazing animal's diet and the partitioning of ingested N within the animal (Williams & Haynes 1994). Generally, between 75-95% of ingested N is returned to the soil, and approximately 70% of this N is excreted in urine (Figure 2.3). Thus, the primary source of NO_3^- leached from grazed pasture is livestock urine (Figure 2.3). Nitrogen in faeces is present in more complex organic forms that are less

rapidly mineralised; therefore, it is not a significant contributor to NO_3^- leaching (Wachendorf et al. 2005).

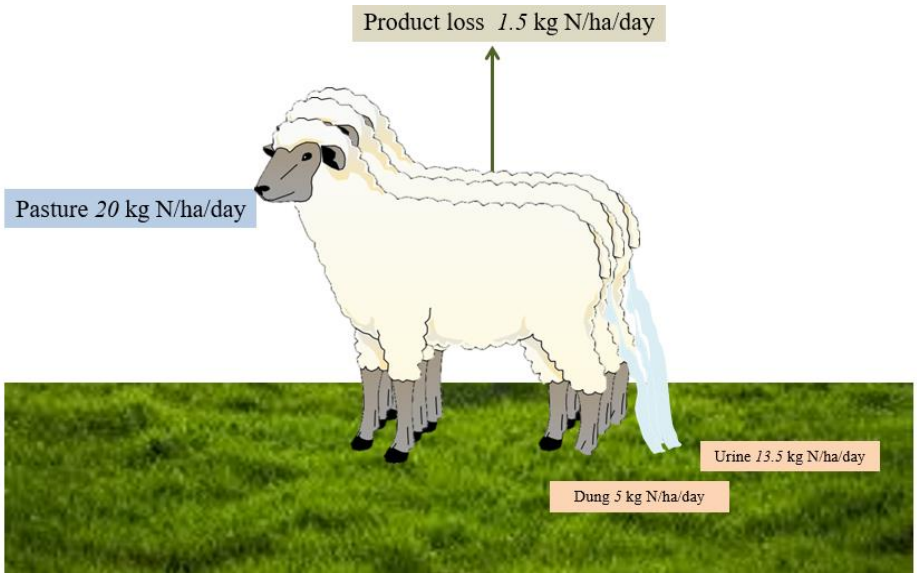

Figure 2.3 Nitrogen (N) transfer in a sheep grazing system, for one day's grazing assuming 533 sheep/ha ingesting 800 kg DM pasture @ 2.5% N. Based on Haynes & Williams (1993).

Nitrate leaching is widely believed to be significantly lower under sheep farming than dairy cow systems (Williams & Haynes 1994; Hoogendoorn et al. 2011). However, in order to remain economically viable, sheep production systems in New Zealand, particularly those on flat or undulating landscapes, have intensified over time, with greater use of N fertiliser and higher stocking rates (Morris & Kenyon 2014). Therefore, it is likely that NO_3^- leaching rates from sheep systems is greater than those previously reported in New Zealand, necessitating a reassessment of N losses, especially under intensive grazing practices. Currently, there are no long-term studies of NO_3^- leaching from modern pastoral-based sheep production systems in New Zealand. Due to the potentially adverse environmental effects of intensive farming on water quality (Cameron et al. 2013), some regional councils have placed NO_3^- leaching caps on dairy farms (Ministry for the Environment 2020). In the future, these restrictions may be extended to other livestock industries, including intensive sheep farming. Therefore, knowledge of potential leaching rates and mitigation measures will be of benefit.

This review aims to explicitly examine the current knowledge of N excretion in sheep urine and how urination behaviour and other factors determine the extent of NO_3^- leaching under sheep grazing systems. Further, it aims to identify the areas of research and development that are required to enhance knowledge and understanding of NO_3^- leaching under modern sheep grazing systems, including N application/loading, grazing management and alternative pastures.

2.3.3 Measurements of nitrate leaching under sheep grazing systems

The few studies to date that have measured NO_3^- leaching under typical sheep grazing conditions have mostly used soil core samples, suction cups or lysimeter methods, and reported NO_3^- leaching rates ranged from 2 to 94 kg N/ha/year (Table 2.15). Nitrate leaching rates greater than 100 kg N/ ha were reported in New Zealand when the N fertiliser level was above 200 kg N/ha (Table 2.15). In contrast, in a UK study (Cuttle et al. 1996), NO_3^- leaching rate remained below 50 kg N/ha even when N fertiliser rate was above 200 kg N/ha. The few studies that measured NO_3^- leaching from mole and pipe drains (Table 2.16) have reported NO_3^- leaching ranging from 8.6 to 50 kg N/ha/year (Heng et al. 1991; Magesan et al. 1996; White et al. 1998). In studies of mole and pipe drainage, NO_3^- leaching under different N fertiliser rates (0, 50 and 120 kg N/ha) were compared (Heng et al. 1991; Magesan et al. 1996; White et al. 1998): no differences in NO_3^- leaching were found between no N fertiliser and the other two moderate N application rates.

Summarising Table 2.15, NO_3^- leaching under sheep grazing varies depending on; the amount of fertiliser applied (Hoogendoorn et al. 2017), the level of soil fertility (Parfitt et al. 2009), the amount of N fixed by legumes in the pasture (Field et al. 1985) and stocking rate (Cuttle & Scholefield 1995). The following sections discuss what is or is not known regarding factors affecting leaching levels under sheep grazing.

2.3.4 Sheep urination

Nitrogen losses under grazing systems are primarily driven by the amount of urinary-N excreted by animals (Williams & Haynes 1994) for a particular soil type, climate context, and the season of the urinary-N return. For example, although the N content in urine in spring may be relatively high, it may not significantly impact NO_3^- leaching due to rapid pasture growth and associated N uptake at this time of the year (Shepherd & Lucci 2013). The total quantity of N deposited in sheep urine patches over a given period is influenced by the concentration of N in the urine, the number of urination events, the volume of urine voided at each urination event and the size of the urination patch (Haynes & Williams 1993). Sheep urination events under grazing conditions are highly variable in volume, concentration and frequency (Betteridge et al. 2010; Hoogendoorn et al. 2010; Marsden et al. 2020). This variation can lead to larger differences in urine-N loading to soils (Li et al. 2012).

Currently, information on sheep urination events under pastoral grazing conditions is limited due to the difficulty of continuously monitoring urination by sheep (David et al. 2015; Marsden et al. 2020). Consequently, most data have been derived from indoor, full-collection studies in which animals are penned for sampling (Jonker et al. 2015; Lindsay 2016; O'Connell et al. 2016; McGusty 2017). However, some studies have utilised sensors (thermistors) in conjunction with GPS to determine the spatial distribution of sheep urination events (Betteridge et al. 2010). In dairy cattle, advanced technologies, including sensors and urine meters, have been successfully utilised to measure urination behaviour, including urine volume and frequency (Marshall et al. 2020). Tri-axial accelerometer sensors are beginning to be trailed on sheep (Lush et al. 2018). If successful, these technologies would allow more comprehensive studies of sheep urination behaviour to be undertaken under field conditions.

Table 2.15 Summary of nitrate-nitrogen (N) leaching in sheep grazing pasture systems measured using lysimeter or ceramic cups at different depths.

Reference	Soil texture	Stocking rate or urine application	Fertiliser-N applied (kg N/ha)	Drainage (mm)	Total Nitrate N leached (kg/ha)
New Zealand					
Monaghan et al. (1989)	Silt loam	Sheep urine applied equivalent to 265 kg N/ ha	0	7-136	19-37
Hoogendoorn et al. (2011)	Sand	Rotational grazing of sheep for many days	0	100	20-31
Hoogendoorn et al. (2017)	Silty clay loam to clay loam	Rotational grazing of non-lactating ewes (200-250 ewes/ha for 3-4-day grazing and 10-14 times/year)	0		47-74
			100		43-67
			200		37-94
			300		113-176
			400		152-227
			500		235-315
			750		238-368
Williams & Haynes (1994)	Silt loam	Sheep urine equivalent to 290 kg N/ ha (5.6 g N/L)			3-16
Di & Cameron (2007)	Silt loam	Cow urine equivalent to sheep urine	20		
		Urine-N applied at the rates of 300 kg N/ha			60
		Urine-N applied at the rates of 300 kg N/ha + DCD			10
Australia					
Melland et al. (2008)	Coarse sand to clay loam and light to heavy clay	26 su/ha at set stocking high P			5.8-7.7
		19 su/ha at set stocking low P			3.5-5.2
		27-28 SU/ha at rotational grazing high P			3.2-5.0
		27-28 SU/ha at rotational grazing high P			4.6-5.1
UK					
Cuttle et al. (1996)	Fine loam	Ewes + PreWL 18.8 unit/ha, PostWL: 24.9 lambs/ha	0		5.4-13.3
		Ewes + PreWL 21.6 unit/ha, PostWL: 25.2 lambs/ha	398		5.6
		Ewes + PreWL 18.8 unit/ha, PostWL: 22.7 lambs/ha	467		13.6
		Ewes + PreWL 17.8 unit/ha, PostWL: 25.7 lambs/ha	462		10.3
Cuttle et al. (1998)	Fine loam	Continuous stocking of ewes and lambs	0	454-692	6-34
			152-197		8-46

SU: stocking unit; PreWL: Pre-weaning lambs; PostWL: Post-weaning lamb.

Table 2.16 Summary of nitrate-nitrogen (N) leaching in sheep grazing pasture systems measured using mole and pipe drainage systems.

Reference	Soil texture	Stocking rate or urine application	Fertiliser-N applied (kg N/ha)	Drainage (mm)	Total Nitrate-N leached (kg N/ha)
New Zealand					
Heng et al. (1991)	Silt loam	No grazing	0	87-304	8.6-12.6
		40-50 sheep grazed one week	50	100-118	14.9-19.7
Magesan et al. (1996)	Silt loam	No grazing	0	304-339	9-23
		No grazing	50	257-300	13-17
		Intensively grazed by sheep for several days	0	118-266	19-50
		Intensively grazed by sheep for several days	50	100-236	15-44
White et al. (1998)	Silt loam	40 sheep for one week	0		35
		40 sheep for one week	120		23
		21 sheep for 5 days	0		43
		21 sheep for 5 days	120		17

Urine volume

Indoor sheep studies utilising metabolic crates report a wide range in total daily urinary volume, ranging from 0.5 L to 6.9 L/day (Marsden et al. 2020). Urine volume is influenced by season and forage type (O'Connell et al. 2016; McGusty 2017; Marsden et al. 2020). Under field conditions, Doak (1952) reported that the average daily urine volume usually varied between 1.7 and 3.8 L. Maximum daily urine volumes (11 L/day) were observed in October (spring); this was a reflection of high foliage water content (Doak 1952). However, in a recent UK study, ewes grazing *ad libitum* on a perennial ryegrass dominated sward had greater individual urine event volumes and daily urine volumes during a hot autumn (mean temperature of 16.4 ℃, 377 ml urine/event and 3.13 L urine/day) compared to a cool summer (mean temperature of 14.5 ℃, 239 ml urine/event and 2.02 L urine/day) or spring (mean temperature of 11.3 ℃, 177 ml urine/event and 2.03 L urine/day) (Marsden et al. 2020). They suggested that feed and water intake could be drivers for these differences in the production of urine volume (Marsden et al. 2020), but these factors were not measured. Recent studies have shown that forage species, such as plantain, influence urinary volume, which is discussed in detail in later sections (Section 2.3.5).

Measuring the urine volume of sheep is a very laborious process and usually involves placing animals in metabolic crates (David et al. 2015). Currently, it is not practical to collect accurate measurements of urine volume from sheep under pastoral grazing conditions and so alternative indicators are often employed. Muscle creatinine (a metabolite formed in muscle by removing water from creatinine phosphate) is produced daily at what is thought to be a constant rate, determined by the sheep's live weight (per kilogram of animal muscle mass), and is exclusively excreted via urine (David et al. 2015). Therefore, measurements of urine creatinine concentration along with the ratio of daily creatinine excretion per unit of live weight (LW) are commonly used to predict daily urine output (L/day). This method has predicted values of 1.4

to 7.6 L/day (Table 2.17). However, Jonker et al. (2021) have recently indicated that creatinine excretion per kg of body weight is not as constant as initially thought, leading to underestimations of the urine output of sheep fed with low DM forage crops. Accordingly, they attempted to develop a model using multiple data inputs to improve the prediction of sheep's daily urine output (Jonker et al. 2021). However, when these equations were applied to creatinine concentrations based on spot urine samples collected in the authors' study (Maheswaran et al. 2022a), predicted urine volumes ranged from 0.5 to 49 L/day (Table 2.17). The equations that use creatinine concentration to predict sheep urinary N output lack consistency and often produce unrealistic values (i.e., well above 10 L/day). If spot urine samples are to be successfully used to predict total urine output (based on creatinine), further studies are required to understand how creatinine excretion is influenced by time of the day, forage type, water intake and animal live weight and physiological stage.

Urine-N concentration

Nitrogen concentration in urine is another primary determinant of soil N loading rates via urine patches (Hoogendoorn et al. 2010). The concentration of N excreted in each urination event depends on the amount of excess metabolised N excreted and urine volume (Hoogendoorn et al. 2010). Therefore, understanding N concentrations in sheep urine helps to explain N leaching rates. Across several studies, the N concentration of sheep urine has been shown to vary from 3 to 13.7 g N/L (Table 2.18). Urine-N concentration appears to vary with: pasture type, N intake, water intake, season, and animal reproductive status (Selbie et al. 2015). Increasing N intake has been shown to increase the excretion of urine-N; however, high N feeds do not necessarily correspond to higher urine-N concentration because it also depends on water intake (Selbie et al. 2015). The effect of forage species on sheep urine-N concentration is explained in Section 2.3.5.

Hoogendoorn et al. (2010) measured the N concentration of urine from ewes (12 to 18-month-old) grazing a ryegrass/cocksfoot-based pasture over three periods for three consecutive days during spring and autumn. They observed variations in N concentration between individual urination events within days, between days and between individual sheep. Their study showed higher urinary N concentration in the afternoon relative to the morning (Hoogendoorn et al. 2010). The reason for this variation was not explained by Hoogendoorn et al. (2010), but previous studies have shown that the water-soluble carbohydrates (WSC) and CP contents of pastures fluctuated throughout the day, with a lower WSC/CP ratio in the morning and higher in the afternoon (Avondo et al. 2008). Therefore, a significant intake of reduced WSC pastures in the morning may lead to an increase in the N concentration in the urine excreted during the afternoon. Variation in morning and afternoon N concentrations are not likely to affect annual leaching. However, it is important to consider this variation when sampling urine and making comparisons between studies.

Urination frequency

There are few data on the average daily urination frequency of sheep under grazing conditions. The available data indicate that urination frequency in sheep varies from 8 to 23 events/day (Betteridge et al. 2010; Marsden et al. 2020). In a field study, the frequency of urination recorded using sensors was between 13 and 23 events/day (Betteridge et al. 2010) while ewes grazing pasture and placed in pens for six-hour periods to facilitate the capture of urination data, had a mean urination frequency of 9.7 events/day, with a range of 8 to 12 events/day (Marsden et al. 2020). However, care is required when interpreting these data and extrapolating to 24 hours under grazing conditions, as penning itself could affect frequency. Betteridge et al. (2010) reported that the frequency of urination in sheep increased from morning (09:00 hrs) and reached a maximum in the evening (20:00 hrs). This variation across the day might be explained by fluctuations in air temperature (Betteridge et al. 2010). In addition, Betteridge et

al. (2010) mentioned that as foraging activity increases, sheep urinate more frequently during the day than at night (Betteridge et al. 2010). More studies are required to measure the urination frequency of sheep when roaming and grazing pastures.

Urine patch area

Many variables determine the size of the urine patch. These factors include urine volume, soil moisture content, soil surface microtopography, the presence and size of pores open to the soil surface, vegetation cover, slope and wind (Haynes & Williams 1993). Williams & Haynes (1994) used bromide to trace the physical movement of sheep urine through a silt loam to determine the shape and size of the soil volume wetted by urine. They reported that sheep urine moved in both horizontal and vertical directions in the soil profile (the soil water content ranged from 20 to 30%), covering an area of 0.043-0.055 m^2 and a maximum depth of 0.15 m (Williams & Haynes 1994). Other studies using ^{15}N-labelled urine reported similar urinary spot areas (0.03-0.05 m^2) (Doak 1952).

If we assume sheep grazing at a set stocking rate of 12 ewes per ha per year, then the area covered in urine spots will be 8.64 m^2 (12 ewes x 16 urination x 0.045 m^2) after 24 hours of grazing. Assuming there is no overlap of urine patches, then after a year of grazing, urine deposition will cover approximately 32% of the pasture area (8.64 m^2 x 365 days x 0.0001 ha/m^2).

Table 2.17 Published equations for predicting daily creatinine excretion (DCE; mg) and urine volume (L/day), including their published ranges (min and max) and calculated DCE values and urine volume using data collected over two years in authors' study (Maheswaran et al. 2022a) based on these equations (average, min and max in parentheses).

Reference	Sheep Description (number of animals)	Equations	Literature values	Calculated values[1]	
				DCE (mg)[2]	Urine volume (L/day)
		Daily creatinine excretion	*DCE (mg)*		
Brody (1945)	Ewes (15)	Mean daily creatinine excretion (mg)= ($12.7LW^{0.896}$)		614.8 (479.1-751.4)	6.7* (0.45-17.8)
Langlands (1966)	Wethers (15)	Mean daily creatinine excretion (mg)=1.825 LW + 305	801-1466	1692 (1354-2039)	18.4* (1.23-48.6)
Langlands (1966)	Ewes (13)	Mean daily creatinine excretion (mg)=1.825LW + 232	728-1393	1619 (1281-1966)	17.6* (1.18-46.8)
Field et al. (1974)	Ewes (59)	Mean daily creatinine excretion (mg)=18.16LW + 93.14	852-1082	1473 (1137-1818)	16* (1.09-43)
		Urine volume	*Urine volume (L/day)*		
McGusty (2017)	Hoggets (20)	Urine output (ml/day) = (-1271.4 * creatinine concentration (mmol/L)) + 6289.9	3.0-7.0		5.0 (0 -10.0)
Jonker et al. (2021)	Ewes (155)	ln urine output (L/day) = 5.474 – 0.8718 ln creatinine concentration (mg/L) + 0.01663 LW	1.4-7.6		15.6 (1.7-41.6)

[1]Data calculated based on the six published equations

[2]Daily creatinine excretion was calculated using the live weight (LW) of the ewes (mixed age between 2 and 4 years old) and the creatinine concentration of the spot urine samples collected over two years in the authors' study (Maheswaran et al. 2022a).

*Calculated urine volume $= \frac{\text{urinary creatinine excretion (mg/kg daily LW)} \times \text{LW (kg)}}{\text{creatinine concentration (mg/deciliter)}}$ (David et al. 2015).

Table 2.18 Individual and total urine volume (L/day), urine-nitrogen (N) concentration (g N/L) and urinary N excretion (g N/day) of sheep grazing pasture measured at various stages using various techniques.

Reference	Type of sheep	Method of urine collection	Urine volume (L/day)	Urine-N concentration (g N/L)	Urinary N excretion (g N/day)
New Zealand					
Doak (1952)	Wethers	Outdoor study (Electrical counters)	2.9	8.68	
Ledgard et al. (2008)	Ram lambs	Indoor study (Metabolic crates)	2		
Hoogendoorn et al. (2010)	Ewe	Outdoor study (Airway obstruction)		5.2-9.6	
Jonker et al. (2015)	Wethers	Indoor study (Metabolic crates)			9.2-20.8
O'Connell et al. (2016)	Ewe lambs	Indoor study (Metabolic crates)	2.9-4.6		
Lindsay (2016)	Ewe lambs	Indoor study (Metabolic crates)	2.5-3.3	3.0-5.0	
McGusty (2017)	Ewe lambs	Indoor study (Metabolic crates)	1.7-3.8		
Al-Marashdeh et al. (2020)	Ram lambs	Outdoor study (Airway obstruction)			17.8-19.7
Australia					
Lynch et al. (1973)	Ewes	Outdoor study (Catheters)	1.75		
UK					
Field et al. (1974)	Ewe				6.0-22.2
Bristow et al. (1992)	Ewes	Outdoor study (Polythene buckets)		3.0-13.7	
Marsden et al. (2020)	Ewes	Partial outdoor study (Pens outside)	0.5-6.9	4.5-7.0	9.8-26.7
David et al. (2015)	Ewe	Indoor study (Metabolic crates)	2.2-2.7		

2.3.5 Impact of climate and pasture uptake on nitrate leaching

Environmental conditions (temperature, soil moisture, rainfall) and soil characteristics (soil texture, drainage class, soil temperature) are among the primary factors that determine the magnitude of N losses from animal excreta (Thomas et al. 1988). Soon after urination, hydrolyses converts urea-N to ammonium, and this is mostly completed within 24 hours of deposition. Some of this ammonium may be recovered by plants, lost as ammonia or immobilised in soil organic matter, but most is nitrified within about two weeks of deposition (Ball & Keeney 1983; Thomas et al. 1988).

The quantity of NO_3^- leached in drainage water depends on the NO_3^- content in the soil profile, the volume of drainage, and how drainage water moves through the pore volume (White & Magesan 1991). The total quantity of drainage is determined principally by climate. In temperate regions, drainage does not typically occur in summer and early autumn, due to the high evaporation rate and lower rainfall, and most leaching occurs over winter and early spring when the volume of drainage is greatest (Magesan et al. 1996). In general terms, for farms of comparable stocking rate and performance, the magnitude of NO_3^- leaching will increase with increasing rainfall (Monaghan et al. 2010). As drainage quantity increases, there is more water movement to depth, which will leach NO_3^- from the profile (Cameron et al. 2013). This is particularly so for coarse-textured soils where larger quantities of drainage will 'flush' the pore space more times than is the case for fine-textured soils (Cameron et al. 2013; Selbie et al. 2015).

In addition, the quantity of NO_3^- available for leaching is also affected by the interactions between season and climate, plant uptake, soil fertility and fertiliser application, stocking rate and urination traits (Di & Cameron 2002).

Pasture production in New Zealand peaks in spring due to good soil moisture levels, increasing soil temperatures and increasing day length (Matthews et al. 1999b) and is lower during late autumn and winter due to cooler temperatures (Matthews et al. 1999a). The rate at which any particular plant takes up NO_3^- depends on plant growth rate, the depth and vigour of its roots, soil temperature and general soil health (Di & Cameron 2007). When the soil temperature is lower than 7°C, the growth of forages slows, and the plant's absorption of N is significantly reduced. If pasture growth is slow and drainage events occur, the potential for NO_3^- leaching is greater (Di & Cameron 2007). Thomas et al. (1988) measured the fate of sheep urine-N (equivalent to 40-52 g N m^{-2}) applied to a grass sward in the United Kingdom (UK) under warm and dry, cool, and cool and wet environmental conditions. They found that when sheep urine was applied under different environmental conditions, the conversion of urine-N in the soil followed the same temporal pattern, and NO_3^- appeared about 14 days after application regardless of season (Thomas et al. 1988). However, the degree of nitrification varied significantly with environmental conditions and was greatest under cool conditions when up to 76% of the inorganic soil N was in the form of NO_3^- while, in all other environmental conditions, NO_3^- levels were relatively lower. Although NO_3^- was relatively low in some circumstances, it was still the major form of inorganic N (Thomas et al. 1988). Further, Thomas et al. (1988) observed that from all the applications, only 10 to 30% of the sheep urine-N (at a rate equivalent to 78 kg N/ha) was recovered in pastures. Similarly Ball & Keeney (1983) studied the effect of season on average apparent recovery of N in a ryegrass/clover pasture after the application of urine-N (at rates equivalent to 300 kg N/ha) under three different environmental conditions (cool-moist, warm-moist and warm-dry) in New Zealand. They reported that the average recovery rate of urinary-N was 30%, with higher (53%) and lower (10%) recovery rates under warm-moist and warm-dry conditions, respectively. A UK study by Cuttle & Bourne (1993) mentioned that the recovery percentage of N in the pastures was

very low (<0.1%) when urine was applied (equivalent to 300 kg N/ha) in the latter part of autumn. Cuttle & Bourne (1993) further reported that approximately 60% (18.6 g N m^{-2}) of applied N (30 g N m^{-2}) was leached under urine treated plots and, in contrast, NO_3^- leaching was negligible (0.7 g N m^{-2}) under untreated plots.

2.3.6 Impact of nitrogen inputs on nitrate leaching

Nitrate leaching under pastoral grazing conditions generally increases with increasing stocking rates and fertiliser rates (Barraclough et al. 1992; Garrett et al. 1992). The direct NO_3^- leaching of fertiliser-N may be low if the timing and rate of fertiliser application are well matched with plant demand (Cameron et al. 2013). However, additional fertiliser input increases dry matter production, N uptake and recycling in animal excreta, which leads to an increased risk of N loss to the environment (Hatch et al. 2007). A recent New Zealand study (Hoogendoorn et al. 2017) has shown that NO_3^- leaching from a sheep-grazed pasture steadily increased as fertiliser-N application increased above 100 kg N/ha (Figure 2.4). It is now prohibited in New Zealand to apply N fertiliser rates above 190 kg N/ha/year to grazed pasture (Ministry for the Environment 2020). For sheep pastures, most N application rates would be less than 100 kg N/ha/year, although, as noted, this rate is increasing on some intensively managed farms. However, the high levels of fertiliser-N, included in Figure 2.4, are now not likely to occur in the near future in New Zealand but can be used to compare with NO_3^- leaching in other temperate countries. In addition, with low to moderate N fertiliser use, urine patches will be the primary source of NO_3^- leaching. The Parfitt et al. (2009) study suggests that even at a fertiliser rate of 300 kg N/ha, that urine patches are still the main source of leaching.

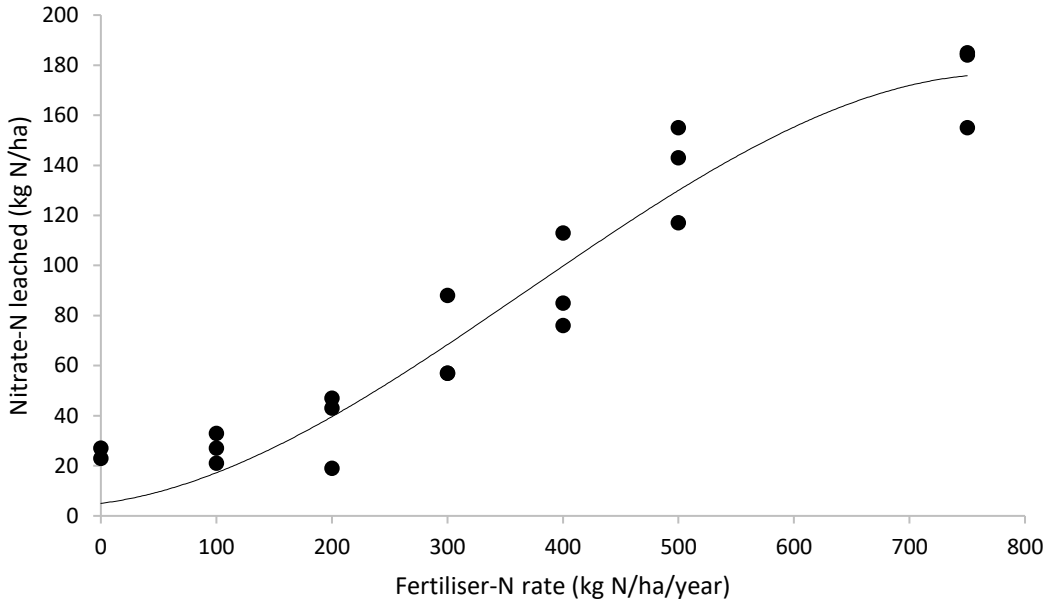

Figure 2.4 Nitrate leached from sheep grazed pasture systems under different nitrogen (N) fertiliser rates. Data adapted from Hoogendoorn et al. (2017).

Although there are numerous suggestions that fertiliser application onto urine-affected areas can lead to an increased risk of NO_3^- leaching and reduce fertiliser-N use efficiency (Cameron et al. 2013), few studies have quantitatively investigated the interactions between the concurrent application of fertiliser-N and urine-N deposition and NO_3^- leaching (Cuttle & Bourne 1993). As shown in Figure 2.5, there is a clear relationship between fertiliser-N rate and stocking rate and NO_3^- leaching. In a six-year study, which compared NO_3^- leaching from a perennial ryegrass pasture that received 150 to 200 kg/ha fertiliser-N to a ryegrass/white clover pasture that received no N fertiliser, there was a positive relationship between stocking rate (15 to 60 lambs/ha) and the quantity of NO_3^- leached from both treatments during the following winter (Figure 2.5). Cuttle & Scholefield (1995) explained that stocking rate and, therefore, the proportion of pasture affected by excreta were the main factors determining the magnitude of NO_3^- leaching. This finding matches the calculation made in Section 2.3.4 (i.e., a greater number of animals leads to more urine patches and more N loss). However, this depends on the seasonality as well: a lot of stock in spring may not have as much influence as a lot of stock in autumn.

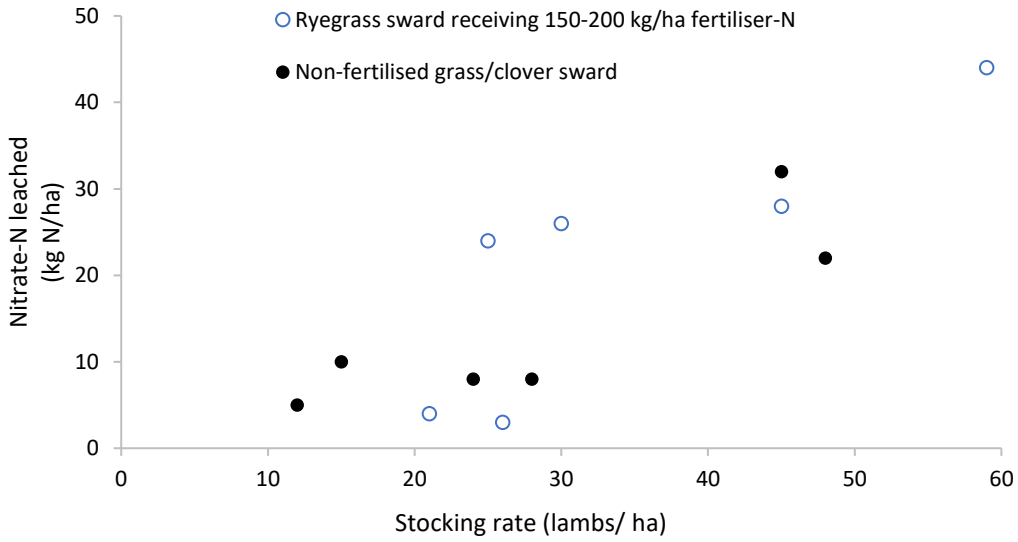

Figure 2.5 Relationship between quantities of annual nitrate-nitrogen (N) leached and mean stocking rates (post-weaning) for a ryegrass sward receiving 150 to 200 kg/ha fertiliser-N (open symbols) and an non-fertilised grass/clover sward (solid symbols): 1987-1993 (Cuttle et al. (1992); Adapted from Cuttle & Scholefield (1995).

It is important to note that, New Zealand pastures also receive N from biological N fixation by clovers (Ruz-Jerez et al. 1995). The amount of N fixed by legumes per year ranges depending on legume content: with pastures containing 3-40% of white clover fixing N at the rates of 20 to 250 kg N/ha/year (Crush et al. 1983; Goh et al. 1996; Kumar & Goh 2000). In a comparative study between sheep grazing a RGWC, and a perennial ryegrass monoculture without N fertiliser, Field et al. (1985) observed that the amount of NO_3^- leaching under the ryegrass/white clover sward was about 50% greater than that of a perennial ryegrass monoculture sward. A UK study of sheep grazed pasture compared NO_3^- leaching under perennial ryegrass/clover pastures (15% clover component) with a perennial ryegrass monoculture fertilised with approximately 200 kg N/ha/year (Cuttle et al. 1992). The monoculture pastures with N fertiliser had slightly higher NO_3^- leaching, which was attributed to the difference in stocking rates (Cuttle et al. 1992; Cuttle et al. 1998). Stocking rates were higher on the fertiliser treatment and, therefore, NO_3^- leaching rates were also higher on the fertiliser treatments. Cuttle et al. (1992) noted that if the stocking rates are similar, NO_3^- leaching rates are similar under non-fertilised ryegrass/clover pastures and highly N-fertilised (150 to 200 kg N/ha /year) ryegrass

monocultures. This suggests that NO_3^- leaching depends on the amount of N consumed by sheep rather than the source of N (fertiliser-N or urinary N or N fixed by legume). In support of this argument Cuttle et al. (1998) found that the NO_3^- leaching was similar in non-fertilised ryegrass/clover pastures treatment and highly N-fertilised treatment under similar rates of N inputs regardless of whether they contained clover or not.

2.3.7 Effect of other forages on reducing nitrate leaching

The use of alternative forage species to minimise or mitigate NO_3^- leaching from pastoral systems is gaining increasing interest and research attention. Forage species with high rumen utilisable protein (RUP), which diverts more of the dietary N away from urine (Woodward et al. 2012), or forages with high WSC to CP ratios (e.g. high sugar ryegrass) can help to reduce urine-N excretion (De Klein et al. 2019). A growing body of evidence indicates that, compared to RGWC swards, the grazing of plantain can help reduce the N concentration of urine, the amount of urea N excretion in urine and potentially the nitrification and denitrification rates in soils (Woodward et al. 2012; Box et al. 2016; Navarrete et al. 2016; Cheng et al. 2017; Gardiner et al. 2018; Bryant et al. 2019). The presence of the secondary compounds (Deaker 1994), smaller DM percentage and higher mineral load (sodium content) of plantain could potentially cause diuresis (Box et al. 2016). The diuretic effect has been shown to reduce urine-N concentration in individual urine patches by increasing the frequency of urination in dairy cows (Box et al. 2016; Cheng et al. 2017). Recent indoor feeding studies in New Zealand have also shown that intake of plantain causes greater water diuresis in sheep when compared with a diet of ryegrass (Lindsay 2016; O'Connell et al. 2016; McGusty 2017). Sheep that were fed plantain produced 0.8 to 1.9 L (7 to 19%) more urine than sheep that were fed ryegrass (Lindsay 2016; O'Connell et al. 2016; McGusty 2017). In addition, Lindsay (2016) noted that sheep that were fed plantain had a lower urine-N concentration (2.98 g N/L) compared to sheep fed ryegrass (4.97 g N/L). The interactions of the aforementioned factors likely explain the lower N

concentration, and higher urinary volume produced by the sheep that were fed plantain by Lindsay (2016). However, the effect of secondary compounds on diuresis was not measured in their study. Although several dairy studies have quantified the benefits of plantain to NO_3^- leaching (Box et al. 2017; Marshall et al. 2020), there is currently limited measurements of the effect of plantain on the amount of NO_3^- leached under sheep grazing.

Italian ryegrass shows greater N uptake during winter due to its faster growth rate which results in reduced NO_3^- leaching (Moir et al. 2012; Malcolm et al. 2014; Malcolm et al. 2015). Studies with dairy cattle have shown that the N uptake by Italian ryegrass in winter was 1.4 to 1.9 times greater than that of perennial ryegrass, and that the NO_3^- leaching was 20 to 50% lower than that of perennial ryegrass (Moir et al. 2013; Malcolm et al. 2014; Woods et al. 2016; Maxwell et al. 2019). However, there are few published data on the effects of Italian ryegrass grazing on N leaching under sheep.

2.3.8 Summary

This review highlights existing knowledge of the factors influencing NO_3^- leaching under sheep grazing pasture systems in New Zealand and other countries, and the factors influencing NO_3^- leaching rates. Further work is required on the following issues:

- Quantifying the excretion of N in sheep urine under grazing conditions.
- Determining the quantity of NO_3^- leaching under modern sheep farming systems.
- Comparisons of NO_3^- leaching under alternative pasture species grazed by sheep.
- Formulating accurate models to determine farm-level NO_3^- leaching under commercial sheep grazing systems.

2.4 Management options to reduce the nitrogen loss to the environment

Various mitigation strategies for reducing the amount of N leached from grazing land to the environment have been investigated. These include split application of N fertiliser, reducing stocking rates, using pasture species with greater winter activity to utilise more soil N for plant growth, applying nitrification inhibitors, decreasing urine inputs to pasture or the concentration of N in urine through reducing crude protein content in animal diets and reducing grazing durations (Smith et al. 1990; Moir et al. 2012; Malcolm et al. 2014). Most of these strategies have been assessed for use on dairy cattle farms and little is known about sheep grazing systems. Some of the practices used to reduce N leaching in dairy systems, for example, housing, are not practical or cost-effective options for sheep farming (Anastasiadis et al. 2012). The use of alternative forages such as plantain and winter active Italian ryegrass, could be beneficial to sheep grazing systems in that their use may result in reduction in N leaching. In addition to the information given in section 2.3.7, this section discusses in greater detail the effects of Italian ryegrass, plantain, and brassicas on N leaching from grazing systems.

Italian ryegrass

Italian ryegrass has the greatest potential for reducing N leaching losses compared to perennial ryegrass because it has faster growth during cooler seasons and can take up more N (Moir et al. 2012; Malcolm et al. 2015). Nitrogen uptake in Italian ryegrass across a range of N application rates has been well studied in New Zealand (Table 2.19). Moir et al. (2012) reported that N uptake of Italian ryegrass was 1.4-1.9 times greater than perennial ryegrass cultivars with the application of 300-700 kg N/ha cow urine (Table 2.19). Similarly, Malcolm et al. (2015) observed 20% greater N uptake in an Italian ryegrass/white clover sward over two winter seasons than a RGWC sward (Table 2.19). The authors suggested that the combination of rooting depth and seasonal growth activity of Italian ryegrass allowed for more N uptake compared with other pasture species (Malcolm et al. 2015; Maxwell et al. 2019).

Table 2.19 Nitrogen uptake, N leaching, drainage volume, and dry matter yield of Italian ryegrass (IR) compared to perennial ryegrass (RG).

Author	Pasture species	N loading rate (kg N/ha)	N leaching loss (kg N/ha)	N uptake (kg N/ ha)	Leachate nitrate (C mg NO_3^-N/L)	Cumulative drainage V (mm)	DM yield (t DM/ha)
New Zealand							
Moir et al. (2012)	RG	0	3-4	11-12		344-348	
	IR	0	3-4	9-10		343-344	
	RG	300	55-66	202-210		233-261	
	IR	300	6-8	261-275		171-181	
	RG	700	280-310	254-322		192-262	
	IR	700	130-134	444-484		72-122	
Malcolm et al. (2015)							
2010-2011	RG and WC	1000	300	473			14.3
	IR and WC	1000	230	675			16.9
2011-2012	RG and WC	1000	416	614			25.2
	IR and WC	1000	317	603			23.6
Woods et al. (2016)							
	RG and WC	700	205	811		275	24.3
	IR	700	133	629		200	20.0
Woods et al. (2018)	RG and WC	No urine		542			18.7
	RG and WC	Urine	112.7	744		332.5	25.0
	RG and WC	700	113.4	744			24.5
	IR, P and WC	No urine		561			20.6
	IR, P and WC	Urine	12.5	679		256.4	24.0
	IR, P and WC	700	61.8	656			23.6
Maxwell et al. (2019)	RG	700	214-267	331	146-228	308	12.5
	IR	700	143	463	119	165	17.2

In addition, Italian ryegrass produced less drainage than perennial ryegrass (Table 2.19). For example, Maxwell et al. (2019) reported 46–31% less cumulative drainage volume from Italian ryegrass than perennial ryegrass (Table 2.19). The ability of Italian ryegrass to produce greater DM yield and uptake more N, combined with lower drainage volume leading to the lower (24-55%; (Cuttle et al. 1998; Moir et al. 2012; Malcolm et al. 2015; Woods et al. 2018; Maxwell et al. 2019) N leaching loss compared to perennial ryegrass (Table 2.19). Although Italian ryegrass has been shown to reduce the N loss, the effectiveness of Italian ryegrass for mitigating N loss over the long term is limited due to its short-lived life cycle (Maxwell et al. 2019).

Plantain

Plantain in pure or mixed swards has the potential to reduce N leaching while maintaining similar herbage yields to standard RGWC (Woodward et al. 2012; Edwards et al. 2015; Box et al. 2016; Cheng et al. 2017). The presence of secondary compounds, minerals and lower DM percentage of plantain may lead to reduce N leaching in plantain (Box et al. 2016).

Secondary metabolites produced by plantain have the potential to reduce the urinary N concentration of the animals (Bowatte et al. 2018; Luo et al. 2018; Carlton et al. 2019). These metabolites include acteoside (syn. Verbascoside), aucubin, catalpol, iridoid and phenylpropanoid glycoside (Navarrete et al. 2016; Gardiner et al. 2018), which are potential sources of biological nitrification inhibition (BNI) (Dietz et al. 2013). Aucubin inhibits rumen fermentation while acteoside increases gas production by acting as an energy source for microbial growth (Navarrete et al. 2016). Thus, these compounds reduce N losses in both soils (De Klein et al. 2019) and rumen fluid (Navarrete et al. 2016). In an in vivo study, Navarrete et al. (2016) observed a reduced level of NH_3 production in rumen fluid. In several soil incubation studies, the application of plantain leaf materials, plantain extract or aucubin inhibited nitrification and N mineralisation (Dietz et al. 2013; Gardiner et al. 2018).

Plantain (7.36 g/kg DM) contains higher sodium content than ryegrass (5.36 g/kg DM; Lindsay (2016)). Sodium content of plantain influenced the urine production in the sheep by osmosis (McGusty 2017). In addition, lower DM content of the plantain may have led to increased water intake and subsequently, increased urine volume and leading to dilute N concentration (Cheng et al. 2017). The presence of secondary compounds (Deaker 1994), lower DM percentage and mineral load (sodium content) of plantain may cause diuresis (Box et al. 2016). Diuretics are substances that increase the production of urine, and its presence interferes with homeostatic mechanisms (i.e. anti-diuretic hormone (ADH) for maintaining body water balance) (Bryant et al. 2018). Common forms of diuresis include water diuresis, which is caused by excessive consumption of water (inhibiting ADH), and osmotic diuresis, which is caused by the presence of high concentrations of solutes restricting the reabsorption of water in the convoluted tubules of the renal system (Mathisen et al. 1981). In an indoor feeding study, O'Connell et al. (2016) investigated the effect of plantain on the diuresis effect on sheep and showed that plantain causes greater water diuresis in sheep compared with ryegrass. Sheep fed plantain produced 1.7 L (59%) more urine on the first day of the experiment compared with sheep fed ryegrass, and 0.5 L (17%) more on 5 subsequent collection days (Table 2.20; O'Connell et al. (2016)). Diuretic properties of plantain had the potential to reduce the urine N concentration in individual urine patches by increasing the frequency of urination (Box et al. 2016; O'Connell et al. 2016; Cheng et al. 2017; De Klein et al. 2019). McGusty (2017) investigated the effect of high plantain sodium content on urine production in sheep and suggested that higher sodium content of plantain was partially responsible for an increase in urine production, due to osmotic diuresis. Sheep fed plantain produced 2 L (54%) more urine compared with sheep fed ryegrass (McGusty 2017).

In sheep, lower urinary N concentration was observed from sheep fed on plantain compared with a diet containing ryegrass (Table 2.20). Similarly, in cattle studies, lower urinary N

concentration was reported in plantain fed dairy cows compared to cows on ryegrass (Table 2.20). For example, Box et al. (2017) found lower N concentration of urine excreted from 100% plantain than RGWC sward or a 50:50 plantain: RGWC sward (Table 2.20). In addition, lower levels of NH_3, urea, and creatinine were observed in the urine of cows grazed on the plantain than on perennial ryegrass (Box et al. 2017). Similarly, in a recent study, Navarrete et al. (2022) reported lower urinary N concentrations and reduced urinary N loads in cows grazing plantain compared with cows grazing RGWC (Table 2.20). In addition, cows grazing on a sward mixture containing 20% Italian ryegrass and 50% plantain showed a reduction in urinary-N excretion of 3.3-8.1% as compared to cows grazing on a standard RGWC sward (Table 2.20; Woods et al. (2018)).

Some studies have shown that the inclusion of condensed tannin (CT) in ruminant feed reduced the urinary N excretion by improving the N retention in the animal and by increasing the N partitioning into dung (Carulla et al. 2005; Misselbrook et al. 2005). Plantain contains CT (Ramirez-Restrepo & Barry 2005) and this could be one of the possible reasons for the reduction of urinary N excreted from cows fed on plantain (De Klein et al. 2019).

Table 2.20 Frequency, volume and components of urine excreted from animals fed on plantain compared to perennial ryegrass.

Author	Pasture species	Urination frequency (times/day)	Urine N (g N/L)	Urinary urea (mmol/L)	Urine ammonia (mmol/L)	Urinary allantoin (mmol/L)	Urinary creatinine (mmol/L)	Urine volume (L/day)	Urinary N excretion (g/day)
Totty et al. (2013)		(Dairy cow)							
	RG				0.66	5.26	1.2		438.3
	HSD				0.36	3.55	0.88		353.8
O'Connell et al. (2016)		(Ewe lamb)							
Day 1	RG							2.9	
	P							4.6	
Day 2-26	RG							3.0	
	P							3.5	
Lindsay (2016)	RG	(Sheep)		75.6	8.02		2.3		97.5
	P			116.6	16.52		3.6		114
McGusty (2017)	RG	(Ewe hogget)		120.4			1.2		
	P			59.6			2.1		
Box et al. (2017)		(Dairy cow)							
Late lactation	RGWC	18.0	5.4	144.3	1.98	5.78	1.67	46.5	251
	50% RG and 50% P	18.3	3.6	94.8	1.35	4.91	1.27	59.1	213
	P	20	2.4	61.5	0.97	3.05	0.88	73.8	177
Early lactation	RGWC	15.5	4.7	113.8	1.17	9.71	1.66	43.6	205
	50% RG and 50% P	11.5	3.4	71.8	1.30	8.81	1.49	33.6	114
	P	18.3	2.2	44.5	1.69	6.63	1.26	54.1	119
Cheng et al. (2017)	50% RG and 50% P	13.2	2.4	51		3.1	1.0		72
	P	9.6	2.8	67		3.6	1.2		80
Spring	RGWC	10.5	4·8	110		5·9	1·8		116
	50% G and 50% P	8.4	3.5	76		4.3	1.3		103
	P	8.7	2.9	65		4.1	1.4		87
Navarrete et al. (2022)		(Dairy cow)							
Spring	RGWC		3.2	43.9					
	P		2.9	27.5					
	PWC and RC		2.9	38.4					
Summer	RGWC		4.6	112.2					
	P		3.9	102					
	PWC and RC		4.4	119.5					
Autumn	RGWC		6.5	158					
	P		4.8	111					
	PWC and RC		6.0	151					

RGWC: Perennial ryegrass/ white clover; HSD: Mix of ryegrass, chicory, plantain, lotus, high-sugar ryegrass, and white clover; P: Plantain; WC mix: White clover mixture; RC: Red clover.

Table 2.21 Nitrogen leaching loss under brassica.

Author	Pasture species	N source	N loading rate (kg N/ha)	N leaching loss (kg N/ha)	Soil type
Shepherd et al. (2012)	Swedes/kale	Dairy cow (Sampling using porous cups)			Freely draining Taupo pumice
2008 (May-Oct)				132	
2009 (Jun-Oct)				173	
Monaghan et al. (2013)	Kale	Cow urine (Hydrologically isolated plots)			Pukemutu silt loam
2006			399	79.22	
2007			528	34.37	
2008			528	44.39	
Malcolm et al. (2016)	Fodder beet	Cow urine (Lysimeter study)			free-draining stony silt loam
2012			0	10.3	
			300	63.5	
2013			0	11.1	
			250	78.7	
Hanly et al. (2017)		Dairy cow (Mole and pipe drainage study)			Tokomaru silt loam
2006	Pasture			11.3	
	*ST			32.8	
2007	Pasture			6.4	
	*ST			14.7	
2008	Pasture			14.6	
	*ST			11.2	

*Summer turnips (ST) forage contribution to farm average 10%.

Brassica (Turnips or Swedes or Kale)

The number of studies directly related to N leaching by sheep grazing brassica forages are limited. Intensive grazing of brassica during winter may cause greater loss of N into drainage (Malcolm et al. 2016). Brassica forages can accumulate large amounts of N at maturity (Shepherd et al. 2012). For example, in the Central North Island, Lucci et al. (2013) reported that a yield of kale-swedes mixes of 10 t DM/ha contained 220 kg N/ha. In Canterbury, kale produced yields of 12–16 t DM/ha, with a CP content of 12.5%, equivalent to 240–300 kg N/ha (Edwards et al. 2015).

Grazing brassica crops returns large amounts of excreted N back to the soil during winter when the risk of leaching is high (Shepherd et al. 2012). Subsequently, it makes a large contribution to annual N leaching despite representing a relatively small area of the farming system (Monaghan et al. 2007). Shepherd et al. (2012) measured leaching of 130–170 kg N from cows grazing a kale-swedes mixed crop. In a lysimeter study, Malcolm et al. (2016) reported that 21–32% of urinary N applied mid-late June could be leached over winter from bare soil in Canterbury (Table 2.21). In a mole and pipe drainage water study, Hanly et al. (2017) reported that cultivation of 10% of a dairy farm area for summer turnips (forage crop) resulted in an annual increase of 6% in average whole-farm N losses in drainage water (turnips contributed 0.7 kg N/ha/year to the weighted farm average of 11.6 kg N/ha/year).

2.5 Summary

This review reveals that sheep production systems in New Zealand, particularly those on flat or undulating landscapes, have intensified over time to remain economically viable, with the greater use of N fertiliser and higher stocking rates than in the past. Interestingly, many New Zealand sheep farmers, like their dairy counterparts, have started to sow a range of alternative forages on their farmland to overcome seasonal pasture shortages to improve their income.

Forages such as brassica, which produce substantial amounts of forage during the winter, have also been widely used to carry more animals during the winter months. However, there is a need to conduct long-term studies to measure the production of these alternative forages across the entire farm on a year-round basis.

There is an increasing concern about quality, particularly N leaching under pastoral farming. However, most studies investigating N leaching under sheep grazing systems were done over 20 years ago, and there is limited recent information regarding N leaching under modern, pasture-based sheep production systems. Due to changes in sheep farming practices, N losses may be greater than previously reported in New Zealand, making it necessary to reassess N losses, especially under intensive sheep farming systems.

Several recent dairy studies have focused on reducing N leaching from dairy farms to reduce the potential adverse environmental impacts of dairy farming on water quality. In these studies, forages such as plantain and Italian ryegrass have been shown to reduce N leaching under dairy systems in comparison to traditional ryegrass due to their ability to reduce urinary N excretion or absorb more NO_3^- during the critical winter months. In contrast, there is a greater risk of N loss under brassica forages. However, there is currently limited information on the effect of alternative forages on the amount of N leached under sheep grazing systems.

This review indicates the need to quantify N leaching under current sheep farming practices. The research objectives of this study seek to fill the knowledge gaps identified above and to extend the understanding of the advantages of alternative forages to environmental protection and to increase sheep productivity. In short, the objectives of this study are to measure forage production, sheep performance, urinary N excretion and N losses to water through drainage.

CHAPTER 3: GENERAL METHODOLOGY

The experiment was undertaken over two and a half consecutive production years (from June 2019 to December 2021). The years will be referred to as "Year 1 (July to December 2019)", "Year 2 (January to December 2020)", and "Year 3 (January to December 2021)", respectively. The study began on 26 June 2019. Each year was divided into four seasons: winter (June to August), spring (September to December), summer (January to March) and autumn (April to June).

3.1 Experimental site and treatments

The study was carried out at Massey University's Keeble farm, 5 km southeast of Palmerston North, Manawatu, New Zealand (40°24'02.0"S 175°35'52.8"E). The site was located in a flat to easy-rolling landscape (c. 3% slope) on the Tokomaru silt loam soil, a Fragic Perch-gley Pallic Soil (Hewitt 1998). The design included four farmlets (total area of 13.28 ha, each approximately 3.32 ha), which had different combinations of forages (Figure 3.1; Tables 3.1 and 3.2). Each farmlet was stocked at 14 ewes/ha for the entirety of the experiment (46 ewes per farmlet) and was reflective of an intensive sheep-farming operation for this farm class and area (Beef + Lamb New Zealand Economic Service 2021).

Table 3.1 Specific and total area of forages included in each farmlet (treatment).

Treatment	Drainage plot area (ha)	Non-drainage plot area (ha)		Total area (ha)	Percentage (%) of area	
		Perennial ryegrass	Alternative forage		Perennial ryegrass	Alternative forage
RGWC	0.40	2.91	0.00	3.31	100	0
IRWC	0.40	2.49	0.41	3.30	75.5	24.5
PWC	0.40	2.51	0.41	3.32	75.6	24.4
Brassica	0.40	2.54	0.41	3.35	75.8	24.2

RGWC: Perennial ryegrass/white clover; IRWC: Italian ryegrass/white clover; PWC: Plantain/white clover; Brassica: turnips or swedes or kale in rotation.

The research site included an area with 20 drainage plots (five plots/treatment; Figure 3.2). Each plot was 40 m × 20 m and contained a hydrologically isolated mole and pipe drainage system. The drainage plots were established in the Autumn of 2019 (21 March 2019) with a roller drill and chain harrows and sown with the pasture species reflective of their treatment (Table 3.2). The plantain/white clover (PWC) and Italian ryegrass/white clover (IRWC) plots were over-sown in Autumn 2020 (9 April 2020). Further in Autumn 2021, the plantain plots were identified as having a significant dock (*Rumex obtusifolius*) infestation, and the IRWC had reduced tiller numbers and, therefore, these forages were sprayed with glyphosate (360 g/litre) at 6 L/ha and harmony 30g/ha. Plantain plots were resown on 15 March 2021, and IRWC plots were resown on 14 April 2021 using a direct drill; however, due to the high percentage of dock weeds identified in PWC plots at the seedling stage, PWC plots were resown again on 14 April 2021. Swedes and kale, which were sown in spring 2019 (23 December 2019) and spring 2020 (15 December 2020) were used as the brassica treatment in Years 2 and 3, respectively (Table 3.2).

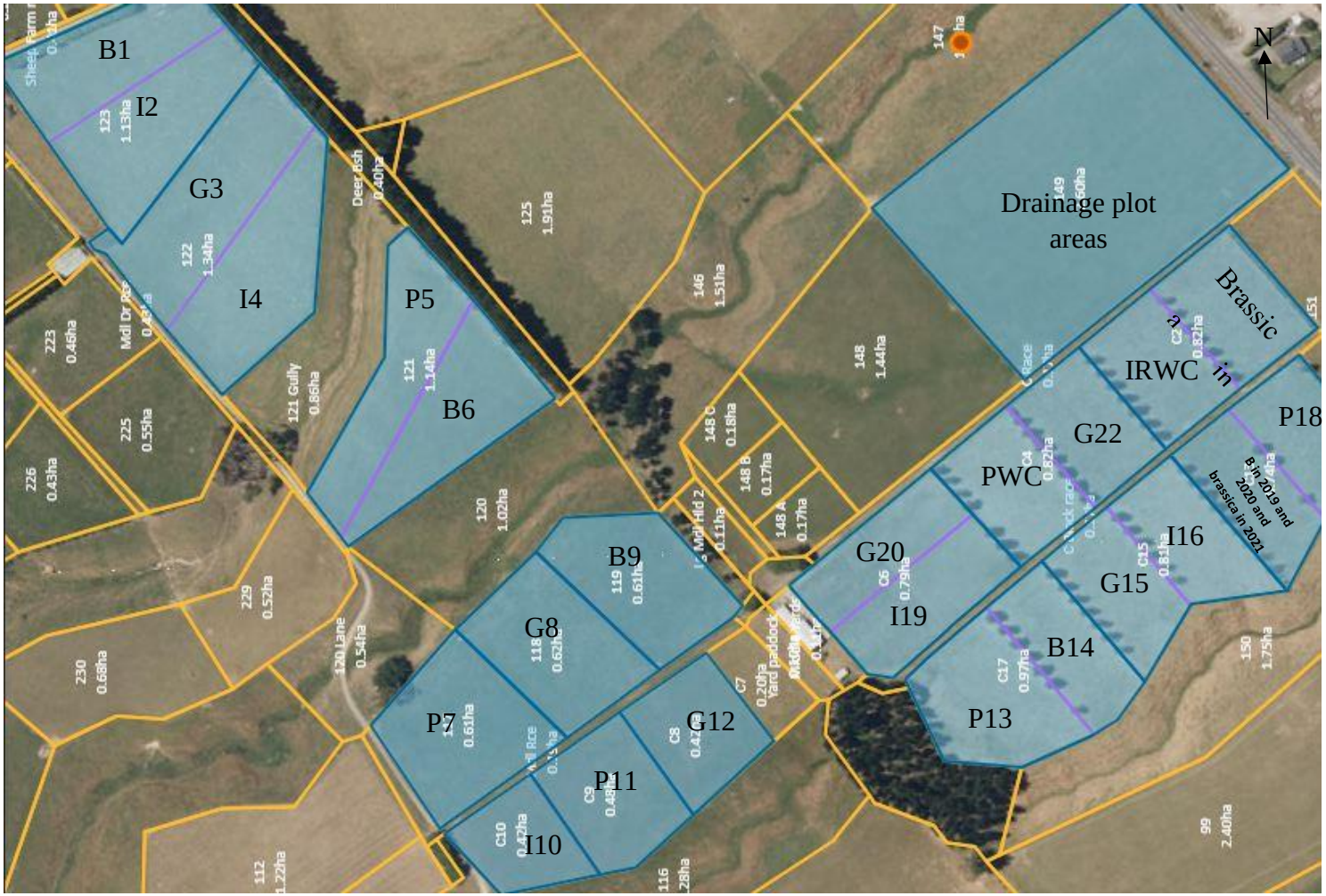

Figure 3.1 Layout of the farmlets (drainage plot and numbers in non-drainage plot area are paddock number; IRWC: Italian ryegrass/white clover; PWC: plantain/white clover; Brassica: turnips or swedes or kale in rotation; G: Perennial ryegrass/white clover (RGWC) for RGWC farmlet; I: RGWC for IRWC farmlet; P: RGWC for PWC farmlet; B: RGWC for brassica farmlet; area in ha also given).

Figure 3.2 Layout of the drainage plots (four treatments with five replicates; RGWC: Perennial ryegrass/white clover sward; IRWC: Italian ryegrass/white clover sward; PWC: Plantain/white clover sward; Brassica: turnips or swedes or kale in rotation; X: drainage water collection site).

Table 3.2 Description of forage treatment, cultivars, and seed rate.

Forage treatment	Species and cultivars	Sowing rate[1] (kg/ha)	Over-sowing rate[2] (kg/ha)	Resowing rate[3] (kg/ha)
RGWC	Perennial ryegrass: Platform AR37 WC: Quartz	19 6		
IRWC	Italian ryegrass: Lush AR37 WC: Quartz	24 6	32	24 6
PWC	Plantain: AgriTonic plantain WC: Quartz	8 6	9	8 6
Brassica Turnips* Swedes[+]	Turnips: Green Globe Swedes: Aparima Gold swedes	1.25 3		
Kale[⊗]	Kale: Kestrel	3.5		

RGWC: Perennial ryegrass/white clover; IRWC: Italian ryegrass/white clover; PWC: Plantain/white clover.
[*]Turnips were autumn sown in Year 1 as brassica treatment; [+]Swedes were spring sown in Year 2 as brassica treatment in place of turnips
[⊗] Kale was summer sown in Year 3 as brassica treatment in place of swedes; [1]Sowing date: 21st March 2019; [2] Over-sown in autumn 9th April 2020
[3]Resown in autumn 14th April 2021.

3.2 Soil fertility and fertiliser application

Soil samples were collected in May 2019 at depth of 7.5 cm. This included 15 soil cores combined to provide a bulked sample from each paddock within each farmlet and a bulked soil sample (15 soil cores per sample) from the entire drainage site. In Year 2, a bulked soil sample (15 soil cores per sample) was collected from four of the paddocks in the farmlets (one per treatment) in October 2020 (Table 3.3). Soil samples were analysed for pH, Olsen P (mg P/L soil), sulphate sulphur (S) (mg S/kg soil), potassium (K) (me/100 g), calcium (Ca) (me/100 g), magnesium (Mg) (me/100 g) and cation exchange capacity (CEC) (me/100 g) at Hill laboratory, Hamilton, New Zealand. These soil fertility data were used to determine the annual fertiliser application rates. The timing and rates of fertiliser applied during the study period are shown in Table 3.4.

Table 3.3 Soil test results for the drainage plot and non-drainage plot areas measured in Years 1 (July to December 2019) and 2 (January to December 2020).

Soil test	Ph	Olsen P (mg/L)	Sulphate S (mg/kg)	Potassium (me/100 g)	Calcium (me/100 g)	Magnesium (me/100 g)	Sodium (me/100 g)	CEC (me/100 g)
Year 1								
Non-drainage plots								
B1 and I2	5.6	19	37	0.18	7.5	0.71	0.12	14
G3 and I4	5.5	14	35	0.18	7.1	0.69	0.12	14
P5 and T6	5.5	12	17	0.20	6.3	0.85	0.15	14
P7	5.5	22	15	0.30	7.5	1.01	0.14	15
G8	5.5	18	16	0.26	5.4	0.88	0.14	12
B9	5.6	22	35	0.28	6.0	0.76	0.11	13
I10	5.4	13	18	0.22	4.7	0.91	0.14	13
P11	5.3	18	24	0.21	3.7	0.79	0.13	11
G12	5.2	16	25	0.20	4.1	0.81	0.15	13
I19 and G20	5.3	37	18	0.33	4.1	0.97	0.14	13
B17 and P18	5.4	30	17	0.36	5.4	1.09	0.17	15
I16 and G15	5.2	30	19	0.52	4.0	1.20	0.14	14
P13 and B14	5.4	34	15	0.33	4.0	0.95	0.13	13
Average of non-drainage plots	*5.4*	*22*	*22*	*0.30*	*5.3*	*0.90*	*0.10*	*13*
P22: PWC	5.3	36	28	0.34	4.9	0.88	0.13	13
G23: RGWC	5.4	32	14	0.43	5.5	1.17	0.15	15
I24: IRWC	5.5	27	20	0.33	4.4	1.02	0.13	12
B25 Brassica	5.3	32	14	0.27	3.6	0.95	0.15	12
Drainage plots	5.7	24	18	0.2	6.7	0.79	0.10	12
Year 2								
T1 and I2	5.5	23	12	0.14	9.2	0.67	0.08	20
P5 and T6	5.7	23	8.9	0.41	7.8	0.92	0.10	19
P7	5.7	31	9.6	0.32	7.8	1.04	0.11	19
I16	5.3	40	7.3	0.51	4.8	1.27	0.09	20
Average of farmlet	*5.6*	*25.7*	*10.2*	*0.3*	*8.3*	*0.9*	*0.1*	*19.3*

IRWC: Italian ryegrass/white clover; PWC: plantain/white clover; Brassica: turnips or swedes or kale in rotation G: Perennial ryegrass/white clover (RGWC) for RGWC treatment; I: RGWC for IRWC treatment; P: RGWC for PWC treatment; B: RGWC for brassica treatment: Numbers: Paddock number.

Table 3.4 Fertiliser applications (kg/ha) to the drainage plot and non-drainage plot areas in Years 1 (July to December 2019), 2 (January to December 2020), and 3 (January to December 2021).

Fertiliser	Year 1 (kg/ha)		Year 2 (kg/ha)		Year 3 (kg/ha)	
	Drainage plots	Non-drainage plots	Drainage plots	Non-drainage plots	Drainage plots	Non-drainage plots
Urea	30 - RGWC, IRWC, PWC (Oct) 30 - PWC (Dec)	30 - PN 22, 23 (Sep) 30 - PN 21(Dec)	30 - B (Mar) 30 - RGWC, IRWC, PWC (Oct)	30 - PN 24 (Apr)	30 - RGWC, IRWC (Mar) 50 - B (Mar)	30 - PN 1 to 16, PN18 to 24 (Mar)
Diammonium phosphate (DAP)	100 - RGWC, IRWC, PWC and B (Mar)	100 - PN 1 to 23 (Mar)				
30% Superphosphate (kg P/ha)		500 - PN 1 to 12 350 - PN 7 and 9 (Dec)				
Nitrophoska			200 - RGWC, IRWC, PWC (Apr)			
Balance Super 10 (N 9.5 K 0 P 10 S 0) (N26 P24 K 25 S 30)			150- RGWC, IRWC, PWC (Apr)	380 - PN 1 to 23 (Apr)		
Flexi N complete (N:P:K:S, 6.9, 6.4, 6.5, 7.8)					350 - RGWC, IRWC, PWC (May)	
Cropmaster 15	250 - B (Dec)	250 - PN24 (Dec)	250 - B (Dec)	250 - PN17 (Dec)		

RGWC: Perennial ryegrass/white clover; IRWC: Italian ryegrass/white clover; PWC: Plantain/white clover; B: Brassica (turnips or swedes or kale in rotation); PN: Perennial ryegrass/white clover paddock number in non-drainage plot areas.

3.3 Animal management

This study was carried out with the approval of the Massey University Animal Ethics Committee (MUAEC 19/20; MUAEC 21/14 amended 05/20).

3.3.1 Ewe management

Set-up for study

A total of 184 pregnant (bred in April 2019) mixed age (range of 2 to 4 years of age) Romney ewes were selected from "Keeble" flock at the start of the study (27 June 2019). The ewes were stratified by live weight (LW) and number of foetuses carried (single/twin/triplet) and allocated to the four forage treatment groups (n=46 at start) to ensure treatments did not differ in LW or number of foetuses carried. Ewes were identified using flexi tags and electronic tags and the treatments were sprayed a coloured mark (perennial ryegrass/white clover (RGWC): green; IRWC: orange; PWC: pink; brassica: yellow).

Romney rams (two rams per farmlet of 46 ewes) were introduced on 9 April 2020 in Year 2 and on 1 April 2021 in Year 3, for a period of 34 days. The rams were rotated among mobs on Day 17, to avoid the potential impact of ram performance on pregnancy rate. All ewes were pregnancy diagnosed (non-pregnant, single, twin- or triplet-bearing) using trans-abdominal ultrasound on 1 July 2020 and 24 June 2021. Ewes which were diagnosed as non-pregnant were replaced by pregnant ewes of similar stage of gestation (n=3 in Year 2, n=5 in Year 3). Ewes which died or those culled were replaced at the next main yarding event with ewes of a similar LW (n= 4 in Year 1, n=14 in Year 2 and, n=9 in Year 3).

3.3.2 Yearly animal management

Grazing management

Each mob of ewes was rotationally grazed within their farmlet. The duration of grazing and interval among paddocks was adjusted based on pasture growth rate and cover, which were

visually assessed every day and estimated with a rising plate meter fortnightly (see 3.4.1 for details of measurement). When the ewes grazed the drainage plots, each mob was separated into five smaller mobs to enable all five replicates to be grazed at the same time (approximately nine ewes/replicate). All replicates within a forage treatment were always grazed for the same number of days and ewes were moved when the minimum target post-grazing residual was reached in any replicate. Therefore, all four treatments were not necessarily grazed for the same duration (i.e., each treatment had its own target post-grazing residual). Where appropriate, treatments on the drainage plots were grazed at the same time to minimise the effect of differing weather conditions (Table 3.5).

Each farmlet was grazed according to best practice for each forage type. Topping of RGWC species was employed when it was necessary to maintain pasture quality in farmlets and drainage plots. Excess pasture from farmlets was also removed by grazing with additional animals or conserved as bales (Table 3.6). Shortages in feed supply were met with supplementation when required (summer and winter) with pasture (off-grazing) or hay (Table 3.6). The PWC sward was not grazed during late pregnancy or early lactation as the high calcium content in plantain can result in an increased occurrence of hypocalcemia and hypomagnesemia in ewes when grazed during late pregnancy or early lactation (Judson & Moorhead 2014). The brassica crop treatment was grazed continuously during winter, after the ewes were gradually adjusted to the diet using on-off grazing with increasing grazing durations of brassica each day (2, 4, 6, 8, hours on days one through four) before continuous grazing.

Table 3.5 Number of days sheep grazed each of the forage treatment (RGWC: perennial ryegrass/white clover; IRWC: Italian ryegrass/white clover; PWC: plantain/white clover; B: brassica (turnips/swedes/kale in rotations) on the drainage plot areas in Years 1 (July to December 2019), 2 (January to December 2020), and 3 (January to December 2021).

	Grazing month	Grazing date	Drainage plots
Year 1			
	August	2 August to 13 August (11 days)	RGWC, IRWC, B
	September	19 September to 23 September (4 days)	RGWC, IRWC, PWC
	October	24 October to 28 October (4 days)	PWC
		24 October to 30 October (6 days)	RGWC, IRWC
	November	25 November to 27 November (2 days)	PWC
		25 November to 28 November (3 days)	RGWC, IRWC
	December	16 December to 19 December (3 days)	RGWC, IRWC, PWC
Year 2			
	January	15 January to 23 January (8 days)	RGWC, IRWC, PWC
		17 March to 20 March (3 days)	PWC
	March	17 March to 21 March (4 days)	RGWC
		17 March to 22 March (5 days)	IRWC
	May	15 May to 18 May (3 days)	RGWC, IRWC
	June	12 June to 16 June (4 days)	RGWC, IRWC, PWC
	July	20 July to 26 July (6 days)	RGWC, IRWC
	August	20 July to 22 August (33 days)	B
		18 August to 22 August (4 days)	RGWC, IRWC
	October	15 October to 20 October (5 days)	PWC
		15 October to 22 October (7 days)	RGWC, IRWC
	November	9 November to 13 November (4 days)	RGWC, IRWC, PWC
	December	7 December to 9 December (2 days)	PWC
		7 December to 11 December (4 days)	RGWC, IRWC
Year 3			
	January	3 January to 8 January (5 days)	IRWC
		3 January to 9 January (6 days)	RGWC, PWC
	February	1 February to 5 February (4 days)	RGWC, IRWC, PWC
	March	6 March to 9 March (3 days)	RGWC
		26 March to 30 March (4 days)	RGWC
	May	5 May to 9 May (4 days)	RGWC
	June	15 June to 18 June (3 days)	IRWC
		15 June to 19 June (4 days)	RGWC
	July	31 May to 5 July (35 days)	B
		12 July to 15 July (3 days)	IRWC, PWC
		12 July to 16 July (4 days)	RGWC
	August	12 August to 14 August (2 days)	RGWC
		12 August to 16 August (4 days)	IRWC
	October	4 October to 12 October (7 days)	RGWC, IRWC, PWC
		22 October to 24 October (2 days)	RGWC, PWC
		22 October to 25 October (3 days)	IRWC

Table 3.6 Supplementary feed balance (kg DM/ha) of treatments (RGWC: Perennial ryegrass/white clover; IRWC: Italian ryegrass/white clover; PWC: plantain/white clover; brassica) in winter and spring of Year 1 (July to December 2019) and summer, late-autumn, winter and spring of Years 2 (January to December 2020) and 3 (January to December 2021). Data are presented as average.

	Conserved or grazed by other animals (kg DM/ha)				Grazing outside of the farmlet (kg DM/ha)				Balance (kg DM/ha)
	Summer	Late-autumn	Winter	Spring	Summer	Late-autumn	Winter	Spring	
Year 1									
RGWC	-	-	-	2394	-	-	-	-	2394
IRWC	-	-	3101	5027	-	-	-	-	8128
PWC	-	-	-	632	-	-	-	-	632
Brassica	-	-	2000	257	-	-	-	-	2257
Year 2									
RGWC	-	-	-	-	-	-	-	-	-
IRWC	-	-	-	-	-	-	-	-	-
PWC	-	-	-	-	-	-	2165	-	-2165
Brassica	-	-	2591*	-	-	644	-	1947	-
Year 3									
RGWC	2240	-	914	-	-	-	-	-	3154
IRWC	3871	-	981	-	569	773	-	-	3510
PWC	2415	-	1148	-	-	214	1485	-	1864
Brassica	496	680	1135	-	658	-	-	-	1653

*6,562 kg DM/ha of brassica was not utilised; Turnips, swedes and kale were used as a brassica treatment in Years 1, 2 and 3, respectively).

Approximately one week before the planned start of lambing (21 August 2019 in Year 1, 26 August 2020 in Year 2, 16 August 2021 in Year 3), pregnant ewes were set stocked on the RGWC area of each farmlet at a stocking rate of 18 ewes/ha until the end of lambing (34 days in Year 1, 46 days in Year 2, 47 days in Year 3). Ewes did not lamb on any of the drainage plots or alternative forage paddocks. Ewes and lambs within each treatment were then rotationally grazed as one mob until weaning (2 December 2019 in Year 1, 12 December 2020 in Year 2 and 1 December 2021 in Year 3).

Ewe live weight and BCS measurements

Ewes were weighed (10 times annually in Years 2 and 3) at approximately monthly intervals (prior to mating, at mid-pregnancy, late-pregnancy, docking, weaning). Ewe body condition score (BCS), scale 1-5 including half units (Jefferies 1961), was assessed at each weighing by a single operator.

3.3.3 Lamb management and measurements

Within 24 hours of birth, all lambs were weighed, ear tagged with electronic ear tags on the right ear and colour flex tag in the left ear, according to the forage treatment. In addition, the dam number, litter size and sex of the lamb were recorded. Dates of lambing began, docking and weaning for the Years 1, 2 and 3 are displayed in Table 3.7. Lambs were weighed at docking and weaning and then removed from the study.

Table 3.7 Lambing, docking and weaning dates for Years 1 (July to December 2019), 2 (January to December 2020), and 3 (January to December 2021).

	Lambing began	Docking	Weaning
Year 1	27 August 2019	8 October 2019	2 December 2019
Year 2	1 September 2020	12 October 2020	12 December 2020
Year 3	25 August 2021	1 October 2021	1 December 2021

Animal health management

Faecal worm egg counts (FEC) were measured (approximately at breeding, docking and weaning) from 10 randomly selected ewes per treatments to monitor the build-up of internal parasites. Drenching was given as required when FEC reached an average of 400 or more eggs/g per 10 random samples. No drenching was required during the study period.

Ewes were vaccinated for clostridial diseases using Ultravac® 5in1 (Zoetis, New Zealand) annually, approximately three weeks prior to lambing.

Ewes within brassica treatment were injected with 1.5 ml of stock iodine 2.5% (FIL, New Zealand) approximately one month before grazing brassica (Year 1) or not less than two months before lambing (Years 2 and 3) to prevent iodine deficiency in the ewe and growing foetus.

Ewes were shorn twice a year, in December and May.

3.4 Forage measurements

3.4.1 Forage dry matter mass measurement

The details of DM mass measurement of each forage in drainage and non-drainage plots areas are summarised in Table 3.8. Ryegrass/white clover herbage within each farmlet was measured fortnightly using a rising plate meter (RPM, Filip's Manual Folding Plate Meter, Development and Prototype Engineering,170 Railway Road, Palmerston North, New Zealand). At least 50 compressed pasture height measurements were recorded in each paddock, from within each farmlet. The formula to determine pasture mass (kg DM/ha) was "average compressed pasture height" (Readings from RPM) x 158 (the multiplier) + 200 (Sanderson et al. 2001).

In the drainage plots, three random quadrat cuts (0.1 m^2 each) were taken to ground level from each of the RGWC, IRWC and PWC plots before and after each grazing using an electric shearing handpiece (Frame 1993). Samples were oven-dried (60 to 70°C) for a minimum of 48 hours to a constant weight to estimate pre- and post-grazing herbage mass (kg DM/ha). Pre-and post-grazing herbage mass (kg DM/ha) of the brassica were determined by pulling all plants from a 1 m length of row with ten samples randomly chosen from each experimental plot (rows were 0.15 m apart, so each 1 m length of row represented 0.15 m^2). Samples were then washed to remove soil contamination and separated to leaves and bulbs and then oven-dried for a minimum of 48 hours (60 to 70°C) to a constant weight. The measurement method of brassica and PWC forages used for adaptation was also the same as explained in the drainage plots of each forage. But for the IRWC, RPM measurement was taken as described for RGWC in the farmlet (Table 3.8).

3.4.2 Botanical and chemical compositions of forages on drainage plots

Pre-grazing samples for botanical composition and nutritive value analysis were collected from each forage treatment once per season (excluding the brassica which was only collected prior to the start of winter grazing; Table 3.9). At each sampling date, one 'hand-plucked' grab sample (Frame, 1993) of approximately 300 g wet weight was collected in each replicate by walking in a random pattern within that plot (five samples per treatment). To determine the botanical composition, the sample was further subsampled (100 g wet sample) and sorted into sown species (perennial ryegrass and white clover for RGWC sward, Italian ryegrass and white clover for IRWC sward and plantain and white clover for PWC sward), weeds and dead material and then oven-dried at 70°C to a constant weight. The botanical composition was calculated on a dry weight basis. The remaining samples (200 g) were freeze-dried and then ground (Wiley mill; Arthur H. Thomas, Philadelphia, PA) to pass through a 1 mm sieve before analysis.

Table 3.8 Method of herbage dry matter mass measurement in drainage and non-drainage plots areas.

Farmlet	Drainage plots	Non-drainage plots	
		Perennial ryegrass	Alternative forages
RGWC	Cutting to ground levels (0.1 m^2 quadrats)	Rising plate meter	-
IRWC	Cutting to ground levels (0.1 m^2 quadrats)	Rising plate meter	Rising plate meter
PWC	Cutting to ground levels (0.1 m^2 quadrats)	Rising plate meter	Cutting to ground levels (0.1 m^2 quadrats)
Brassica	Pulling all plants (1 m length, 15 cm row width)	Rising plate meter	Pulling all plants (1 m length, 15 cm row width)

RGWC: Perennial Ryegrass/white clover; IRWC: Italian ryegrass/white clover; PWC: Plantain/white clover sward; Brassica: Turnips/ swedes/ kale in rotation: -: not applicable.

Table 3.9 Forage and animal (blood, urine, and faeces) sample collection dates in winter and spring of Year 1 (July to December 2019) and summer, late-autumn, winter and spring of Years 2 (January to December 2020), and 3 (January to December 2021).

Seasons	Forage samples collection date	Animal sample collection date
Year 1		
Winter	1 August 2019 (RGWC, IRWC and B)	16 August 2019 (RGWC, IRWC and B)
Spring	23 October 2019 (RGWC, IRWC and PWC)	01 November 2019 (RGWC, IRWC and PWC)
Year 2		
Summer	16 March 2020 (RGWC, IRWC and PWC)	20 March 2020 (RGWC, IRWC and PWC)
Late-Autumn	11 June 2020 (RGWC, IRWC and PWC)	16 June 2020 (RGWC, IRWC and PWC)
Winter	19 July 2020 (RGWC, IRWC and B)	22 July 2020 (RGWC, IRWC and B)
Spring	8 November 2020 (RGWC, IRWC and PWC)	10 November 2020 (RGWC, IRWC and PWC)
Year 3		
Summer	31 January 2021 (RGWC, IRWC and PWC)	5 February 2021 (RGWC, IRWC and PWC)
Late-Autumn	14 June 2021 (RGWC, IRWC and B)	17 June 2021 (RGWC, IRWC and B)
Winter	1 July 2021 (B) 11 July 2021 (RGWC, IRWC and PWC)	2 July 2021 (B) 14 July 2021 (RGWC, IRWC and PWC)
Spring	3 October 2021 (RGWC, IRWC and PWC)	5 October 2021 (RGWC, IRWC and PWC)

RGWC: Perennial ryegrass/white clover; IRWC: Italian ryegrass/white clover treatment; PWC: Plantain/white clover treatment; B: Brassica (turnips/ swedes/ kale in rotations).

Freeze-dried ground samples were scanned by near infra-red spectrophotometer (NIRS) to estimate metabolisable energy (ME), organic matter digestibility (OMD), ash, dry matter (DM), crude protein (CP), acid detergent fibre (ADF) and neutral detergent fibre (NDF), and starch and soluble sugars (SSS) (Corson et al. 1999). Calibrations for each component have been developed (Massey University Nutrition Laboratory, Palmerston North, New Zealand) using NIRS after scanning finely-ground pasture samples in the range of 400 to 2500 nm. A Bruker MPA NIRS (Ettlingen, Germany) was used to scan the samples, and the resulting NIRS spectra were analysed using Optic user software version 5.0. (Ettlingen, Germany). The resulting NIRS calibration typically had a correlation of 0.90 when compared to the wet chemistry results for each component.

At each sampling date, a grab sample of approximately 400 g (pulling 8-10 whole plants) brassica sample was collected from each plot by walking in a random pattern within that plot (five samples per treatment). Samples were then washed for removing soil contamination and separated into leaves and bulbs. Leaves and bulbs were freeze dried and then ground to pass

through a 1 mm sieve before analysis. Freeze-dried, ground samples were analysed for *in vitro* organic matter digestibility (OMD; Roughan & Holland (1977)) and CP (Dumas' procedure, AOAC method 968.06 using a Leco total combustion method, LECO Corporation, St. Joseph, MI, USA; AOAC, 2007), ADF and neutral detergent fibre NDF (AOAC method 973.18 using Tecator Fibretec System, AOAC, 2002.04), DM (AOAC 925.10, 930.16) and ash (Furnace 550°C, AOAC 942.05). Metabolisable energy content was calculated (MJ ME/kg DM = 0.16 × DOMD) using the organic matter digestibility (Roughan & Holland 1977).

A sub-sample of all forages was also wet acid digested with nitric and perchloric acid mixture to determine concentrations of minerals (calcium: Ca; sodium: Na; phosphorus: P) by Inductively Coupled Plasma-Optical Emission Spectroscopy (ICP-OES) using a Thermo Jarrell Ash IRIS instrument (Thermo Jarrell Ash Corporation, Franklin, MA) (Rubio et al. 2012). In addition, secondary compounds (catalpol, aucubin, and acteoside) of the pure plantain samples were also analysed using high-performance liquid chromatography (HPLC) by adopting procedures outlined by Tamura & Nishibe (2002).

CHAPTER 4: EFFECT OF ALTERNATIVE FORAGES ON BREEDING EWE AND THEIR LAMB PERFORMANCES

4.1 Abstract

A system-scale field study was conducted at Massey University's 'Keeble' farm over a period of two and a half years to evaluate the effects of integrating alternative forages into a perennial ryegrass/white clover system on the performance of breeding ewes and their lambs. The four treatments, or farmlets, included forages on the drainage and non-drainage plot areas. On three of the farmlets, 0.81 ha (0.4 ha of drainage plots and 0.41 ha of non-drainage plots) were sown (~24% of grazing area) in one of three alternative forages (i.e., plantain/white clover or Italian ryegrass/white clover or brassica). The remaining area (~2.5 ha) of each farmlet was in perennial ryegrass/white clover (RGWC) and was not drained. The fourth farmlet was entirely (100%) RGWC (including both the 0.4 ha drainage plots and 2.91 ha of non-drainage plots). Each farmlet was stocked with 14 ewes per hectare (46 ewes per treatment) over the entire study period. Each mob of ewes was rotationally grazed within their farmlet according to the best practices for each forage type. Dry matter (DM) masses of RGWC in non-drainage plot areas were measured fortnightly using a rising plate meter, whereas pre-and post-grazing DM masses of various alternative forages in drainage plot areas were measured using standard methods at each grazing event. The chemical and botanical compositions of forages in drainage plot areas were also measured once in each season. Results indicated that neither DM nor nutrients were restricted in any of the treatments; in addition, the forage treatments had no impact ($P>0.05$) on the performance traits of ewes or their lambs. The lack of positive or negative effects on animal performance suggests that farmers should not be concerned about adverse impacts on animal performance when the used alternative forages are incorporated into 24% of the land area, with the remaining area being RGWC, and lambs are sold at weaning.

Keywords: Brassica, dry matter intake, perennial ryegrass, Italian ryegrass, plantain.

4.2 Introduction

New Zealand sheep pastoral-based farming systems, which are grazed year-round, have been somewhat focused on intensification to increase profitability and to help meet the growing demand for food, especially protein (Morris & Kenyon 2014; Farrell et al. 2020). Thus, productivity on New Zealand sheep farms has increased over time in terms of both lambing percentage and lamb growth rates (Morris & Kenyon 2014). Profitability of a sheep system can be improved by increasing the total lamb carcass weight per ewe per year: by either increasing the number of weaned lambs and/or individual carcass weight (Morris & Kenyon 2014; Farrell et al. 2020). The quantity and quality of pastures grown drive the performance of the sheep flock; therefore it is important to ensure an adequate supply of high quality forage throughout the production year (Raeside et al. 2014).

Sheep farming systems in New Zealand are typically based on grass-clover mixes, with perennial ryegrass (*Lolium perenne* L.)/white clover (*Trifolium repens*) the predominant mix in the improved intensive farming pasture systems (Kemp et al. 1999; Cranston et al. 2017). However, these pasture-based systems can be characterised by fluctuations in both quantity (dry matter production) and quality (nutritive value) of available pasture across seasons (Kemp et al. 1999; Cranston et al. 2017). Pasture production generally peaks in spring (Matthews et al. 1999a) and can be limited during summer due to moisture stress and in winter due to cold temperature (Matthews et al. 1999a). Lambing is often timed to occur in late winter/early spring to ensure the high feed demand requirements of the ewes, and her lambs coincide with the spring pasture peak and, therefore, it can be challenging to ensure adequate pasture availability in late winter to meet the nutritional requirements of ewes (Young et al. 2011; Kenyon et al. 2014). Dry summer conditions can result in suboptimal herbage growth and quality, negatively influencing ewe body condition scores (BCS) and resulting reproductive performance (Young

et al. 2011; Kenyon et al. 2014) and the growth of weaned lambs (Somasiri et al. 2015). Therefore, farmers often utilise alternative forages to fill these feed gaps in summer and late winter/early spring to provide an adequate and high-quality feed. As a result, the use of alternative forages have gained increasing interest (Valentine & Kemp 2007; Kemp et al. 2010). New Zealand sheep farmers sow a varying percentage of their farm area in alternative forages to flatten the pasture supply curve and minimise times of seasonal pasture shortage (Kemp et al. 2010; Beef + lamb 2018; Stevens et al. 2021).

Forages such as plantain (Judson et al. 2009), Italian ryegrass (Rae et al. 1964) and brassica species (Drew et al. 1974; Barry & Drew 1978) or a mix of forages such as plantain and white clover (Judson et al. 2009; Raeside et al. 2014; Cranston et al. 2015a), or a brassica and Italian ryegrass (Stevens et al. 1994) have been shown to improve sheep performance. Plantain (*Plantago lanceolata* L.) is a summer active alternative perennial forage (Powell et al. 2007) and, when mixed with white clover, is generally associated with higher feeding value and metabolisable energy (ME) compared to a perennial ryegrass/white clover mix (Kemp et al. 2010). Grazing a plantain/clover mix compared to perennial ryegrass/clover mix can improve ewe and lamb weaning weights (Judson et al. 2009; Raeside et al. 2014; Cranston et al. 2015a) and post-weaning lamb growth rates (Somasiri 2014; Kenyon et al. 2017). Italian ryegrass (*Lolium multiflorum* Lam.) has a slightly higher nutritive value and greater winter growth rate than perennial ryegrass (Charlton & Stewart 1999; Burns et al. 2015). Consequently, Italian ryegrass can support greater live-weight gains of sheep during winter, compared to perennial ryegrass (Rae et al. 1964). Brassica species such as swedes (*Brassica napus* ssp. *napobrassica*) turnips (*Brassica rapa* ssp. *Rapa)* and kale (*Brassica oleracea* ssp. *Acephala*) sown in spring or early summer can also provide a large amount of feed in winter (Cranston et al. 2017). Brassicas typically yield 8-14 t/ha (Valentine & Kemp 2007) and can be energy-dense (ME

typically >12.0 MJ/kg DM; Litherland & Lambert (2007)). Therefore, a small area of these crops can be grazed with a high stocking rate during winter (Cranston et al. 2017).

While these studies indicate the benefits of these forages, their growth profile is somewhat limited to certain seasons/periods of the year. Therefore, in a pastoral system, only a proportion (10-20%) of the farm can be planted in alternative forages to achieve specific roles such as increasing ewe milk production and lamb survival (Kemp et al. 2010). However, a farmer needs to consider production on a year-round basis across the entire farm, so it is important to consider the impacts of these alternative forage species over the entire year. The objective of the present study was to compare the long-term (July 2019 to December 2021) effects of incorporating alternative forages (Italian ryegrass, plantain and/or a brassica) on approximately 24% of the overall grazing area, within a perennial ryegrass/white clover farmlet system, on the performance of breeding ewes and their lambs compared to a perennial ryegrass/white clover system.

4.3 Materials and methods

4.3.1 Brief summary

The experimental site and farmlet systems are fully described in Chapter 3. Briefly, the design of this study included four self-contained farmlets or "treatments;" each of approximately 3.32 ha, representing different forages (Table 4.1). Each treatment included forages from both the drainage and non-drainage plot areas (Figure 4.1 and Table 4.1). Briefly, three of the treatments had 0.81 ha (0.4 ha of drainage plots and 0.41 ha of non-drainage plots), and were sown in one of three alternative forages, i.e., plantain/white clover (PWC) or Italian ryegrass/white clover (IRWC) or brassica (turnips in 2019 or swedes in 2020 or kale in 2021). The remaining area (approximately 2.5 ha) of each treatment was in perennial ryegrass/white clover (RGWC) and was not drained. The fourth treatment was entirely RGWC (0.4 ha drainage plots and 2.91 ha

of non-drainage plots). Each treatment was stocked at 14 ewes/ha for the entirety of the experiment (46 ewes per treatment), which was reflective of an intensive sheep-farming operation for this farm class and area (Beef + Lamb New Zealand Economic Service 2022a).

The data collected over two and a half consecutive production years (from June 2019 to December 2021) are presented in this chapter. The years will be referred as "Year 1 (July to December 2019)", "Year 2 (January to December 2020)", and "Year 3 (January to December 2021)".

Table 4.1 Specific and total area of forages included in each treatment.

Treatment	Drainage plots[x] (ha)	Non-drainage plots (ha)		Total area (ha)
		Perennial ryegrass	Alternative forages[*]	
RGWC	0.40	2.91	0.00	3.31
IRWC	0.40	2.49	0.41	3.30
PWC	0.40	2.51	0.41	3.32
Brassica	0.40	2.54	0.41	3.35

RGWC: perennial ryegrass/white clover; IRWC: Italian ryegrass/white clover; PWC: plantain/white clover; brassica: turnips or swedes or kale in rotation over two and a half years. [x]Drainage area consisted of RGWC, IRWC, PWC and brassica for RGWC, IRWC, PWC and brassica treatments. [*]Alternative forage is the forage specific to the treatment.

Each mob of ewes was rotationally grazed within their treatment (see Chapter 3 for details of grazing management). Each treatment was grazed according to best practice for each forage type. Mowing of RGWC species was employed when it was necessary to maintain pasture quality by removing seedheads in the non-drainage plot areas. Excess forages from non-drainage plot areas were also removed by grazing with additional animals or conserved as baleage or hay. Shortages in feed supply were met with supplementation when required (summer and winter) with pasture (off-grazing) or hay. The PWC in drainage and non-drainage plot areas were not grazed during late pregnancy or early lactation as the high calcium content in plantain can increase hypocalcemia and hypomagnesemia in ewes grazing plantain at these times (Judson & Moorhead 2014). The brassica crops in drainage and non-drainage areas were

grazed continuously during winter after the ewes were gradually adjusted to the diet using the on-off grazing method, with increasing grazing durations of brassica in non-drainage plot areas each day (2, 4, 6, 8, hours on days one through to days four) before continuous grazing started on day five.

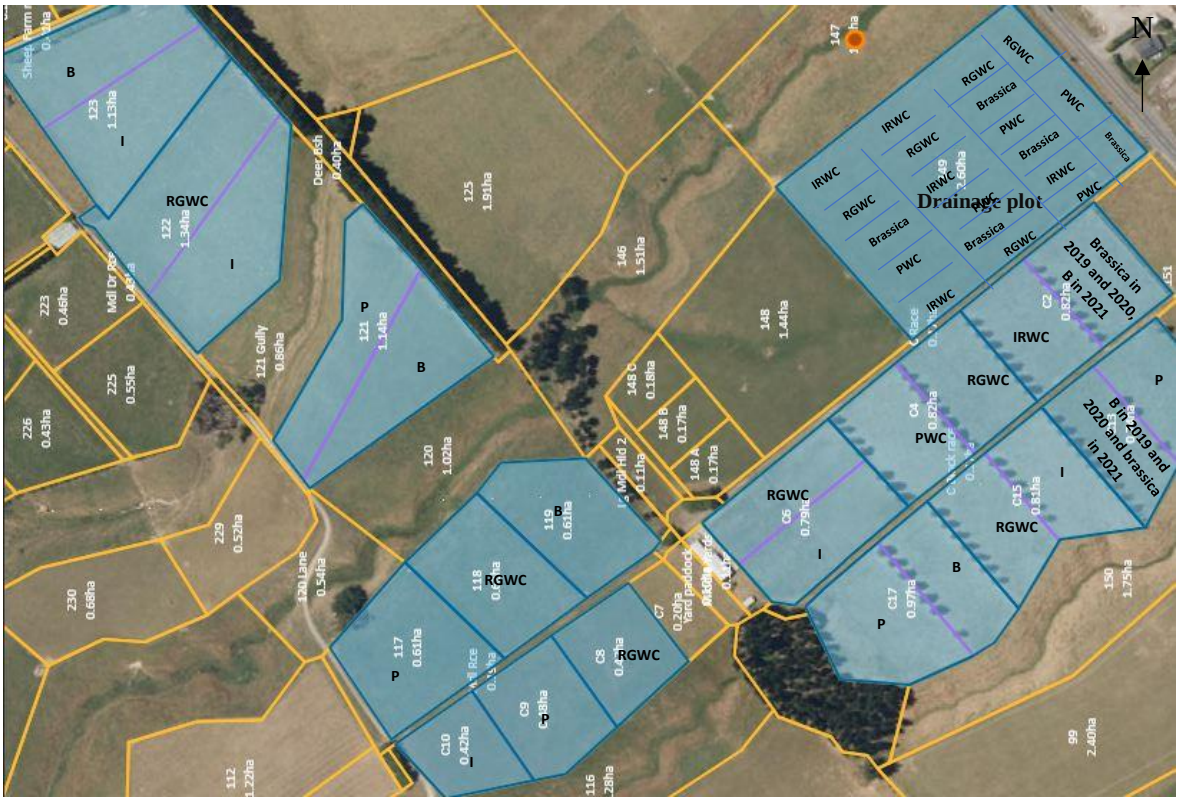

Figure 4.1 Layout of the treatments (RGWC: perennial ryegrass/white clover; IRWC: Italian ryegrass/white clover; PWC: plantain/white clover; brassica in drainage and non-drainage plot areas and I: RGWC in IRWC treatment; P: RGWC in PWC treatment; B: RGWC in brassica treatment in non-drainage plot areas).

4.3.2 Forage measurements

The details of DM mass measurement of each forage in drainage and non-drainage plot areas are summarised in Table 4.2. Dry matter masses (kg DM/ha) of RGWC and IRWC in non-drainage plot areas were measured fortnightly using a rising plate meter (see Chapter 3 for measurement details). Pre-and post-grazing herbage mass (kg DM/ha) of RGWC and IRWC in drainage plot areas and PWC in drainage and non-drainage plot areas were determined by

cutting to ground level using random quadrat (0.1 m^2 each, 20 quadrats per treatment). Pre-and post-grazing herbage mass (kg DM/ha) of the brassica in drainage and non-drainage plot areas were determined by pulling all plants from a 1 m length of the row (20 sticks per treatment). Chemical and botanical compositions of forages were measured in drainage plot areas only with samples collected from each plot (see Chapter 3 for details and sampling frequency).

Table 4.2 Method of herbage dry matter mass measurement in drainage and non-drainage plots.

Treatment	Drainage plots	Non-drainage plots	
		Perennial ryegrass	Alternative forages
RGWC	Cutting to ground level (0.1 m^2 quadrats)	RPM	n/a
IRWC	Cutting to ground level (0.1 m^2 quadrats)	RPM	RPM
PWC	Cutting to ground level (0.1 m^2 quadrats)	RPM	Cutting to ground levels (0.1 m^2 quadrats)
Brassica	Pulling plants (1 m length, 15 cm row width)	RPM	Pulling all plants (1 m length, 15 cm width)

RGWC: perennial ryegrass/white clover; IRWC: Italian ryegrass/white clover; PWC: plantain/white clover; RPM: Rising plate meter; n/a: not applicable.

4.3.3 Animal measurements

Ewes were weighed and body condition scored at breeding, pre-lambing, docking and weaning. Ewe body condition score (BCS) was assessed using a 1-5 scale including half units (Jefferies 1961), undertaken at each weighing by a single operator. Lambs were weighed at birth, docking and weaning (see Chapter 3 for details of animal management and measurements).

4.3.4 Statistical analysis

All data were tested for normality and outliers using Minitab 16 statistical software before analysis in SAS version 9.4 (Statistical Analysis System, version 9.4; SAS Institute Inc., Cary, NC, USA). Data were subjected to analysis of variance using the MIXED or GLM procedure in SAS version 9.4 (SAS Institute 2015).

Forage mass in non-drainage and drainage plot areas

Forage mass measured fortnightly in non-drainage plot areas were analysed using a model containing fixed effects of treatment (RGWC, IRWC, PWC or brassica), measurement time (day), Year (1, 2 and 3) and their three-way interaction. The model also included paddocks nested within treatment as a random effect.

Pre- and post-grazing DM mass of forages in the drainage plot areas were analysed using a model containing the fixed effects of treatment (RGWC, IRWC, PWC or brassica), measurement time (months), Year (1, 2 and 3) and their three-way interaction. The models also included plots (replicates) nested within treatment as a random effect.

Herbage quality and composition of forages in the drainage plot areas

Herbage CP, OMD, ME, NDF, ADF and ash content of forages in the drainage plot areas were analysed using a mixed model that contained the fixed effects of year, treatment, and the season of sample collection (winter and spring in Year 1, summer, late-autumn, winter and spring in Years 2 and 3) and their three-way interaction. The botanical composition of forages in the drainage plot areas were analysed using a mixed model that contained the fixed effects of year, treatment, the season of sample collection (spring in Year 1, summer, late-autumn, winter and spring in Years 1 and 2) and their three-way interaction. The models also included replicates nested within treatment as a random effect.

Ewe measures

The effect of different treatments on ewes' LW was analysed using a mixed model that contained the fixed effects of year, treatment, event (pre-mating, scanning, pre-lambing, docking and weaning) and their two-and three-way interactions. The model also contained the covariate of ewe LW at scanning each year. Ewe body condition score was analysed using a Poisson distribution and logit transformation using the GENMOD procedure in SAS. The

Poisson distribution was chosen as it is a nonlinear regression model for discrete outcomes. The model included the fixed effects of year, treatment and event and their two-and three-way interactions. The ewe LW and BCS models included ewe nested within treatment as a random effect.

Lamb measures

Lamb LW measured at docking (approximately 5 weeks after the start of lambing), and weaning (approximately 13 weeks after the start of lambing) were adjusted based on their actual average age (docking weight adjusted for 35 days and weaning weight adjusted for 90 days) using date of birth and measurement date data. The model of lamb LW contained the fixed effect of year, treatment, event (birth, docking and weaning), birth rank (i.e., number of siblings born *i.e*., single or twins or triplets), and the two-way interaction of treatment and year, treatment and event and their three-way interaction of treatment, year and event. Ewe LW at pre-lambing was included as a covariate in the model. The model also included lamb nested within treatment as a random effect.

Lamb liveweight gains (LWG) from birth to docking, docking to weaning and birth to weaning were calculated using their LW-adjusted for actual average age. The models of lamb LWG from birth to docking and birth to weaning included the fixed effects of year, treatment, birth rank, rearing rank (i.e., number of lambs reared to weaning – single or twin or triplet), and two-way interaction of treatment and year. The model of lamb LWG from docking to weaning contained the fixed effect of treatment, year, rearing rank, and two-way interaction of treatment and year. Ewe LW at pre-lambing was included as a covariate in the models of LWG from birth to weaning and docking to weaning but not included in the LWG from birth to docking model as it was not significant ($P>0.05$). The models also included lamb nested within treatment as a random effect.

Number of foetuses per ewe put to the ram, number of lambs born per ewe put to the ram and number of lambs weaned per ewe put to the ram were analysed using mixed models that contained the fixed effects of treatment, year and two-way interaction of treatment and year. The models included animal nested within treatment as a random effect. Ewe LW at pre-lambing was not included as a covariate in the models as it was not significant ($P>0.05$).

Lamb survival to weaning (i.e., number of lambs weaned alive per number of lambs born) was analysed as a binominal trait after logit transformation using SAS for categorical data modelling (GENMOD). Main effects of treatment, year, and their interactions were tested. Data are also presented as back-transformed percentages.

The total weight of lamb weaned per ewe put to the ram was calculated as the combined LW of all lambs alive at weaning (actual LW approximately 90 days after mid-point of lambing) plus a nominal value of 0 kg for lambs that had died. The total weight of lamb weaned per ewe was analysed using a mixed model that included the fixed effects of treatment, year, birth rank, rearing rank and two-way interactions of treatment and year. The model included ewe nested within treatment as a random effect. Ewe LW at pre-lambing was not included as a covariate in the model as it was not significant ($P>0.05$).

4.4 Results

Table 4.3 Ten-year average (2008-2018) monthly rainfall (mm) and mean air temperatures (°C) from NIWA/Ag Research, Palmerston North meteorological station (approximately 2 km from study site), and actual monthly rainfall and mean air temperatures during the trial period (Year 1: July to December 2019; Year 2: January to December 2020; Year 3: January to December 2021).

	Month											
	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
Rainfall (mm)												
10-year average	62.4	68.9	57.6	95.0	98.7	89.9	78.2	80.6	92.9	85.2	79.2	79.2
Year 1	-	-	-	-	-	111.4	115.4	117	60.6	97.6	78.8	124.2
Year 2	31.6	34.8	48.8	65.6	66.4	64.4	65	40	137.6	63	107.4	101.8
Year 3	85.8	24.2	102.6	59	45	105.8	43.8	115.4	120.8	76.4	76.8	228.2
Mean air temperature (°C)												
10-year average	18.3	18.6	16.7	14.4	11.6	9.6	8.7	9.9	11.1	12.6	14.4	17.2
Year 1	-	-	-	-	-	8.8	10	9.3	10.9	12.3	15.4	16.9
Year 2	17.8	19.4	16.4	14	11.9	11.7	9.2	10.3	11.1	13.7	15.2	16.4
Year 3	17.8	18.2	16.6	13.9	11.5	9.3	8.7	9.4	11	12.5	14.2	16.4

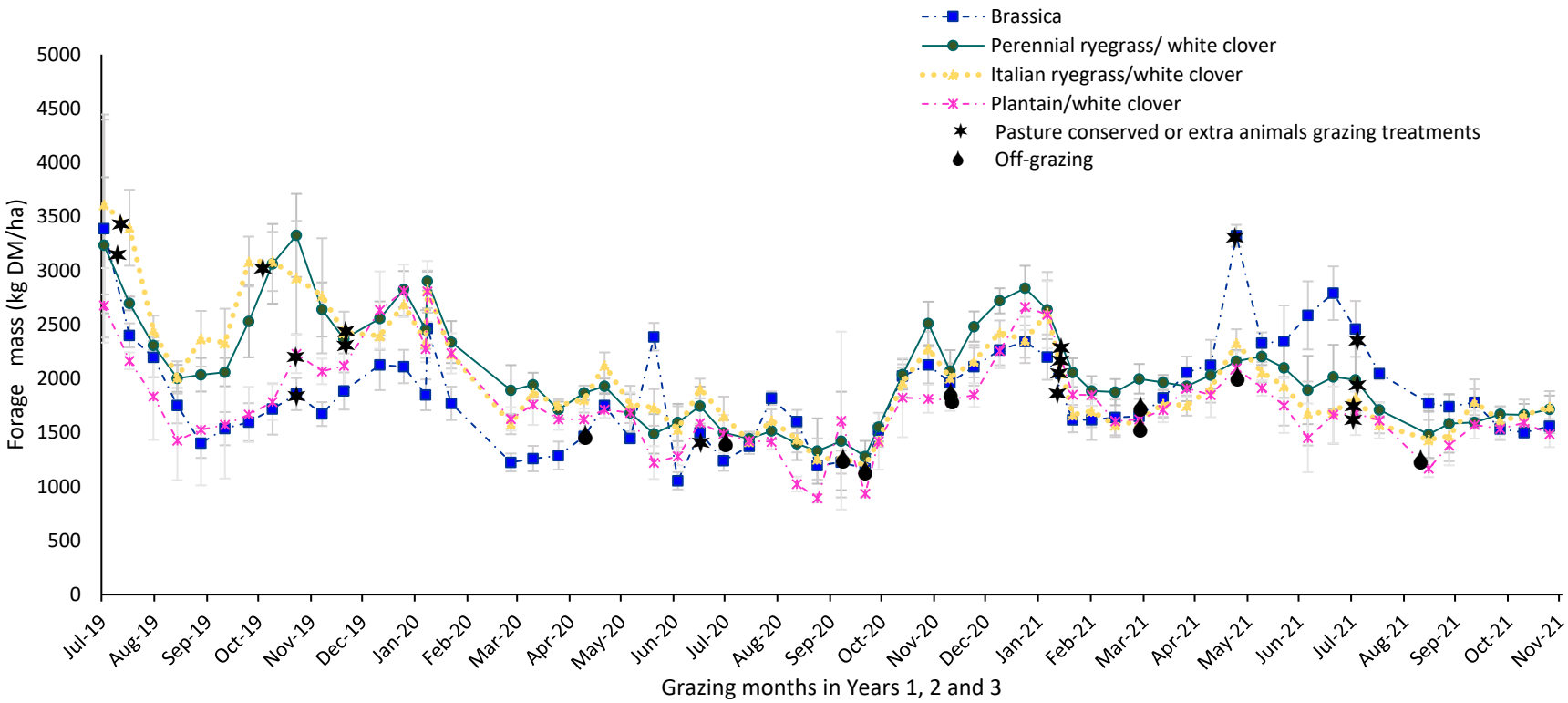

Figure 4.2 Mean forage mass of treatments (approximately 2.9 ha) in non-drainage plot areas (within non-drainage plots, each treatment included the following areas: perennial ryegrass/white clover (RGWC): 2.91 ha of RGWC; Italian ryegrass/white clover (IRWC): 2.49 ha of RGWC plus 0.41 ha of IRWC; plantain/white clover (PWC): 2.51 ha of the area of RGWC plus 0.41 ha of PWC; brassica: 2.54 ha of RGWC plus 0.41 ha of brassica) over three years (Year 1: July to December 2019; Year 2: January to December 2020; Year 3: January to December 2021). Decision process involved that resulted in extra animals grazing the treatment area (✶) or off grazing (i.e., grazing sheep outside of the treatment area; ⧫); Values are least-squares means ± SE bars.

Table 4.4 Supplementary feed balance (kg DM/ha) for treatments (RGWC: Perennial ryegrass/white clover; IRWC: Italian ryegrass/white clover; PWC: plantain/white clover; brassica) in winter and spring of Year 1 (July to December 2019) and summer, autumn, winter and spring of Years 2 (January to December 2020), and 3 (January to December 2021). Values are presented as average.

	Conserved or grazed by other animals (kg DM/ha)				Grazing outside of the treatment area (kg DM/ha)				Overall yearly balance
	Summer	Autumn	Winter	Spring	Summer	Autumn	Winter	Spring	(kg DM/ha)
Year 1									
RGWC	x	x		2394	x	x	x	x	2394
IRWC	x	x	3101	5027	x	x	x	x	8128
PWC	x	x		632	x	x	x	x	632
Brassica	x	x	2000	257	x	x	x	x	2257
Year 2									
RGWC	x	x	x	x	x	x	x	x	x
IRWC	x	x	x	x	x	x	x	x	x
PWC	x	x	x	x	x	x	2165	x	-2165
Brassica	x	x	2591[1]	x	x	644	x	1947	x
Year 3									
RGWC	2240	x	914	x	x	x	x	x	3154
IRWC	3871	x	981	x	569	773	x	x	3510
PWC	2415	x	1148	x	x	214	1485	x	1864
Brassica	496	680	1135	x	658	x	x	x	1653

x: no supplementation or grazing outside of the treatment area or overall yearly balance. [1]6,562 kg DM of brassica was not utilised in winter of Year 2: Perennial ryegrass/white clover (RGWC), Italian ryegrass/WC (IRWC), plantain/WC (PWC), and brassica (turnips in Year 1, swedes in Year 2 and kale in Year 3).

4.4.1 Rainfall and Temperature

Actual monthly averages for rainfall and temperature over the study period are shown in Table 4.3, in addition to 10-year (2008-2018) mean values. In most months, especially during winter and spring, there was substantial year to year variation in rainfall. Compared to other years, Year 2 had less rainfall in June, July, and August.

4.4.2 Forage mass

Forage mass in non-drainage plot areas (approximately 2.9 ha for each treatment: 88% of the total area of each treatment)

The pattern of forage mass in non-drainage plot areas of each treatment over the study period is shown in Figure 4.2 (see Appendix 2 for numerical values). Mean forage mass varied little among treatments, but all treatments showed similar trends of forage mass over the period. The average forage covers of all treatments were above 1200 kg DM/ha in the non-drainage plot areas during the study with the exception of the PWC treatment, which was lower than 1200 kg DM/ha in September and early October of Year 2.

In winter (July 2019) of Year 1, IRWC and brassica had excess forage cover, resulting in extra animals (it refers to animals other than those in the trial) grazing those treatments (Figure 4.2 and Table 4.4). In the spring of Year 1, all treatments had excess forage yield, resulting in extra animal grazing in those treatments (IRWC, PWC and brassica in October 2019) or conserved as baleage (RGWC and IRWC in December 2019). In November or December of 2020, surplus forages from all treatments were not conserved (due to a malfunction in the machinery), therefore excess forages were carried forward to Year 3 which resulted in extra animals grazing in January 2021 (summer). In Year 3, all treatments had excess forages in winter (July 2021) and had been grazed by extra animals. In the late-autumn (May 2021) of Year 3, brassica paddocks were grazed by the extra animals (Figure 4.2 and Table 4.4).

In winter (July 2020) of Year 2, the ewes in PWC treatment grazed outside of their treatment area due to the low forage covers (Figure 4.2 and Table 4.4), while ewes on brassica treatment grazed outside of their area in late-autumn (April 2020) and spring (October and December 2020). Ewes in PWC grazed outside of the treatment area in late-autumn (May 2021) and winter (August 2021) of Year 3, while ewes in the IRWC and brassica treatments grazed outside of their treatment areas in the summer (March 2021).

Forage mass in drainage plot areas

The pre-and post-grazing DM mass of treatments in drainage plot areas fluctuated from month to month, with the higher ($P>0.05$) DM harvested at the beginning of the experiment for RGWC, IRWC and PWC (Figure 4.3). There were no differences ($P>0.05$) in the total DM harvested among RGWC, IRWC and PWC in Years 1 and 2, but in Year 3, greater ($P<0.05$) total DM yield was harvested from RGWC compared to IRWC and PWC (Table 4.5). Across the study period, the lowest ($P<0.05$) pre-grazing (1380, 923 and 1039 kg DM/ha) and post-grazing mass (483, 447 and 650 kg DM/ha) were observed in Year 3 for RGWC, IRWC and PWC, respectively (Figure 4.3). The total DM harvested from IRWC and PWC in Year 3 was one third ($P<0.05$) of that in Years 1 and 2 (Table 4.5).

The three-year mean indicated that total DM harvested differed significantly among treatments, with higher ($P<0.05$) DM harvested in RGWC and IRWC and lower DM harvested in brassica (Table 4.5). Likewise, averaged across the four treatments, total DM harvested in Years 1 (9180 kg DM/ha/year) and 2 (9473 kg DM/ha/year) were twice ($P<0.05$) that in Year 3 (4364 kg DM/ha/year). Compared to the RGWC, the PWC had fewer grazing days over the trial period (Table 4.5). Perennial ryegrass/WC and IRWC had nearly the same number of grazing days in Years 1 and 2, but IRWC had fewer grazing days in Year 3. It should be noted that, in Year 2

of the study, ewes began grazing the brassica in drainage areas in the latter part of the winter and had not completely utilised the crop before set-stocking for lambing.

Quality of the forages measured in drainage plot areas (approximately 0.4 ha for each treatment: 12% of total area of each treatment)

Forage chemical composition showed a significant ($P<0.05$) three-way interaction between treatment, season and year (Tables 4.6 and 4.7; Appendix 3). The CP concentrations of RGWC and IRWC did not differ ($P>0.05$) within seasons for a given year except in the winter of Year 1 and the late-autumn and spring of Year 3. Italian ryegrass/WC had higher ($P<0.05$) CP concentration in the late-autumn of Year 3, compared to other treatments and or seasons. In the summer of Years 2 and 3, PWC had greater ($P<0.05$) CP concentrations compared to RGWC. Crude protein content in brassica leaves were greater ($P<0.05$) than in either the bulb or stem in each season it was measured. Ryegrass/WC, IRWC and PWC had lower ($P<0.05$) CP and ME in the summer of Year 2, compared to all other seasons. Compared to RGWC and IRWC, brassica had lower ($P<0.05$) NDF concentrations in all years.

Botanical composition of forages measured in drainage plot areas (approximately 0.4 ha for each treatment: 12% of total area of each treatment)

Perennial ryegrass/WC and IRWC had lower ($P>0.05$) crop (i.e., green forage) percentages in the summer of Year 2 compared to the crop percentages of RGWC and IRWC in other seasons (Figure 4.4). Overall, compared with other seasons of the experiment, PWC had the highest ($P<0.05$) percentage of weed in the spring of Year 3, which resulted in the lowest ($P>0.05$) crop percentage spring of Year 3. Overall, the WC percentages of RGWC and IRWC were less than 4%, while PWC had a greater ($P<0.05$) WC percentage in the spring of Year 2 and summer of Year 3.

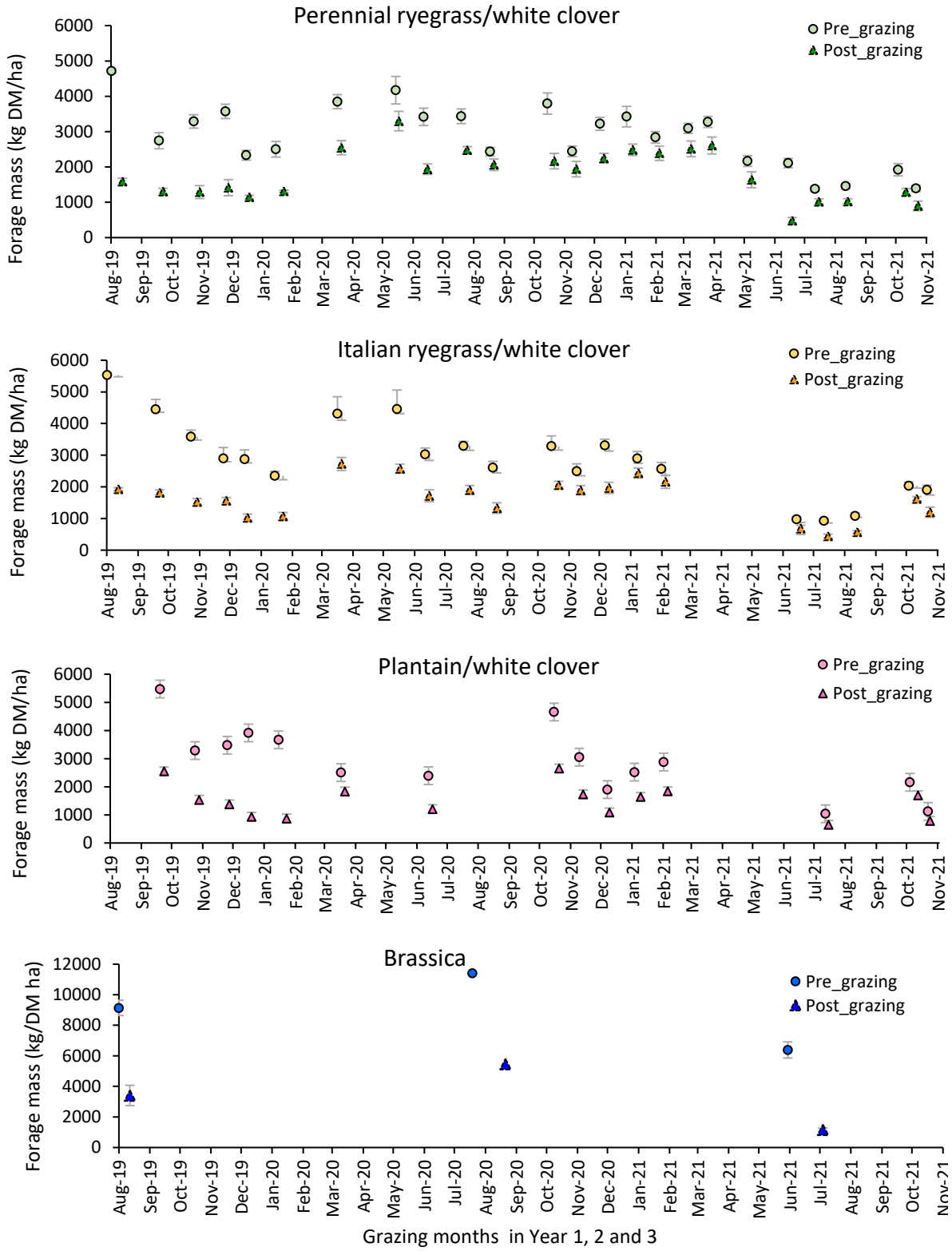

Figure 4.3 Pre- and post-grazing forage mass (kg DM/ha) in drainage plot areas over three years (Year 1: July to December 2019; Year 2: January to December 2020; Year 3: January to December 2021). Values are least-squares means ± SE.

Table 4.5 Total DM harvested (kg DM/ha), number of grazing days on the drainage plot areas over three years (Year 1: July to December 2019; Year 2: January to December 2020; Year 3: January to December 2021). Values are least-squares means ± SEM.

	RGWC	IRWC	PWC	Brassica
Total DM harvested per year [1]				
Year 1	9909^{bc}±702	11479^{ab}±702	9753^{bc}±702	5815^{d}±1551
Year 2	10438^{abc}±702	12345^{a}±702	9162^{c}±702	5944^{d}±702
Year 3	5822^{d}±702	3203^{e}±702	2929^{e}±702	5501^{d}±702
3-year mean	8723^{x}±491	9009^{x}±491	7281^{y}±491	5754^{z}±674
Number of grazing days per year[2]				
Year 1	27	27	13	11
Year 2	46	47	26	33
Year 3	40	29	22	35
3-year average	38	34	20	26

[a-d]Superscripts with different letters indicate that means are significantly different among treatments (P<0.05). [1]Total DM harvested per year (kg DM/ha/year) was calculated as the sum of differences between post- and pre-grazing per grazing event. [2]The number of grazing days/ewes was calculated as the sum of the grazing days the ewes grazed on drainage plot areas during each grazing event.

Table 4.6 P-value of main effects of treatment (T), season (S) and year (Y) and their three-way interaction (T*Y*S) on forage chemical composition (CP: crude protein; ash; NDF: neutral detergent fibre; ADF: acid detergent fibre; OMD: organic matter digestibility ME: metabolisable energy), and botanical composition (crop, dead, white clover and weed) determined in drainage plot areas in winter and spring of Year 1 (July to December 2019) and summer, late-autumn, winter and spring of Years 2 (January to December 2020), and 3 (January to December 2021).

	Treatment (T)	Season (S)	Year (Y)	T*S*Y
CP (g kg/DM)	<.0001	<.0001	0.0024	<.0001
Ash	<.0001	<.0001	<.0001	<.0001
NDF (g kg/DM)	<.0001	<.0001	<.0001	<.0001
ADF (g kg/DM)	<.0001	<.0001	<.0001	<.0001
OMD (g kg/DM)	<.0001	<.0001	<.0001	<.0001
ME (MJ/kg)	<.0001	<.0001	<.0001	<.0001
Crop (% of total DM)	<.0001	<.0001	<.0001	<.0001
Dead materials (% of total DM)	<.0001	<.0001	<.0001	<.0001
White clover (% of total DM)	<.0001	0.0003	<.0001	<.0001
Weed (% of total DM)	<.0001	<.0001	<.0001	<.0001

Table 4.7 Crude protein (CP; g kg/DM), neutral detergent fibre (NDF; g kg/DM), and metabolisable energy (ME; MJ kg/DM) content of RGWC (perennial ryegrass/white clover), IRWC (Italian ryegrass/WC), PWC (plantain/WC), BL (brassica leaf) and BB (bulb or stem) measured in drainage plot areas in winter and spring of Year 1 (July to December 2019) and summer, late-autumn, winter and spring of Years 2 (January to December 2020), and 3 (January to December 2021).Values are least-squares means ± SEM.

		Year 1		Year 2				Year 3				
		Winter	Spring	Summer	Late-Autumn	Winter	Spring	Summer	Late-Autumn	Winter	Spring	SEM
CP	RGWC	193^{ij}	210^{hi}	113^{o}	213^{ghi}	284^{bc}	244^{ef}	190^{ij}	303^{b}	280^{cd}	204^{i}	6.6
	IRWC	151^{klm}	203^{i}	124^{no}	222^{ghi}	288^{bc}	243^{ef}	182^{jk}	341^{a}	277^{cd}	168^{kl}	
	PWC	ns	219^{ghi}	143^{mn}	228^{fgh}	ns	262^{de}	229^{fgh}	ns	269^{cd}	182^{jk}	
	Brassica											
	BL	267^{cd}	-	-	-	229^{fg}	-	-	109^{o}	137^{mn}	-	
	BB	148^{lm}	-	-	-	64^{p}	-	-	67^{p}	71^{p}	-	
NDF	RGWC	424^{ef}	467^{cd}	578^{b}	481^{c}	382^{g}	437^{e}	456^{de}	393^{fg}	360^{gh}	409^{ef}	8.1
	IRWC	481^{c}	357^{hi}	627^{a}	283^{k}	384^{g}	436^{e}	473^{cd}	357^{hi}	321^{j}	383^{g}	
	PWC	-	486^{c}	401^{fg}	471^{cd}	-	328^{j}	309^{j}	-	258^{l}	357^{hi}	
	Brassica											
	BL	211^{m}	-	-	-	161^{no}	-	-	218^{m}	132^{n}	-	
	BB	216^{m}	-	-	-	147^{op}	-	-	175^{n}	204^{m}	-	
ME	RGWC	11.1^{ij}	10.8^{jk}	9.2^{m}	10.6^{k}	11.9^{efg}	11.3^{hi}	10.9^{jk}	11.6^{gh}	12.1^{cdef}	12.1^{cdef}	0.12
	IRWC	10.5^{kl}	10.0^{l}	8.6^{n}	11.5^{h}	12.0^{ef}	11.3^{hi}	11.1^{ij}	12.4^{bc}	12.4^{bc}	12.2^{bcd}	
	PWC	ns	10.7^{k}	9.4^{m}	10.8^{jk}	ns	10.8^{jk}	11.4^{hi}	ns	12.2^{bcde}	11.8^{fg}	
	Brassica											
	BL	11.9^{defg}	-	-	-	12.5^{b}	-	-	12.3^{bc}	12.1^{cdef}	-	
	BB	12.0^{bcdef}	-	-	-	13.2^{a}	-	-	12.2^{bcd}	12.0^{cdef}	-	

[a-p]Superscripts with different letters indicate that means are significantly different among treatments within each parameter across years (P<0.05); ns: not sampled as PWC were not grazed in winter of Years 1 and 2, and late-autumn of Year 3; -: no forage was present to sample as brassica was only present in winter of Years 1 and 2, and late-autumn and winter of Year 3.

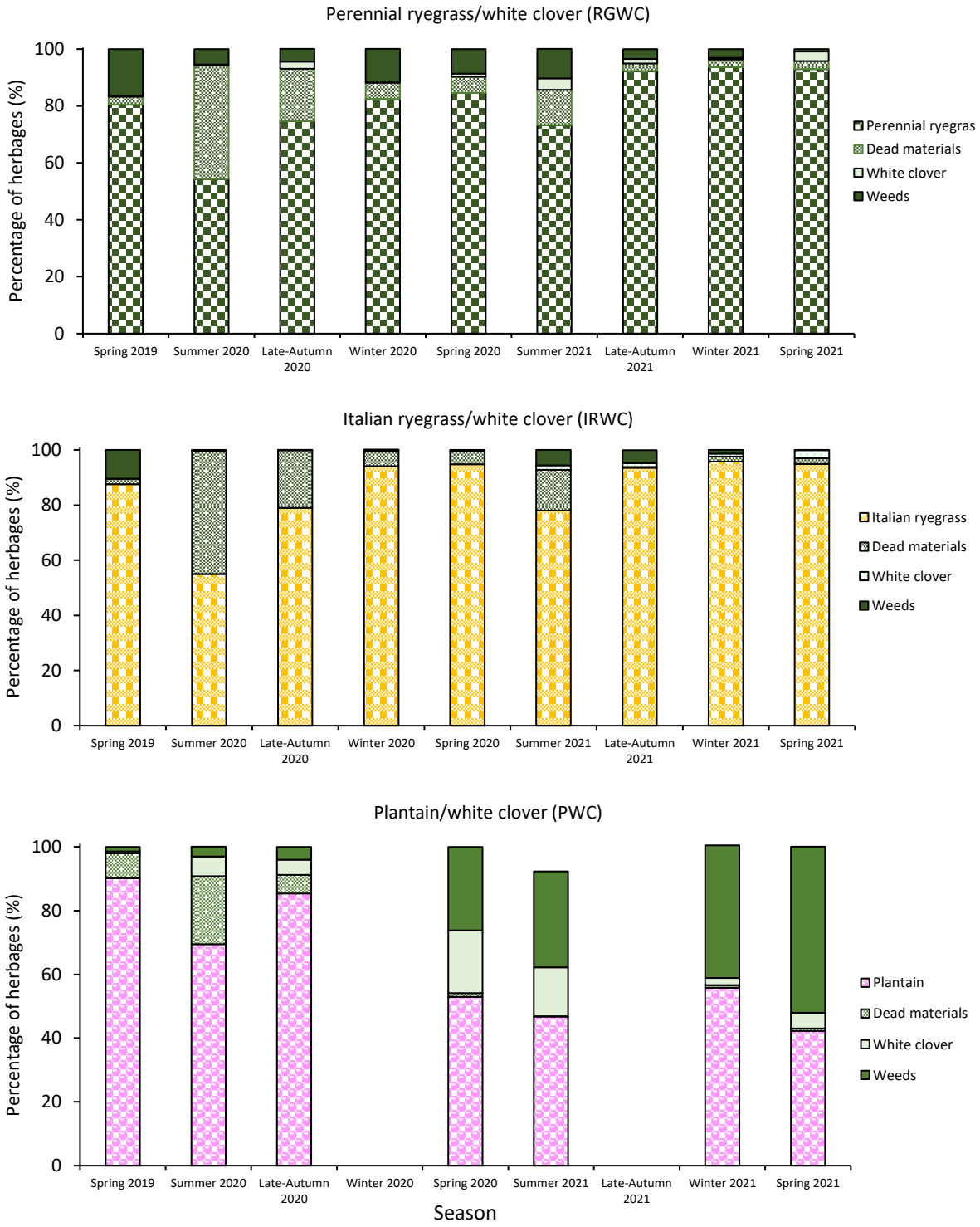

Figure 4.4 Botanical composition of the perennial ryegrass/white clover (RGWC), Italian ryegrass/WC (IRWC) and plantain/WC (PWC) swards measured in drainage plot areas in spring of Year 1 (July to December 2019) and summer, late-autumn, winter and spring of Years 2 (January to December 2020), and 3 (January to December 2021). Values are least-squares means. Plantain/WC was not grazed in winter of Year 2 and late-autumn of Year 3, therefore, PWC was not sampled in those seasons.

4.4.3 Animal performance

Ewe live weight and body condition score

Overall, the LW and BCS of ewes did not differ (P>0.05) within the treatments (see Appendix 4). However, ewe LW showed a significant (P<0.05) three-way interaction within year, treatment and monitoring events (Table 4.8). In Year 1, at pre-lambing, ewes grazing PWC were heavier (P<0.05) than the ewes grazing RGWC and IRWC, but no difference was observed (P>0.05) among the treatments at docking and weaning (Figure 4.5). In Year 2, ewes in PWC were heavier (P<0.05) than all other treatments at breeding and were heavier (P<0.05) than the ewes in brassica at pre-lambing but not (P>0.05) at docking and weaning. In Year 3, ewes grazing brassica were heavier (P<0.05) than the ewes grazing PWC at pre-lambing, in contrast, at docking and weaning, ewes grazing PWC were heavier (P<0.05) than ewes grazing brassica. Further, in Year 3, ewes grazing RGWC were heavier (P<0.05) than ewes fed brassica at docking and weaning but not (P>0.05) at breeding and pre-lambing.

Table 4.8 P-value of main effects of treatment (T), animal monitoring event (E), year (Y), and their three-way interaction (T*E*Y) on ewe live weight (LW), ewe performances and lamb LW and liveweight gain (LWG) measured at each event in Year 1 (July to December 2019) Year 2 (January to December 2020) and Year 3 (January to December 2021).

	Treatment (T)	Event (E)	Year (Y)	T*E*Y
Ewe				
Ewe LW[1]	0.9036	<.0001	<.0001	<.0001
Number of foetuses per ewe put to the ram	0.9326	-	0.3274	-
Number of lambs born per ewe put to the ram	0.9451	-	0.5077	-
Lamb survival to weaning	0.2726	-	0.0011	
Number of lambs weaned per ewe mated	0.3778	-	0.0694	-
Total weight of lamb per ewe at weaning (kg)	0.5570	-	0.0254	-
Lamb				
Lamb LW[2]	0.0248	<.0001	0.0001	<.0001
LWG birth to weaning	0.0040	-	<.0001	-

[1]Ewe LWs were measured at breeding, pre-lambing, docking and weaning. [2]Lamb LWs were measured at birth, docking and weaning. P-value less than 0.05 (typically ≤ 0.05) is statistically significant.

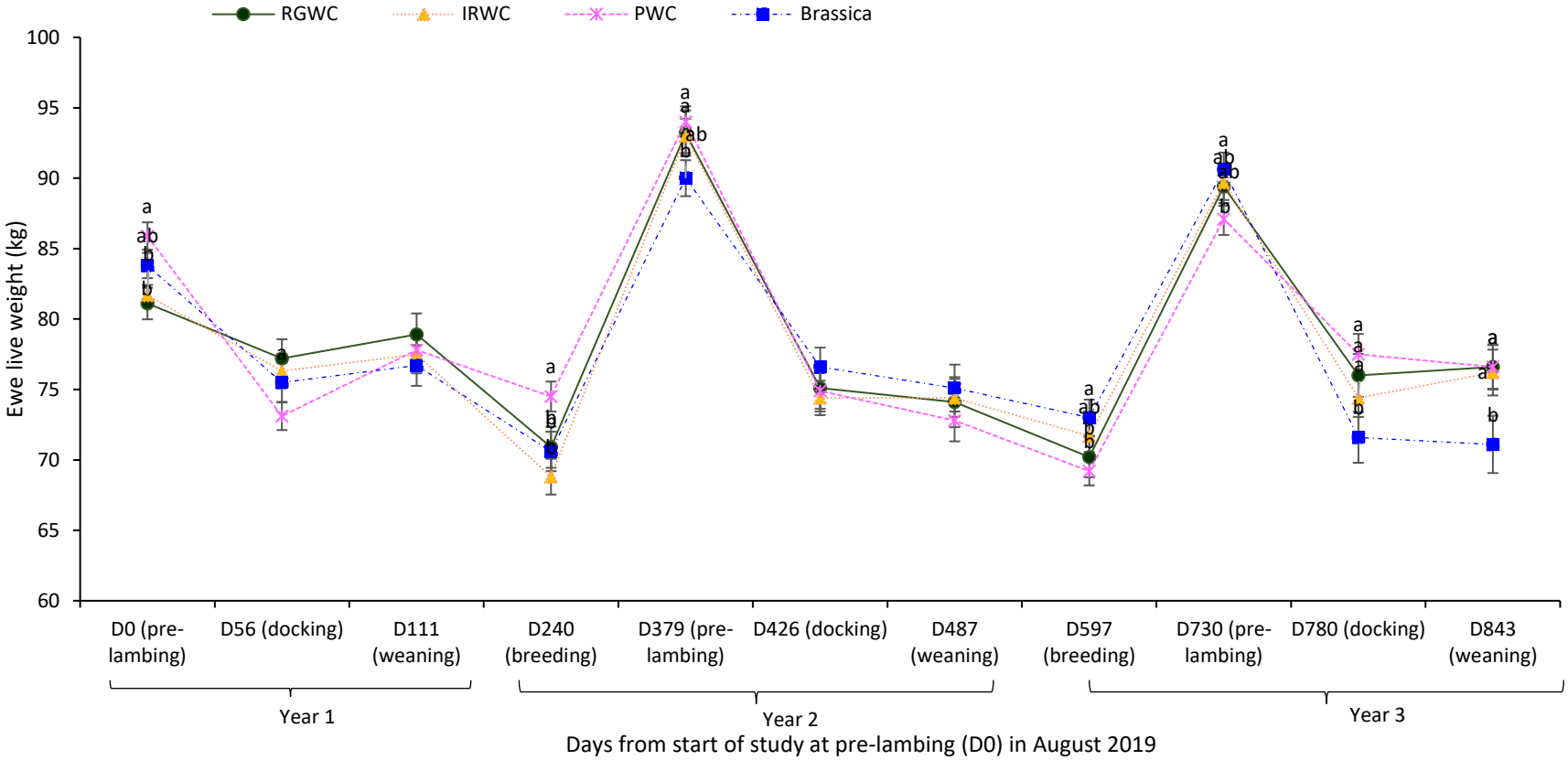

Figure 4.5 Effect of forage treatment (perennial ryegrass/white clover (RGWC): 100% of grazing area was RGWC; Italian ryegrass/white clover (IRWC): 24% of grazing area was IRWC and remaining 76% of area was RGWC; plantain/white clover (PWC): 24% of grazing area was PWC and remaining 76% of area was RGWC; brassica: 24% of grazing area was brassica and remaining 76% of area was RGWC) on live weight (kg) of ewes at breeding, pre-lambing, docking and weaning in Year 1 (July to December 2019) Year 2 (January to December 2020) and Year 3 (January to December 2021). Live weight data are presented as least-squares means ± SE. ^{abc}Superscripts with different letters indicate that means are significantly different among treatments within measuring day (D; $P<0.05$).

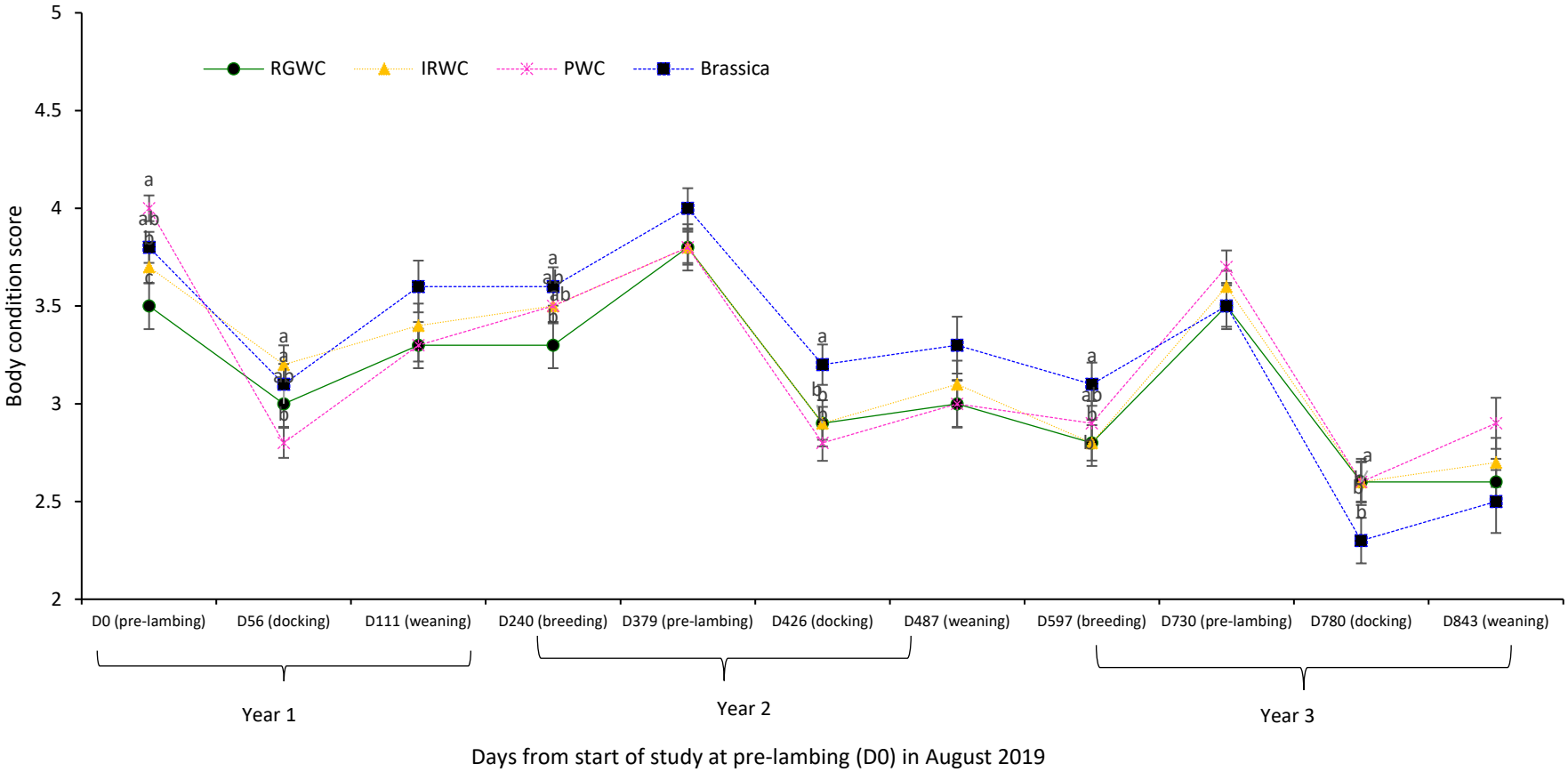

Figure 4.6 Effect of forage treatment (perennial ryegrass/white clover (RGWC): 100% of grazing area was RGWC; Italian ryegrass/white clover (IRWC): 24% of grazing area was IRWC and remaining 76% of area was RGWC; plantain/white clover (PWC): 24% of grazing area was PWC and remaining 76% of area was RGWC; brassica: 24% of grazing area was brassica and remaining 76% of area was RGWC) on body condition score of ewes at breeding, pre-lambing, docking and weaning in Year 1 (July to December 2019) Year 2 (January to December 2020) and Year 3 (January to December 2021). Results displayed as back-transformed logit mean ± SE; ^{abc}Superscripts with different letters indicate that means are significantly different among treatments within measuring day (D; $P<0.05$).

Table 4.9 Effect of forage treatment (perennial ryegrass/white clover (RGWC): 100% of grazing area was RGWC; Italian ryegrass/white clover (IRWC): 24% of grazing area was IRWC and remaining 76% of area was RGWC; plantain/white clover (PWC): 24% of grazing area was PWC and remaining 76% of area was RGWC; brassica: 24% of grazing area was brassica and remaining 76% of area was RGWC) on live weight (LW; kg) of lambs at birth and adjusted LW[1] at docking and weaning, and liveweight gain (LWG; g/day) from birth to weaning[2] in Year 1 (July to December 2019) Year 2 (January to December 2020) and Year 3 (January to December 2021). Values are least-squares means ± SEM.

	LW (kg)						*n*	LWG (g/day) from birth to weaning[2]
	n	Birth weight	*n*	Docking weight[1]	*n*	Weaning weight[1]		
Year 1								
RGWC	*87*	5.1±0.8	*68*	14.7^{bcd}±0.8	*68*	26.8^{abcd}±0.8	*68*	245.6^{ab}±5.5
IRWC	*85*	5.5±0.8	*64*	14.8^{abcd}±0.8	*62*	27.5^{ab}±0.8	*62*	250.9^{a}±5.6
PWC	*89*	5.4±0.8	*73*	14.6^{cd}±0.8	*72*	28.0^{a}±0.8	*72*	253.0^{a}±5.3
Brassica	*88*	5.4±0.8	*64*	15.1^{abc}±0.8	*63*	27.2^{abc}±0.8	*63*	246.3^{ab}±5.6
Year 2								
RGWC	*87*	4.8±0.8	*66*	14.5^{bcd}±0.8	*63*	26.7^{bcd}±0.8	*63*	223.4^{de}±5.7
IRWC	*87*	5.1±0.8	*62*	14.0^{d}±0.8	*55*	24.6^{e}±0.8	*55*	193.8^{f}±5.7
PWC	*93*	4.9±0.8	*74*	14.9^{abcd}±0.8	68	23.8^{e}±0.8	68	197.1^{f}±5.3
Brassica	*92*	5.0±0.8	*67*	15.5^{a}±0.8	*64*	25.8^{d}±0.8	*64*	217.0^{e}±5.4
Year 3								
RGWC	*93*	4.9±0.8	*62*	15.9^{a}±0.8	*53*	27.3^{abc}±0.8	*53*	243.0^{abc}±5.7
IRWC	*93*	5.0±0.8	*61*	14.6^{cd}±0.8	56	26.2^{d}±0.8	56	229.1^{cde}±5.5
PWC	*84*	4.9±0.8	*60*	13.9^{d}±0.8	58	25.8^{d}±0.8	58	227.0^{de}±5.5
Brassica	*91*	5.2±0.8	*65*	15.0^{bc}±0.8	*64*	26.5^{cd}±0.8	*64*	235.6^{bcd}±5.2

Data within columns with different letters differ significantly ($P<0.05$). [1]Weights were adjusted based on their average age (docking weight adjusted for 35 days and weaning weight adjusted for 90 days) using date of birth and measurement date data. [2]Liveweight gains were calculated based on the adjusted LW at docking (docking weight adjusted for 35 days) and weaning (weaning weight adjusted for 90 days).

Table 4.10 Effect of forage treatment (RGWC: 100% of grazing area was perennial ryegrass/white clover (RGWC); IRWC: 24% of grazing area was Italian ryegrass/white clover and remaining 76% of area was RGWC; PWC: 24% of grazing area was plantain/white clover and remaining 76% of area was RGWC; brassica: 24% of grazing area was brassica and remaining 76% of area was RGWC) on ewe reproductive traits (number of foetuses per ewe exposed to the ram, number of lambs born per ewe exposed to the ram, number of lambs weaned per ewe exposed to the ram and total weight of lamb weaned per ewe exposed to the ram) and lamb survival over three years (Year 1: July to December 2019; Year 2: January to December 2020; Year 3: January to December 2021). Values are least-squares means ± SEM.

	Number of foetuses per ewe exposed to the ram	Number of lambs born per ewe exposed to the ram	Lamb survival to weaning[1] (%) (no of lamb weaned alive/no of lamb born)	Number of lambs weaned per ewe exposed to the ram	Total weight of lamb weaned per ewe exposed to the ram[2] (kg)
Year					
1	1.91±0.05	1.90±0.05	1.16^{x}±0.13 (76.1)	1.44±0.06	38.7^{x}±1.47
2	1.96±0.05	1.95±0.05	0.83^{xy}±0.12 (69.7)	1.36±0.06	35.5^{xy}±1.47
3	2.01±0.05	1.97±0.05	0.55^{y}±0.11 (63.3)	1.27±0.06	33.4^{y}±1.47
Treatment					
RGWC	1.98±0.06	1.95±0.06	0.84±0.14 (69.9)	1.34±0.07	35.7±1.90
IRWC	1.93±0.06	1.92±0.06	0.65±0.13 (65.7)	1.27±0.07	33.7±1.92
PWC	1.96±0.06	1.93±0.06	1.02±0.14 (73.6)	1.43±0.07	37.4±1.90
Brassica	1.97±0.06	1.96±0.06	0.87±0.13 (70.5)	1.39±0.07	36.7±1.93
Year X Treatment					
Year 1					
RGWC	1.91±0.10	1.89±0.10	1.28^{ab}±0.26 (78.2)	1.48±0.11	38.7^{ab}±2.94
IRWC	1.87±0.10	1.85±0.10	0.99^{abc}±0.24 (72.9)	1.35±0.11	36.8^{ab}±2.94
PWC	1.93±0.10	1.93±0.10	1.44^{a}±0.27 (80.9)	1.56±0.11	42.4^{a}±2.94
Brassica	1.91±0.10	1.91±0.10	0.92^{abc}±0.24 (71.6)	1.37±0.11	36.8^{ab}±2.94
Year 2					
RGWC	1.93±0.10	1.89±0.10	0.97^{abc}±0.24 (72.4)	1.37±0.11	37.0^{ab}±2.94
IRWC	1.89±0.10	1.89±0.10	0.54^{cd}±0.22 (63.2)	1.20±0.11	31.4^{b}±2.94
PWC	2.02±0.10	2.02±0.10	1.00^{abc}±0.23 (73.1)	1.48±0.11	36.9^{ab}±2.94
Brassica	2.00±0.10	2.00±0.10	0.83^{abcd}±0.23 (69.6)	1.40±0.11	36.7^{ab}±2.94
Year 3					
RGWC	2.09±0.10	2.07±0.10	0.28^{d}±0.21 (57.0)	1.18±0.11	31.2^{b}±2.97
IRWC	2.02±0.10	2.02±0.10	0.41^{cd}±0.21 (60.2)	1.23±0.11	33.0^{b}±2.94
PWC	1.94±0.10	1.83±0.10	0.76^{bcd}±0.22 (68.1)	1.26±0.11	33.0^{b}±2.94
Brassica	1.98±0.10	1.98±0.10	0.86^{abcd}±0.23 (70.3)	1.39±0.11	36.5^{ab}±2.94

Within column and within year or treatment or the tested interaction of year and treatment data with different letters differ significantly ($P<0.05$); [1]Data for lamb survival to weaning are shown as the logit-transformed mean ± SEM, with the back-transformed percentage given in parentheses. [2]Total weights of lamb weaned per ewe put to the ram were calculated from the live weight of the lambs at weaning.

At pre-lambing in Year 1, ewes grazing PWC had greater (P<0.05) BCS than the ewes grazing RGWC and IRWC; however, at docking, the BCS of ewes in PWC was lower than the BCS of the ewes grazing IRWC and brassica. There were no differences (P>0.05) among the treatments at weaning (Figure 4.6). In Year 2, at breeding, ewes grazing brassica had greater (P<0.05) BCS compared to the ewes grazing RGWC but did not differ (P>0.05) at pre-lambing or weaning. Similarly, in Year 3, ewes grazing brassica had greater (P<0.05) BCS compared to the ewes grazing RGWC at breeding but not (P>0.05) at pre-lambing and weaning.

Lamb live weight and liveweight gain

Overall, lamb LW of RGWC and brassica treatments were heavier (P<0.05) compared to IRWC lambs (see Appendix 5). However, there was a significant three-way interaction of year, treatment and event (Table 4.8). In Year 1, the LW of the lambs did not differ (P>0.05) within the treatments at birth, docking or weaning (Table 4.9). Lamb LW at birth did not differ (P>0.05) among treatments in Year 2, but lambs in the brassica treatment had heavier (P<0.05) adjusted LW at docking compared to RGWC and IRWC. However, lambs in brassica and RGWC treatments had greater (P<0.05) adjusted LW than those in the other treatments at weaning. In Year 3, lambs in the RGWC treatment were heavier (P<0.05) than all other treatments at docking. Further, brassica lambs were heavier (P<0.05) than IRWC and PWC at weaning.

Overall, lamb LWG from docking to weaning did not differ (P>0.05) among treatments; however, lambs in RGWC and brassica had greater (P<0.05) LW gain from birth to weaning compared to the lambs in IRWC and PWC (see Appendix 5). Lamb LWG from birth to weaning did not differ (P> 0.05) among treatments in Year 1 (Table 4.9). In Year 2, lambs in RGWC and brassica had greater (P<0.05) LW gain from birth to weaning compared to the lambs in

PWC and IRWC. In Year 3, the lambs in PWC gained less (P<0.05) LW from birth to weaning compared to the lambs in RGWC.

Ewe reproductive traits and lamb survival

The number of foetuses, lambs born, number of lambs weaned, and the total weight of lamb weaned per ewe exposed to the ram did not differ (P>0.05) among treatments (Table 4.10). Lamb survival was also not affected (P>0.05) by treatments. The number of foetuses, lambs born per ewe and lambs weaned per ewe put to the ram did not differ (P>0.05) between years. However, lamb survival to weaning and the total weight of lamb weaned per ewe put to the ram were greater (P<0.05) in Year 1 compared to Year 3. No differences (P>0.05) were found among treatments for any of the traits measured within a given year.

4.5 Discussion

The objective of this study was to determine that on both an individual and on a per ha basis, the performance of ewes and their lambs on the treatments with 24% of alternative forages (IRWC, PWC and brassica) would be similar to or greater than that of the RGWC treatment due to the herbage quality traits of the forages and their seasonal growth pattern. Overall, all treatments generally showed similar performance over two and a half years period. The level of performance in terms of lambing percentage was high (*i.e.*, higher than 120%) in all treatments across years and well above the top Quintile for North Island finishing farms in 2021, 126% (Class 5; Beef + Lamb New Zealand (2021).

Ewe nutrition, especially during gestation and lactation, can affect lamb growth rates (Lambert et al. 1999; Raeside et al. 2014). Overall, the herbage mass and quality data measured in the present study indicated that ewes were not under nutritional restriction. Previous studies have shown that, under ryegrass white clover grazing conditions, to ensure ewe intake is not restricted, grazing mass should not go below 1200 kg DM/ha (Morris & Kenyon 2004). Given

that the perennial ryegrass/white clover area was at least 76% of the total grazed area in all treatments and that the average perennial ryegrass/white clover covers measured were consistently above 1200 kg DM/ha, the results indicate animal intakes were not restricted for extended periods. However, in New Zealand, after weaning, lambs are typically raised to slaughter weight; therefore, there is a high demand for forages during the summer and early autumn because of the moisture and temperature stress. Consequently, pasture cover is reduced during the autumn months, but in this study, the lambs were sold at weaning.

In the present study, pre-and post-grazing masses of the alternative forages were only measured in the drainage plots. The pre-grazing masses of plantain/white clover in the drainage-plot areas were typically greater than 1500 kg DM/ha. Previous studies have shown that to maximise sheep production, plantain should be grazed at a sward height of at least 70 mm or 1500-1700 kg DM/ha (Somasiri et al. 2015; Somasiri et al. 2020) and grazing avoided during the late-autumn and winter months (Cranston et al. 2015b). These recommendations were followed in the current study, ensuring animal performance was not limited. Forage cover of Italian ryegrass/white clover on the drainage areas was also typically greater than 1500 kg DM/ha, which is higher than the minimum optimal level reported for ryegrass/white clover (Morris & Kenyon 2004), a similar sward. In mid-to-late pregnancy, ewes (bearing twins with the average lamb weight of 4 kg) should be offered 14 to 18 MJ ME/ewe/day (Nicol & Brookes 2007). In the brassica forage, ewes were allocated feed at a rate of 1.8 kg DM/day, with an average forage utilisation rate of 85%, indicating intake was not restricted when ewes were on the various brassica forages.

Forages containing a ME content of 10-12 MJ ME/kg DM (Nichol 2007; Cranston et al. 2017) and CP content of 8-16% (National Research Council 2007; Hynd 2019) are adequate for sheep production. The ME and CP values of the various forages were only recorded in the drainage

area for each treatment. Perennial ryegrass/white clover and Italian ryegrass/white clover forages remained within the recommended levels for both quality traits in all periods except the summer of Year 2, while for the plantain/white clover forage, CP and ME levels were higher or equivalent to the optimum level in most seasons. The ME content of the brassica forages in drainage areas remained above 12.0 MJ ME/kg DM over the study period. It is well-known that brassicas (whole crop) have a lower CP content than that which is required for optimum rumen microbial protein synthesis (Cranston et al. 2017), and it is known to be considerably lower than for perennial ryegrass/white clover (Dalley et al. 2017). The CP levels of brassica in Years 2 (bulb) and three were below the optimal, but not in Year 1. Brassica leaves are known to contain a greater level of CP than their bulbs (De Ruiter et al. 2007; Dalley et al. 2017). Given that the ewes had eaten all the brassica leaves in Year 2, but residual bulb biomass was substantial (approximately 45% of the bulbs were not utilised), the CP may not have been limited in Year 2. It is also important to note that ewes were on brassica forages for approximately two, seven and ten weeks, in Years 1, 2 and 3, respectively. Overall, the results indicate that ME and CP levels observed would not have limited ewe performance on any of the forage treatments.

The forage covers, feed allocations and forage quality utilised in this study indicate that ewes in all treatments were well-fed. This probably explains the very good BCS of ewes in all treatments (Kenyon et al. 2014). Previous studies have suggested that sheep BCS should be above 2.5-3.0 throughout the year for maximum overall performance (Kenyon & Webby 2007; Kenyon et al. 2014; Kenyon et al. 2019). This was achieved in all treatment groups.

The CP and OMD values of plantain/white clover mix were higher compared to RGWC during summer while ME was similar across seasons. Previous studies have reported plantain, white and/or red clover mix to have higher nutritive value during summer, compared to

ryegrass/white clover (Cranston 2014; Somasiri et al. 2016). Both perennial ryegrass/white clover and Italian ryegrass/white clover had lower CP, ME and OMD levels during summer, compared to other seasons. It has also been previously reported that Italian ryegrass has higher levels of CP and ME in winter than in summer (Ryan-Salter & Black 2012). Perennial ryegrass/white clover has also been shown to have lower levels of CP and ME levels in summer compared to the other seasons in previous studies (Fulkerson et al. 2007; Raeside et al. 2014; Somasiri et al. 2016).

In the present study, brassica forages showed a greater CP concentration in Year 1 than in Years 2 and 3. It is important to note, in this study, that different varieties of brassica were sown in Years 1 (turnips), 2 (swedes) and 3 (kale). Crops were established by ploughing permanent pastures in the first year; this process can release significant amounts of mineral N in the soil (Cameron et al. 2013) as a result of organic matter decomposition, which may have contributed to the higher CP of the turnips (brassica) in Year 1, compared to swedes and kale in the later years. The CP concentrations of turnip bulbs and leaves observed in this study were within the range reported in previous studies (De Ruiter et al. 2007; Dalley et al. 2017). In contrast, the CP concentrations of swede bulbs (Dalley et al. 2017; Winkler 2017) and kale were lower than that reported previously (De Ruiter et al. 2007). The N fertiliser applied to the brassica forages (30-80 kg N/ha/year) in this study was much lower than the N requirement (250 to 500 kg N/ha) recommended for brassica crops in New Zealand (De Ruiter et al. 2009), which may explain why the CP concentrations in Years 2 and 3 were lower.

Significant growth of broad-leaved dock (*Rumex obtusifolius*) was observed in plantain/white clover from the spring of Year 2. Chemical and mechanical control methods were utilised without successful eradication. The presence of this weed may have affected plantain growth and perhaps decreased the proportion of plantain in the mix of plantain and white clover. When

the sown species is replaced by a weed species, it results in a decline in the plant density of the sown species, which eventually reduces the DM contribution of the sown species (Tozer et al. 2011a; Tozer et al. 2011b). The presence of high levels of broad-leaved dock in the spring of Year 2 and in all seasons of Year 3 likely contributed to the reduced DM production of plantain in drainage areas in Year 3.

In the summer of Year 3, the number of tillers in the Italian ryegrass had reduced. Therefore, the Italian ryegrass/white clover in drainage areas was sprayed-out and resown using a direct-drill in April 2021. However, the Italian ryegrass/white clover was patchy following resowing. Consequently, this likely drove the reduction in DM production in Italian ryegrass/white clover in Year 3.

Ewe reproductive traits and lamb survival were not affected by forage treatment. Under both pastoral and indoor conditions, Kenyon et al. (2019) reported average lamb survival rates of 89.5 (range from 71 to 100%), 85.5 (range from 74 to 100%), and 67.5% (range from 45 to 89%) for singles, twins, and triplets, respectively. It has been reported previously that triplets are more likely to be born when the prolificacy increases from 1.5 to 2.1 lambs per ewe lambing (Amer et al. 1999). In this study, the number of foetuses carried and, therefore, lambs born to each ewe exposed to a ram ranged between 1.9 and 2.1. Based on a scanning percentage of 2.0, the modelling of Amer et al. (1999) suggests approximately 20% triplets and 60% twin lambs. This high rate of triplets likely contributed to the low lamb survival rates in all treatments (Kenyon et al. 2019).

Finally, it is important to note that under the conditions of the study, feed intake could not be restricted, or ewe stocking rates could not be increased as this would not have allowed for testing of the environmental impacts (see later Chapters 5 and 6) over the two-and-a-half-year

period under consistent management conditions. Further studies are required to examine how sheep would respond to lower feed levels and/or a higher stocking rate when grazing these forage species.

4.6 Conclusions

Based on the results of the current study, it can be concluded that, in a farmlet system that consists of 24% of the land area in alternative forage and the remaining area being perennial ryegrass/white clover, and where lambs are sold at weaning, the ewes and lambs performed no differently from those grazing perennial ryegrass/white clover only when stocked at 14 ewes per hectare. This suggests that farmers should not be concerned about adverse effects on animal performance; however, further studies would likely be required to gain an understanding of how sheep might perform at lower feeding levels with these alternative forages.

CHAPTER 5: URINARY NITROGEN EXCRETION OF SHEEP GRAZING FORAGES

5.1 Abstract

The use of some alternative forages can reduce urinary nitrogen (N) loads to soil and subsequently to the environment. The potential extent to which a forage reduces N loads in comparison to traditional ryegrass/white clover pastures can be assessed by quantifying the N excretion in urine and faeces. The effects of four forages (perennial ryegrass/white clover: RGWC; Italian ryegrass/white clover: IRWC; plantain/ white clover: PWC; brassica) on N partitioning and excretion in breeding ewes were studied at Massey University's 'Keeble' farm over two and a half years (Year 1: July to December 2019; Year 2: January to December 2020; Year 3: January to December 2021). Perennial ryegrass/WC and IRWC were grazed in all seasons, whereas PWC was only grazed in the spring of Years 1, 2 and 3, summer of Years 2 and 3, and autumn of Year 2 and winter of Year 3. Brassica crops were only grazed in the winter of Years 1, 2 and 3 and in the autumn of Year 3. The apparent N intakes of the ewes were calculated as the difference between pre-and post-grazing herbage mass and forage N concentrations. The N concentrations of urine and faeces were determined using spot samples collected from five ewes per treatment during each season. There were no ($P>0.05$) differences in apparent dry matter intake among treatments, but ewes grazing brassica had a lower ($P<0.05$) apparent N intake compared with ewes grazing other treatments. Overall, the estimated daily urinary N excretion levels (based on spot samples) across all seasons and forages ranged from 9 to 27 g N/sheep/d. The urinary N concentrations of ewes grazing on PWC, and brassica treatments were lower ($P<0.05$) than those grazing on RGWC in all seasons where it was compared. The results suggest that PWC and brassica can reduce urinary N load per animal in the soil via a diuretic effect and lower herbage N concentrations, respectively.

Keywords: Alternative forages, breeding ewes, faeces N, N intake, plasma N, urinary N.

5.2 Introduction

Nutrient leaching from agricultural soils, particularly from intensively grazed pasture, is a significant cause of water quality deterioration in many parts of the world (Ruz-Jerez et al. 1995; Silva et al. 1999; Di & Cameron 2002; Houlbrooke et al. 2004). Nitrogen (N) cycling in a grazing system is influenced by the grazing animal's diet and the partitioning of ingested N within the animal (Williams & Haynes 1994). In sheep, approximately 7-17% of ingested N is partitioned into products, including; meat, milk, fibre (Gillingham & During 1973; Williams & Haynes 1990; Kohn et al. 2005). Most of the remaining N is excreted in urine (55-67%) and faeces (17-28%) (Gillingham & During 1973; Williams & Haynes 1990; Kohn et al. 2005). Urinary N typically consists of 70 to 90% urea, which is rapidly hydrolysed to NH_4^+ and subsequently nitrified to NO_3^- within the soil, where it can be readily leached, particularly during periods of high rainfall (Whitehead & Bristow 1990). Faecal N is in a stable form (organic), and consequently, it is not a major contributor to N leaching (Wachendorf et al. 2005).

Sheep production systems in New Zealand are predominantly based on grazing a grass (perennial ryegrass: *Lolium perenne* L.) and white clover (*Trifolium repens*) mix (Cranston et al. 2017). However, this grass-based mix has a high N content relative to dietary energy requirement, especially in spring and late-autumn, resulting in low efficiency of incorporating feed N into animal product N, consequently which results in high urinary N excretion (Cheng et al. 2013; Jonker et al. 2015). Therefore, alternative forages have been sought to mitigate leaching losses (Bryant et al. 2018). For example, forage species with high rumen-utilisable protein (RUP), which divert the dietary N away from urine (Woodward et al. 2012), or forages with lower crude protein (CP) concentrations, can reduce the excretion of urinary N (Miller et al. 2001). Forage species with more readily available water-soluble carbohydrates (WSC)

relative to slowly fermentable neutral detergent fibre (NDF) or CP have also been shown to improve the energy-to-N balance within the rumen and reduce urinary N excretion (Edwards et al. 2007; Jonker et al. 2015). For example, brassicas such as swedes (*Brassica napus* ssp. *napobrassica*) have a low CP (10 to16%) and a high WSC (25 to 50%) content (De Ruiter et al. 2007), which reduce N excretion on an individual animal basis by maximising N utilisation efficiency in the rumen (Dalley et al. 2017). In contrast, ryegrass has CP and WSC contents of 17-27% (Litherland & Lambert 2007) and 16-22% (Edwards et al. 2007), respectively.

Forages such as plantain (*Plantago lanceolata* L.) contain secondary metabolites (including acteoside syn. verbascoside, aucubin, catalpol, iridoid and phenylpropanoid glycosides) (Navarrete et al. 2016; Gardiner et al. 2018), which may reduce urinary N losses by inhibiting biological nitrification (Dietz et al. 2013). Aucubin inhibits rumen fermentation, while acteoside increases gas production by acting as an energy source for rumen microbial growth (Navarrete et al. 2016). In addition, the presence of secondary metabolites (Deaker 1994), mineral load (e.g., sodium content) and the higher water content of plantain can result in diuresis, and consequently, reduce urinary N concentration (Box et al. 2016; De Klein et al. 2019; Dineen et al. 2021). In addition to the above attributes, plantain contains condensed tannins (CT), which have been shown to reduce the urinary N excretion (De Klein et al. 2019) by increasing the portioning of faecal N relative to urine (Carulla et al. 2005).

Winter-active forages such as Italian ryegrass (*Lolium multiflorum* Lam.) (Moir et al. 2012; Malcolm et al. 2015) can reduce the accumulation of NO_3^- in the soil profile through high uptake of NO_3^-, which otherwise would be susceptible to leaching during winter (Nichols & Crush 2007; Moir et al. 2013; Woods et al. 2016; Maxwell et al. 2019). However, little is known about N partitioning in animals grazing Italian ryegrass.

The use of alternative forages to reduce the urinary N excretion load from grazing dairy cows is well recognised (De Klein et al. 2019); however, little is known about their ability to reduce N excretion from sheep in New Zealand. The quantification of total urinary and faecal N excretion is essential to determine the potential of a given forage to reduce urinary N excretion and N loading to the soil (Marsden et al. 2020). Due to the difficulty of continuous monitoring of urination events of sheep under grazing conditions (David et al. 2015; Marsden et al. 2020), indirect methods such as urinary creatinine excretion have been suggested as an alternative method to determine the urine volume produced by grazing sheep (David et al. 2015). In addition, the partitioning of N between urine and dung can be measured from the urine and faeces N (Kohn et al. 2005). This study aimed to determine the quantity of N ingested by sheep grazing four forage types/mixes (perennial ryegrass/white clover, plantain/white clover, Italian ryegrass/white clover, brassica) and to compare the impact of these forages on N partitioning and excretion by sheep as assessed by spot sampling.

5.3 Materials and methods

This study was carried out with the approval of the Massey University Animal Ethics Committee (MUAEC 19/20; MUAEC 21/14 amended 05/20). See Chapter 3.0 for details of the experimental site, treatments, and forage sample analysis. Forage data collected from drainage plots, and animal samples (urine, blood and faeces) collected from ewes while grazing on the forages for a minimum of 48 hours (refer to Table 3.9 for sampling dates) over two and a half consecutive production years (from June 2019 to December 2021), were used for this study. The years will be referred to as “Year 1 (July to December 2019)”, “Year 2 (January to December 2020)”, and “Year 3 (January to December 2021)”.

5.3.1 Blood, urine and faeces sample collection and analyses

The number of samples collected over the grazing experiments to evaluate the effects of perennial ryegrass/white clover (RGWC), Italian ryegrass/white clover (IRWC), plantain/ white clover (PWC) and brassica on N partitioning of sheep are summarised in Table 5.1. Once in each season (winter, spring, summer, and late-autumn), in the late morning (between 10 a.m. and 11.30 a.m.), after the ewes had been grazing the forages for a minimum of 48 hours, blood, faeces and urine samples were collected from a random subset of five ewes, from each forage treatment (i.e., five samples per treatment). The ewe's vulva was wiped prior to sampling to clean off any faecal material before its nasal and oral passages were obstructed (manually). A urine sample would generally be produced within 5 to 10 seconds (30 seconds maximum) of airway obstruction (transitory apnoea) (Benech et al. 2015). Obstruction of the ewe's airways was halted as soon as approximately 40 ml of midstream urine was obtained. The samples were separated into two parts (about 20 ml each) and then placed in polypropylene screw-capped containers. One part of each sample (for analysis of N and NH_3) was acidified to a pH of 4.0 or below with concentrated sulphuric acid (6N) to prevent NH_3 volatilisation. Acidified and non-acidified samples were stored at -20℃ until analysis.

Table 5.1 Description of the number (n) of samples (forage, urine, blood and faeces) collected in winter and spring of Year 1 (July to December 2019) and summer, late-autumn, winter and spring of Years 2 (January to December 2020), and 3 (January to December 2021) to evaluate the effects of perennial ryegrass/white clover (RGWC), Italian ryegrass/white clover (IRWC), plantain/white clover (PWC) and brassica on N partitioning in sheep.

	Year 1		Year 2				Year 3			
	Winter	Spring	Summer	Late-Autumn	Winter	Spring	Summer	Late-Autumn	Winter	Spring
Treatment included	RGWC	RGWC	RGWC	RGWC	RGWC	RGWC	RGWC	RGWC	RGWC	RGWC
	IRWC	IRWC	IRWC	IRWC	IRWC	IRWC	IRWC	IRWC	IRWC	IRWC
	-	PWC	PWC	PWC	-	PWC	PWC	-	PWC	PWC
	Brassica	-	-	-	Brassica	-	-	Brassica	Brassica	-
Number of samples[1]										
Forages	n=15	n=15	n=15	n=15	n=15	n=15	n=15	n=15	n=20	n=15
Urine	n=15	n=15	n=15	n=15	n=15	n=15	n=15	n=15	n=20	n=15
Blood	n=15	n=15	n=15	n=15	n=15	n=15	n=15	n=15	n=20	n=15
Faeces	n=15	n=15	n=15	n=15	n=15	n=15	n=15	n=15	n=20	n=15

[1]Five samples per forage treatment were collected

The N content of thawed urine subsamples was determined by an autoanalyser (Dumas' procedure, AOAC method 968.06 using a Leco total combustion method, LECO Corporation, St. Joseph, MI, USA; AOAC, 2007). Creatinine concentration in urine samples was determined by colorimetric analysis (Bartels & Bohmer 1972; Henry et al. 1974) using a Daytona Plus clinical analyser (Randox, Crumlin, Co. Antrim, UK) (Box et al. 2017; Bryant et al. 2017). Urine uric acid concentration was determined using an enzymatic colorimetric technique (Barham & Trinder 1972; Fossati et al. 1980) in a Daytona Plus clinical analyser (Randox, Crumlin, Co. Antrim, UK) and urea concentration was determined using a commercial enzymatic kinetic technique (Ulrich 1965; Tietz 1976) in a Daytona Plus clinical analyser (Randox, Crumlin, Co. Antrim, UK) (Box et al. 2017; Bryant et al. 2017). Allantoin concentration was measured using a colorimetric analysis by adapting the procedure outlined by Chen & Gomes (1992) (Chen & Ørskov 2004; Ben Salem & Makkar 2009). Ammonia concentration of acidified urine samples was analysed using the Glutamate dehydrogenate enzymatic method as described by Neeley & Phillipson (1988) (Marshall et al. 2020). During the winter of 2019, urine samples were not acidified; therefore, non-acidified urine samples were used for NH_3 analysis.

Faecal samples were collected by rectal stimulation or as the animal defecated, and frozen at -20℃ until analysis. Faeces were then freeze-dried and ground through a 1-mm sieve. Faecal N content was determined by an autoanalyser (Dumas' procedure, AOAC method 968.06 using a Leco total combustion method, LECO Corporation, St. Joseph, MI, USA (AOAC 2007). In Year 1 (winter 1 and spring 2), faeces samples were not freeze-dried, and therefore the results are not included here.

Blood samples were collected by jugular venepuncture using 10 mL of K2E (EDTA) Vacuette blood collection tubes (BD Vacutainer, Plymouth, PL6 7BP, UK). Blood samples were placed

on ice and then centrifuged at 3,000 × g at 4°C for 15 min. The separated plasma was stored at −20°C until analysis. Urea and creatinine concentrations of the plasma samples were determined as per the method used for the urine samples.

All chemical analyses of plasma, faeces and urine were undertaken at the Nutrition Laboratory, Massey University (Palmerston North, New Zealand).

5.3.2 Calculations

Chemical composition and mineral components of brassica treatment

The chemical composition and mineral contents of leaves and bulb or stem of the brassica forage were measured separately. Therefore, the yield of leaf and stem or bulb components were used to calculate the chemical composition of the whole crop.

Apparent dry matter intake (DMI) and apparent nitrogen (N) intake

The apparent daily DM intake (DMI) per ewe per day was calculated according to Matthews et al. (1999a) by dividing the difference between pre-and post-grazing DM mass for each grazing event by the number of sheep grazing days. In the calculation of the DMI of brassica, it was assumed that the ewes grazing brassica consumed 100% of leaves.

Apparent N intake was calculated by multiplying DMI by the N% of each forage type.

Urine output and daily urinary N excretion measurements using urine creatinine concentration

Urine output (L/day) and total urinary N excretion (g/day) were estimated using the following equations as described by Pacheco et al. (2007).

$$\text{Urine volume (L/day)} = \frac{\text{CEF (mg/kg LW)} \times \text{LW (kg)}}{\text{Urinary creatinine concentration in the spot urine sample (mg/L)}}$$

or

$$\text{Urine volume (L/day)} = \frac{\text{Mean daily creatinine excretion (mg)}}{\text{Urinary creatinine concentration in the spot urine sample (mg/L)}}$$

$$\text{Urinary N excretion } \left(\frac{\text{g}}{\text{day}}\right) = \left(\frac{\text{CEF (mg/kg LW)} \times \text{LW (kg)}}{\text{Urinary creatinine concentration in the spot urine sample (mg/kg)}}\right) \times \text{urine N concentration in the spot urine sample (g/kg)}$$

or

$$\text{Urinary N excretion } \left(\frac{\text{g}}{\text{day}}\right) = \left(\frac{\text{Mean daily creatinine excretion (mg)}}{\text{Urinary creatinine concentration in the spot urine sample (mg/kg)}}\right) \times \text{urine N concentration in the spot urine sample (g/kg)}$$

Creatinine excretion factor (CEF; mg/kg LW) was calculated using Brody's equation: CEF (mg/kg LW) = Mean daily creatinine excretion (mg)/LW where mean daily creatinine excretion = ($12.7 \times LW^{0.896}$) (Brody 1945; David et al. 2015); LW: live weight.

Daily N excretion measurement using plasma urea concentration

Daily urinary N excretion (g/day) was estimated using the following equation as described by Kohn et al. (2005):

$$\text{Urinary N excretion } \left(\frac{\text{g}}{\text{day}}\right) = \text{CR } \left(\frac{\text{L}}{\text{day kg BW}}\right) \times \text{PUN (g/L)} \times \text{LW (kg)}$$

In the calculation, CR content was assumed to be 1.2 based on a regression equation developed by Kohn et al. (2005); CR: N clearance rate, L of blood cleared of PUN/day kg BW; PUN: Plasma urea concentration; LW: Live weight.

Estimation of microbial N supply using urine purine derivatives

Rumen microbial activity was calculated from the ratio of purine derivatives (PD) to creatinine in spot samples of urine (Chen & Gomes 1992). The resulting PD index is a relative measure of microbial protein synthesis.

$$\text{PD index} = \frac{\text{total PD (mmol/L)}}{\text{creatinine (mmol/L)}} \times \text{LW}^{0.75}$$

Where total PD = allantoin + uric acid + xanthin + hypoxanthine (Chen & Gomes 1992); In sheep, allantoin, uric acid and xanthin plus hypoxanthine range between 60 and 80%, 30 and 10% and 10 and 5%, respectively (Chen & Gomes 1992). In the present study, as the xanthine plus hypoxanthine were not measured in the spot samples, the combined xanthin and hypoxanthine contents were estimated based on the allantoin concentration ratio given in Chen & Gomes (1992).

$$\text{Xanthine plus hypoxanthine} = \frac{\text{allantoin concentration (mmol/L)} \times (5/100)}{(80/100)}$$

5.3.3 Statistical analysis

All data were tested for outliers and normality using Minitab 16 statistical software before analysis was carried out in SAS version 9.4 (Statistical Analysis System, version 9.4; SAS Institute Inc., Cary, NC, USA).

This study was carried out over a three-year period: the first year of the study included the winter and spring while the second and third years included all four seasons (summer, late-autumn, winter and spring). Four forage types were investigated, but not all forages were

grazed in all four seasons. For example, RGWC and IRWC were grazed in all seasons, whereas PWC was grazed in spring of Years 1, 2 and 3, summer of Year 2 and 3, and late-autumn of Year 2 and winter of Year 3. Brassica crops were grazed in winter of Years 1, 2 and 3 and late-autumn of Year 3. The herbage and animal components were analysed using a mixed model containing the fixed effects of year (1 or 2 or 3), forage treatment, and season (winter and spring in Year 1, summer, late-autumn, winter and spring in Years 2 and 3). Where appropriate, the three-way interaction among year, season and forage treatment was included in the model. Because of missing data, two-way interactions were not included in the analysis. The results of three-way interactions are presented in this chapter, and the main effects are presented in Appendices 6 and 7. However, the key results from analysis of the main effects are introduced in this chapter. Ewes were not allocated to the same plot (replicate) at each grazing event (due to culling, deaths and subsequent replacement); therefore, repeated measures could not be undertaken.

Herbage measures

Herbage pre-and post-grazing DM mass, soluble sugars and starch (SSS), SSS:CP, SSS:NDF, Na, Ca and P, and botanical composition data were analysed using a mixed model. The model included replicates (plots) nested within the treatment as a random effect. Means were separated using the LSD procedure in proc GLM.

Animal measures

Non-normally distributed data (urine N and urea N concentration of urine and plasma uric acid concentration) were log-transformed prior to analysis to comply with the assumption of residuals normality for the analysis of variance. Back transformed means are reported.

Effect of forage treatment on urine, faecal, plasma composition and urinary N excretion were analysed using a mixed model. The model included ewe nested within the treatment, season

and year as a random effect. For the analysis of urine volume, urinary N excretion, and ammonia, ewe live weight at the sample collection time (± 15 days) was included as a covariate. Means were separated using the LSD procedure in proc GLM.

5.4 Results

5.4.1 Dry matter intake and pasture quality

The results of the main effects for DM intake and chemical composition of forages (Appendix 6), show that, overall, there was no difference ($P>0.05$) in DMI among treatments ($P>0.05$), and that the CP content of brassica was significantly lower ($P<0.05$) than the other forages. Therefore, lower ($P<0.05$) estimate of N intake was most often observed in ewes grazing brassica compared to the N intakes of ewes grazing other treatments. The brassica had higher ($P<0.05$) ME, SSS, SSS:CP and SSS:NDF concentrations than other forages but there were no differences ($P>0.05$) among other forages. Plantain/WC had the lowest ($P<0.05$) DM% and highest ($P<0.05$) Na and P concentrations compared to other forages. The lowest ($P<0.05$) CP and highest DM% ($P<0.05$) were observed in summer.

Forage parameters showed a significant ($P<0.05$) three-way interaction among treatment, season and year (Table 5.2). Herbage characteristics and chemical compositions of the forage treatments are shown in Tables 5.3 and 5.4 and Figures 5.1 to 5.4. The pre-grazing masses of the RGWC and IRWC did not differ ($P>0.05$) within seasons of a given year, except ($P<0.05$) the winter of Year 1 and late-autumn of Year 3. The pre-grazing masses of PWC and RGWC did not differ ($P>0.05$) in spring of Year 1 and summer, winter, and spring of Year 3, whereas in Year 2, pre-grazing masses of PWC were lower ($P<0.05$) compared to RGWC in summer and late-autumn. In contrast, PWC had greater ($P<0.05$) pre-grazing mass in spring. Ewes grazing PWC had greater ($P<0.05$) apparent DMI compared to the ewes grazing RGWC and

IRWC in the spring of experimental Year 2 and summer of Year 3 but did not differ (P>0.05) in other seasons.

Table 5.2 P-value of main effects (forage treatment, season, year) and their three-way interaction (Treatment: T* Season: S* Year: Y) of forage characteristics (DM: dry matter; apparent DMI: dry matter intake; apparent dietary N intake), pre-grazing chemical (CP: crude protein; N: Nitrogen; ME: metabolisable energy; SSS: soluble sugars and starch; NDF: neutral detergent fibre; SSS:CP; SSS:NDF; ADF: acid detergent fibre; OMD: organic matter digestibility), mineral (calcium: Ca; phosphorus: P; sodium: Na), bioactive glycoside contents (catalpol, aucubin, acteoside) and botanical composition (crop; dead materials; white clover; weed) of forage treatments measured in winter and spring of Year 1 (July to December 2019) and summer, late-autumn, winter and spring of Years 2 (January to December 2020), and 3 (January to December 2021).

	Forage treatment (T)	Season (S)	Year (Y)	T*S*Y
Pre-grazing pasture mass (kg DM/ha)	<.0001	<.0001	<.0001	<.0001
Apparent DMI (kg/ewe/d)	0.1095	0.0197	<.0001	0.0203
CP (g/kg DM)	<.0001	<.0001	<.0001	<.0001
Apparent dietary N intake (g/d)	0.0117	<0.0001	<0.0001	<0.0001
DM (%)	<.0001	<.0001	<.0001	<.0001
ME (MJ/kg)	<.0001	<.0001	<.0001	<.0001
SSS (g/kg DM)	<.0001	<.0001	<.0001	<.0001
SSS:CP	<.0001	<.0001	0.0003	<.0001
SSS:NDF	<.0001	<.0001	0.0003	<.0001
ADF (g/kg DM)	<.0001	<.0001	<.0001	<.0001
NDF (g/kg DM)	<.0001	<.0001	<.0001	<.0001
OMD (g/kg DM)	<.0001	<.0001	<.0001	<.0001
Ca[1] (mg/kg DM)	<.0001	<.0001	<.0001	<.0001
P[1] (mg/kg)	<.0001	<.0001	0.4571	<.0001
Na[1] (mg/kg)	<.0001	<.0001	<.0001	<.0001
Catalpol[1]	n/a	<.0001	<.0001	n/a
Aucubin[1]	n/a	0.0003	0.9933	n/a
Acteoside[1]	n/a	0.0208	0.7380	n/a
Crop[2] (% of total DM)	<.0001	<.0001	<.0001	<.0001
Dead materials[2] (% of total DM)	<.0001	<.0001	<.0001	<.0001
White clover[2] (% of total DM)	<.0001	<.0001	<.0001	<.0001
Weed[2] (% of total DM)	<.0001	<.0001	<.0001	<.0001

n/a: not applicable as it was measured only in one treatment; [1]Not measured in winter and spring of Year 1 and summer, late-autumn, winter and spring of Year 3; [2]Not measured in winter of Year 1.

Perennial ryegrass/WC had higher (P<0.05) DM% compared to IRWC in the spring of Year 1 and summer, late-autumn and winter of Year 3 (Figure 5.1). The PWC had lower (P<0.05) DM% compared to RGWC in all seasons it was measured. Compared to RGWC, brassica had lower (P<0.05) DM% in the winter of Years 2 and 3.

The CP concentrations in RGWC and IRWC did not differ (P>0.05) within seasons for a given year except winter of Year 1 and late-autumn and spring of Year 3. Compared to IRWC, RGWC had greater (P<0.05) CP concentration in spring and lower (P<0.05) CP concentration in late-autumn of Year 3 (Table 5.4). In the summer of Years 2 and 3, PWC had higher (P<0.05) CP concentrations compared to RGWC and IRWC, whereas in the spring of Year 3, compared to RGWC, PWC had a lower (P<0.05) CP concentration. Brassica had lower (P<0.05) CP concentrations compared to other treatments in winter of Year 2 and winter and late-autumn of Year 3. The CP concentration of brassica in winter of Year 1 was double (P<0.05) than that in winter of Year 2 and late-autumn and winter of Year 3.

Apparent N intakes of ewes grazing RGWC and IRWC did not differ (P>0.05) within the seasons of a given year (Figure 5.2). Ewes grazing PWC had greater (P<0.05) apparent N intake compared to ewes grazing RGWC in the spring of Year 2 and summer of Year 3 and compared to ewes grazing IRWC in late-autumn and spring of Year 2 and summer of Year 3. Ewes grazing brassica had greater (P<0.05) apparent N intakes compared to the ewes grazing IRWC and RGWC in the winter of Year 1. Further, in contrast, the apparent N intakes of ewes grazing brassica were lower (P<0.05) compared to the apparent N intakes of ewes grazing RGWC and IRWC in winter of Year 2 and late-autumn of Year 3.

There were no differences (P>0.05) in SSS:CP ratios among RGWC, IRWC and PWC treatments in all seasons it was measured, except late-autumn of Year 2 and summer and spring of Year 3 (Figure 5.3). On the other hand, the brassica treatment had higher (P<0.05) SSS: CP and SSS: NDF ratios compared to other treatments in all seasons it was measured (Figures 5.3 and 5.4).

Within the seasons of a given year, PWC had greater ($P<0.05$) Na concentrations compared to other forage treatments. Compared to RGWC and IRWC, PWC had greater ($P<0.05$) Ca concentrations within the seasons of a given year (Table 5.5). Brassica had higher ($P<0.05$) Ca concentrations in the late-autumn and winter of experimental Year 3, with the Ca concentration of brassica only measured in the third year of the experiment.

The concentration of bioactive compounds naturally present in plantain samples harvested in Years 2 and 3 are presented in Table 5.5. Among the measured compounds, catalpol was detected at low concentrations ranging from 0.06 to 0.60 mg/g DM, whereas acteoside was detected at high concentrations ranging from 8 to 25 mg/g DM.

The botanical composition (grass, clover, weeds and dead material) for the RGWC, IRWC and PWC treatments is given in Table 5.6. Perennial ryegrass/WC and IRWC had high grass (>69%) contents in most seasons (except the summer of Year 2). Initially (spring of Year 1 and summer and late-autumn of Year 2), the PWC treatment had high (>69%) plantain content, but weed content increased significantly for the remainder of Year 2 and all of Year 3. Overall, the WC percentages of RGWC and IRWC were small and accounted for less than 4% of botanical composition. Although, PWC had around 2-6% of WC in summer and late-autumn of Year 2 and winter and spring of Year 3, relatively greater (approximately 15-20%) percentage of WC was observed in the spring of Year 2 and summer of Year 3.

Table 5.3 Pre-grazing forage mass (kg DM/ha) and apparent dry matter intake (DMI; kg DM ewe/day) of forages[1] (RGWC: perennial ryegrass/white clover; IRWC: Italian ryegrass/white clover; PWC: plantain/white clover; brassica) in winter and spring of Year 1 (July to December 2019) and summer, late-autumn, winter and spring of Years 2 (January to December 2020), and 3 (January to December 2021). Values are least-squares means.

Measure	Forage type	Year 1		Year 2				Year 3				SEM
		Winter	Spring	Summer	Late-Autumn	Winter	Spring	Summer	Late-Autumn	Winter	Spring	
Pre-grazing forage mass (kg DM/ha)	RGWC	4711^{e}	3288^{ghi}	3848^{fg}	3420^{ghi}	3434^{gh}	2442^{lm}	2837^{ijkl}	2109^{mn}	1380^{o}	1920^{n}	177
	IRWC	5529^{d}	3584^{g}	4312^{ef}	3023^{hijk}	3296^{ghi}	2496^{klm}	2563^{ijklm}	974^{o}	923^{o}	2027^{mn}	
	PWC	nm	3034^{hij}	2501^{jklmn}	2390^{lmn}	nm	3050^{hi}	2879^{ijkl}	nm	1039^{o}	2157^{mn}	
	Brassica	8012^{b}	-	-	-	11406^{a}	-	-	6386^{c}	-	-	
Apparent DMI[2] (kg/ewe/d)	RGWC	2.47^{bcd}	2.88^{ab}	2.80^{abc}	2.13^{bcdef}	2.40^{bcde}	1.49^{defghi}	0.80^{ghi}	2.06^{bcdef}	0.98^{hi}	0.81^{ghi}	0.34
	IRWC	2.85^{ab}	2.98^{ab}	2.06^{bcdef}	1.87^{bcdef}	2.0^{bcdef}	1.69^{defg}	0.60^{hi}	1.57^{efgh}	1.50^{efghi}	0.52^{i}	
	PWC	nm	3.16^{a}	1.91^{bcdef}	2.57^{abcd}	nm	2.87^{ab}	2.18^{bcdef}	nm	1.12^{ghi}	0.58^{hi}	
	Brassica	3.62^{a}	-	-	-	1.56^{efgh}	-	-	1.36^{fghi}	-	-	

[a-o]Superscripts with different letters indicate that means are significantly different among forage treatments within each parameter ($P<0.05$); nm: not measured as PWC were not grazed in winter of Years 1 and 2, and late-autumn of Year 3; -: no forage was present to sample as brassica was only present in winter for Years 1 and 2, and late-autumn and winter in Year 3.

[1]The pre-grazing forage mass and DMI reported here are only from one of several grazing events per season.

[2]Apparent DMI = this was calculated based on Mathews et al., (1999) by dividing the difference between pre-and post-grazing DM mass for each grazing event by the number of grazing days.

Table 5.4 Chemical composition[1] (CP: crude protein g/kg DM; ME: metabolisable energy MJ/kg DM; SSS: soluble sugars and starch g/kg DM; ADF: acid detergent fibre g/kg DM; NDF: neutral detergent fibre g/kg DM) of forages (RGWC: perennial ryegrass/white clover; IRWC: Italian ryegrass/white clover; PWC: plantain/white clover; brassica) in winter and spring of Year 1 (July to December 2019) and summer, late-autumn, winter and spring of Years 2 (January to December 2020), and 3 (January to December 2021). Values are least-squares means.

Measure	Forage type	Year 1		Year 2				Year 3				SEM
		Winter	Spring	Summer	Late-Autumn	Winter	Spring	Summer	Late-Autumn	Winter	Spring	
CP (g/kg DM)	RGWC	194^{ij}	210^{ghi}	113^{n}	213^{ghi}	284^{c}	244^{ef}	190^{ij}	303^{b}	280^{cd}	204^{hi}	6.62
	IRWC	151^{klm}	203^{hi}	122^{n}	222^{gh}	288^{bc}	243^{ef}	182^{j}	341^{a}	277^{cd}	168^{kl}	
	PWC	nm	219^{gh}	143^{m}	228^{fg}	nm	262^{de}	229^{fg}	nm	269^{cd}	182^{jk}	
	Brassica	224^{fgh}	-	-	-	85^{p}	-	-	89^{op}	107^{no}	-	
ME (MJ/kg DM)	RGWC	11.1^{ij}	10.8^{jk}	9.19^{m}	10.6^{k}	11.9^{efg}	11.3^{hi}	10.9^{jk}	11.6^{gh}	12.1^{bcdef}	12.1^{cdef}	0.13
	IRWC	10.5^{kl}	10.0^{l}	8.57^{n}	11.4^{h}	12.0^{defg}	11.3^{hi}	11.1^{ij}	12.4^{b}	12.4^{bc}	12.2^{bcde}	
	PWC	nm	10.7^{jk}	9.43^{m}	10.8^{jk}	nm	10.8^{jk}	11.4^{hi}	nm	12.2^{bcde}	11.8^{fg}	
	Brassica	12^{cdefg}	-	-	-	13.1^{a}	-	-	12.3^{bcd}	12.1^{cdef}	-	
SSS (g/kg DM)	RGWC	164^{ef}	119^{h}	86^{jkl}	89^{jk}	72^{klm}	114^{hi}	127^{gh}	81^{klm}	162^{ef}	150^{f}	6.10
	IRWC	201^{d}	71^{lm}	66^{m}	138^{g}	69^{lm}	119^{hi}	121^{gh}	70^{lm}	194^{d}	182^{ed}	
	PWC	nm	124^{gh}	99^{ij}	72^{lm}	nm	64^{m}	101^{ij}	nm	169^{e}	174^{e}	
	Brassica	na	-	-	-	436^{a}	-	-	342^{b}	311^{c}	-	
ADF (g/kg DM)	RGWC	231^{ef}	233^{ef}	320^{b}	239^{ef}	173^{kl}	205^{gh}	237^{ef}	174^{kl}	176^{kl}	194^{ghi}	5.31
	IRWC	264^{cd}	207^{g}	348^{a}	167^{l}	171^{l}	201^{gh}	242^{ef}	136^{o}	146^{no}	191^{hij}	
	PWC	nm	247^{ef}	269^{c}	231^{e}	nm	178^{ijkl}	180^{ijkl}	nm	147^{no}	186^{ijk}	
	Brassica	167^{lm}	-	-	-	108^{p}	-	-	152^{mn}	132^{o}	-	
NDF (g/kg DM)	RGWC	424^{def}	467^{c}	578^{b}	481^{c}	382^{gh}	437^{d}	456^{d}	393^{ef}	360^{gh}	407^{ef}	8.39
	IRWC	481^{c}	357^{h}	627^{a}	284^{j}	384^{fg}	436^{d}	472^{c}	357^{h}	321^{i}	383^{fg}	
	PWC	nm	486^{c}	401^{ef}	471^{c}	nm	328^{i}	309^{i}	nm	258^{k}	357^{h}	
	Brassica	213^{l}	-	-	-	149^{n}	-	-	179^{m}	189^{lm}	-	

[a-p]Superscripts with different letters indicate that means are significantly different among forage treatments within each parameter across years (P<0.05); nm: not measured as PWC was not grazed in winter of Years 1 and 2, and late-autumn of Year 3; -: no forage was present to sample as brassica was only present in winter of Years 1 and 2, and late-autumn and winter of Year 3.

[1]The chemical composition of forages was determined only once per season.

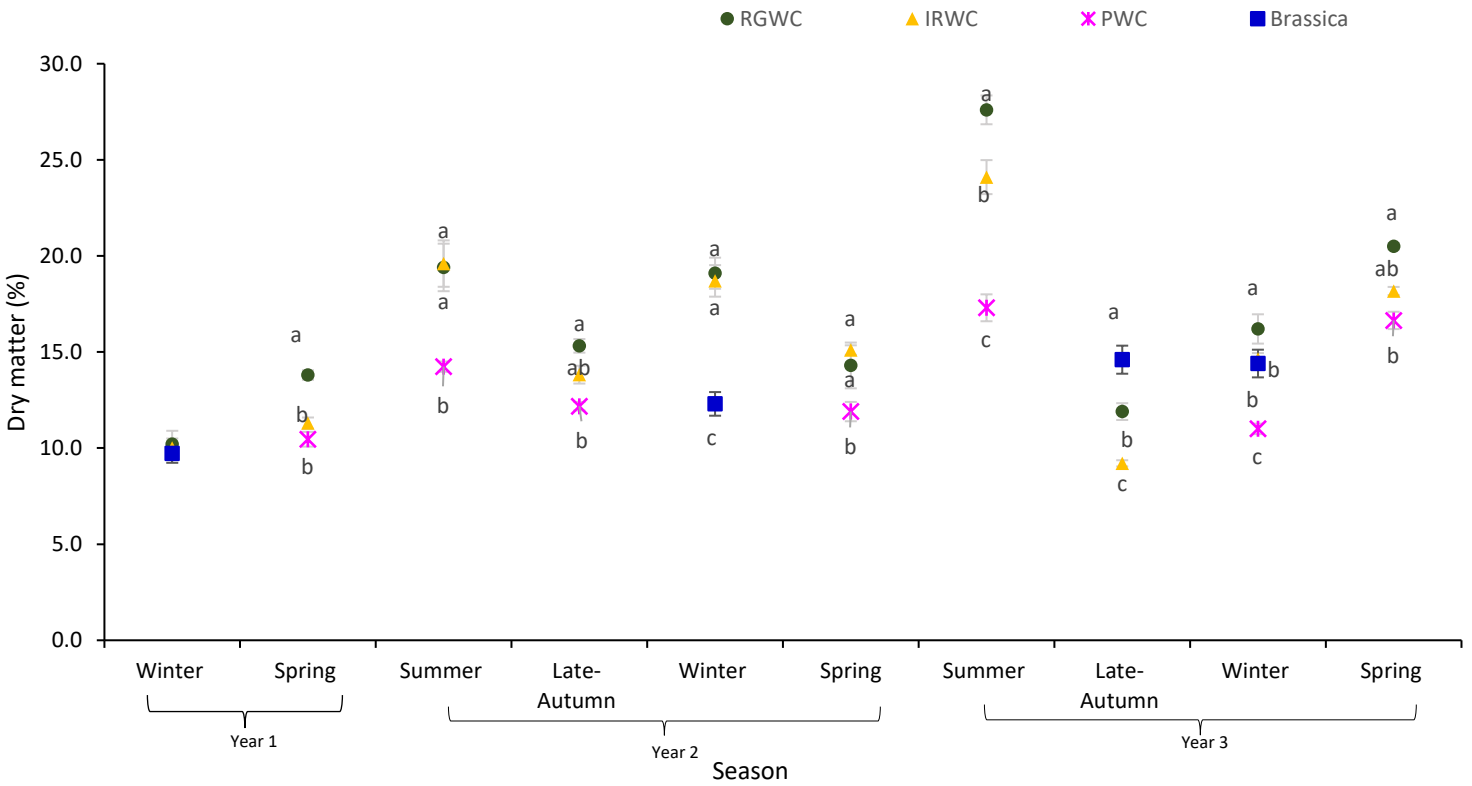

Figure 5.1 Dry matter percentages (least-squares mean ± SE) of forages (RGWC: perennial ryegrass/white clover; IRWC: Italian ryegrass/white clover; PWC: plantain/white clover; brassica) in winter and spring of Year 1 (July to December 2019) and summer, late-autumn, winter and spring of Years 2 (January to December 2020), and 3 (January to December 2021). [abc]superscripts with different letters indicate that means are significantly different among forage treatments within each season ($P<0.05$). The DM % of forages were determined only once per season. Brassica was only present in the winter of Years 1 and 2 and late-autumn and winter of Year 3, and ewes did not graze PWC in the winter of Years 1 and 2 and late-autumn of Year 3.

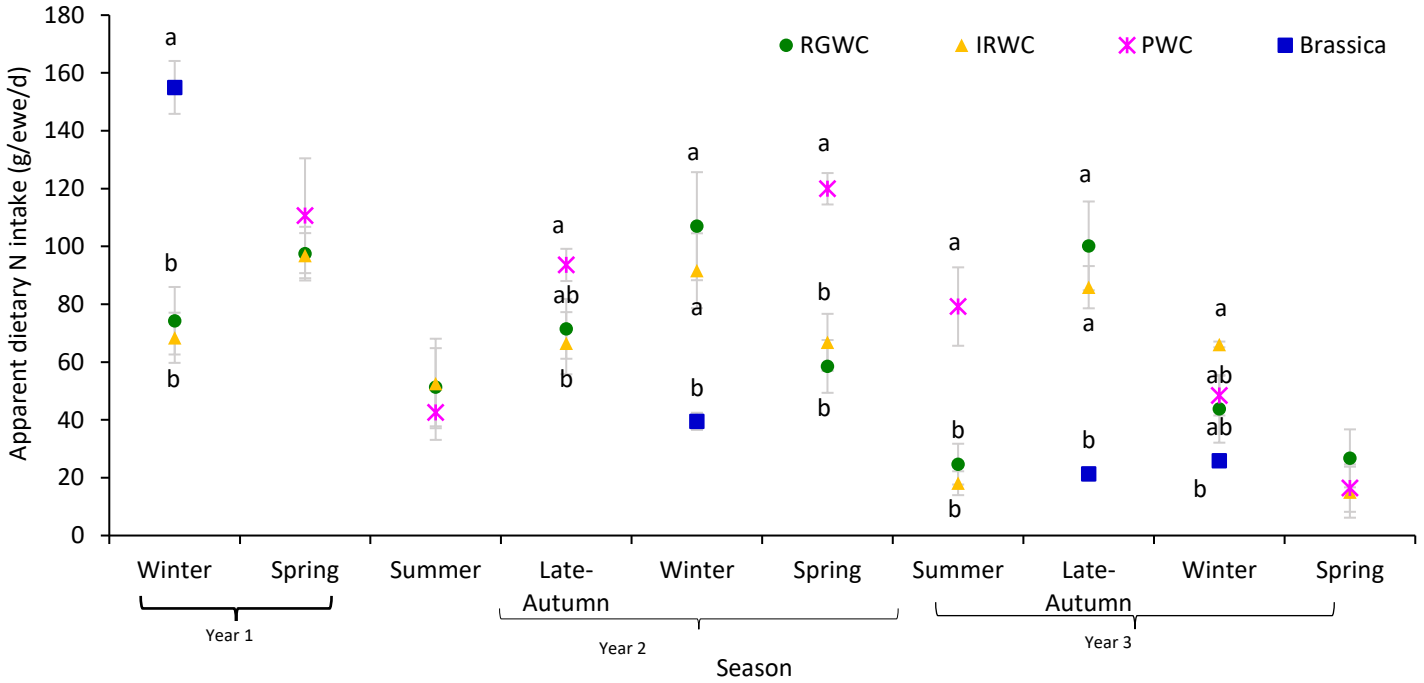

Figure 5.2 Apparent dietary nitrogen (N) intake (g/ewe/d; least-squares mean ± SE) of forages (RGWC: perennial ryegrass/white clover; IRWC: Italian ryegrass/white clover; PWC: plantain/white clover; brassica) in winter and spring of Year 1 (July to December 2019) and summer, late-autumn, winter and spring of Years 2 (January to December 2020), and 3 (January to December 2021). ^{abc}superscripts with different letters indicate that means are significantly different among forage treatments within each season ($P<0.05$). The apparent dietary N intake reported here are only from one of several grazing events per season. Brassica was only present in the winter of Years 1 and 2 and late-autumn and winter of Year 3, and ewes did not graze PWC in the winter of Years 1 and 2 and late-autumn of Year 3.

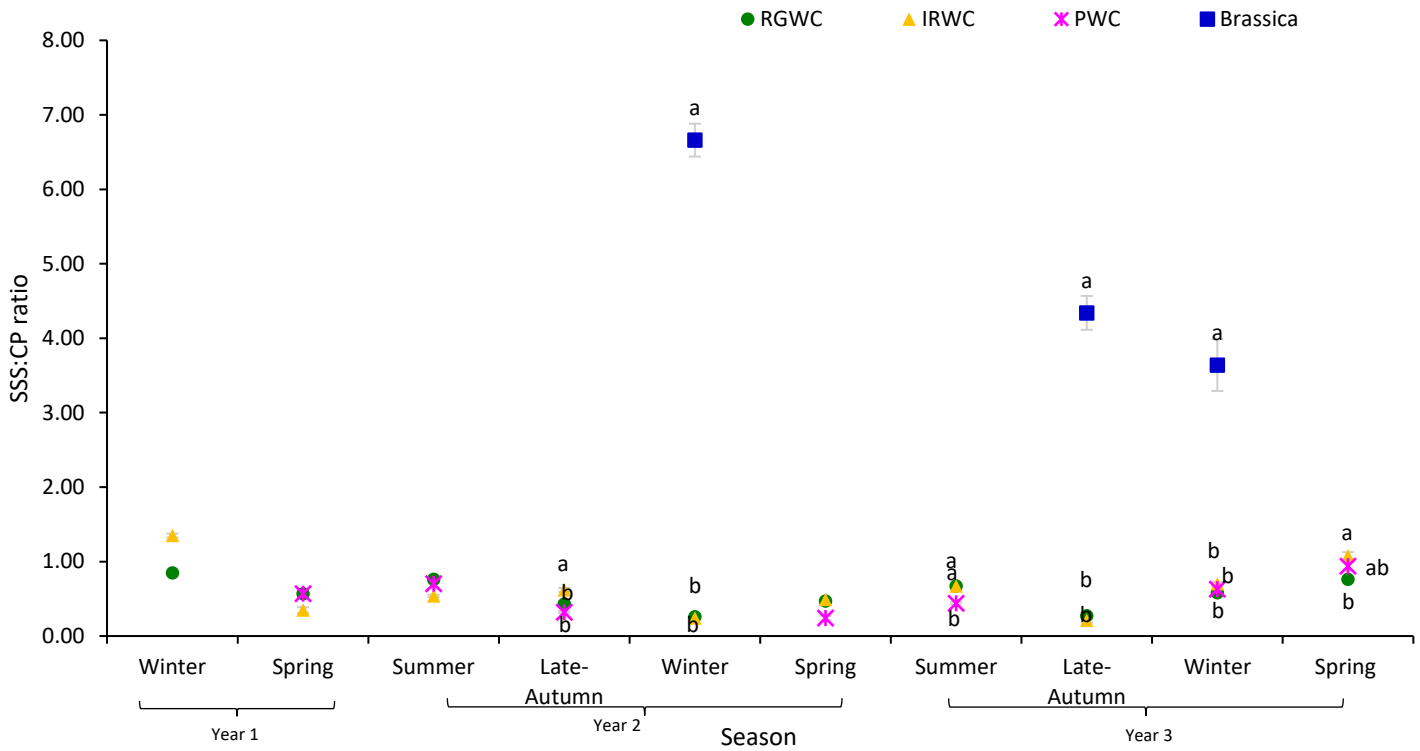

Figure 5.3 Soluble sugars and starch (SSS):crude protein (CP) ratio (least-squares mean ± SE) of forages (RGWC: perennial ryegrass/white clover; IRWC: Italian ryegrass/white clover; PWC: plantain/white clover; brassica) in winter and spring of Year 1 (July to December 2019) and summer, late-autumn, winter and spring of Years 2 (January to December 2020), and 3 (January to December 2021). [abc]superscripts with different letters indicate that means are significantly different among forage treatments within each season ($P<0.05$). The SSS:CP ratio of forages were determined only once per season. The SSS content of brassica was not measured in Year 1. Brassica was only present in the winter of Years 1 and 2 and late-autumn and winter of Year 3, and ewes did not graze PWC in the winter of Years 1 and 2 and late-autumn of Year 3.

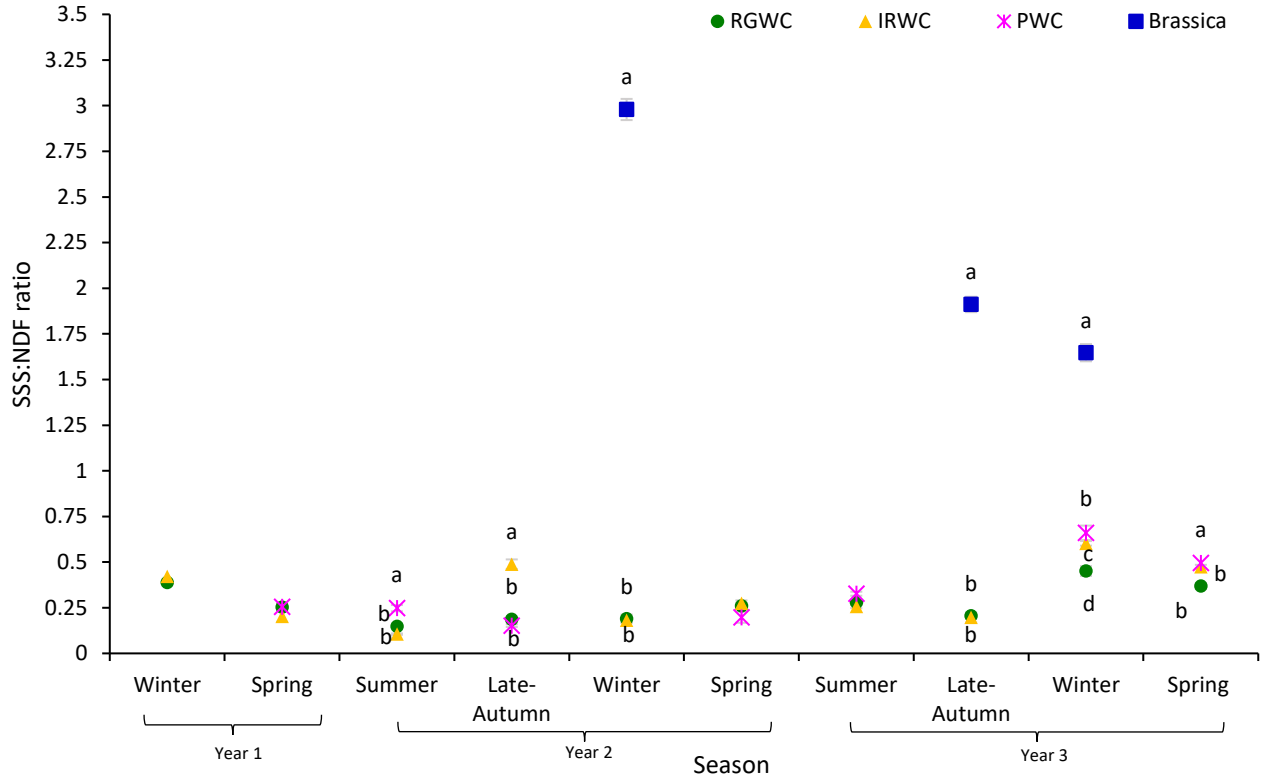

Figure 5.4 Soluble sugars and starch (SSS):neutral detergent fibre (NDF) ratio (least-squares mean ± SE) of forages (RGWC: perennial ryegrass/white clover; IRWC: Italian ryegrass/white clover; PWC: plantain/white clover; brassica) in winter and spring of Year 1 (July to December 2019) and summer, late-autumn, winter and spring of Years 2 (January to December 2020), and 3 (January to December 2021). [abc]superscripts with different letters indicate that means are significantly different among forage treatments within each season ($P<0.05$). The SSS:NDF ratio of forages were determined only once per season. Brassica was only present in the winter of experimental Years 1 and 2 and the late-autumn and winter of Year 3, and ewes did not graze PWC in winter of Years 1 and 2 and late-autumn of Year 3.

Table 5.5 Mean forage mineral concentrations[1] (calcium: Ca; phosphorus: P; sodium: Na; mg/g DM) for all forages (RGWC: perennial ryegrass/white clover; IRWC: Italian ryegrass/white clover; PWC: plantain/white clover; brassica) and bioactive glycoside (catalpol; aucubin; acteoside; mg/g DM) concentrations[1] of PWC only in late-autumn and spring of Year 2 (January to December 2020) and summer, late-autumn, winter and spring of Year 3 (January to December 2021). Values are least-squares means.

		Year 2		Year 3				SEM
		Late-Autumn	Spring	Summer	Late-Autumn	Winter	Spring	
Ca	RGWC	6.2^{ef}	6.0^{ef}	6.6^{e}	5.3^{ef}	4.6^{fg}	6.5^{e}	0.55
	IRWC	5.4^{ef}	5.8^{ef}	5.9^{ef}	4.6^{fg}	3.6^{g}	6.5^{e}	
	PWC	17.3^{b}	16.9^{c}	19.0^{a}	nm	10.5^{d}	9.2^{d}	
	Brassica	-	-	-	13.2^{c}	14.1^{c}	-	
P	RGWC	4.8^{c}	4.5^{cd}	3.8^{e}	6.1^{a}	5.3^{b}	4.0^{e}	0.10
	IRWC	5.3^{b}	4.8^{c}	3.9^{e}	6.0^{a}	4.5^{d}	3.8^{e}	
	PWC	4.6^{cd}	4.8^{cd}	4.5^{cd}	nm	4.7^{cd}	3.9^{e}	
	Brassica	-	-	-	2.8^{g}	3.3^{f}	-	
Na	RGWC	4.3^{e}	3.4^{fg}	2.7^{gh}	2.7^{gh}	2.7^{gh}	2.1^{hij}	0.30
	IRWC	4.3^{ef}	2.5^{ghi}	1.7^{jkl}	5.8^{d}	4.0^{ef}	1.8^{ijk}	
	PWC	8.0^{ab}	7.6^{bc}	8.9^{a}	nm	6.9^{c}	4.5^{e}	
	Brassica	-	-	-	1.1^{kl}	0.8^{l}	-	
Catalpol	PWC	0.1^{c}	0.6^{a}	0.1^{bc}	nm	0.3^{b}	0.1^{bc}	0.06
Aucubin	PWC	4.3^{bc}	5.9^{b}	2.8^{c}	nm	8.5^{a}	4.0^{bc}	0.79
Acteoside	PWC	8.3^{c}	24.8^{a}	23.0^{ab}	nm	12.2^{bc}	18.2^{abc}	4.00

[a-c]Superscripts with different letters indicate that means are significantly different among forage treatments within each parameter across years ($P<0.05$); nm: not measured as PWC was not grazed in late-autumn of Year 3; -: no forage was present to sample as brassica was only present in winter of Year 2, and late-autumn and winter of Year 3.

[1] Mineral composition and bioactive glycoside concentrations of forages were determined only once per season.

Table 5.6 Botanical composition[1] (% of total DM) of the forages (RGWC: perennial ryegrass/white clover; IRWC: Italian ryegrass/white clover; PWC: plantain/white clover) in spring of Year 1 (July to December 2019) and summer, late-autumn, winter and spring of Years 2 (January to December 2020), and 3 (January to December 2021). Values are least-squares means.

		Year 1	Year 2				Year 3				SEM
		Spring	Summer	Late-Autumn	Winter	Spring	Summer	Late-Autumn	Winter	Spring	
Grass/herb	RGWC	80.5^{defg}	54.3^{i}	74.7^{fgh}	82.5^{bcdef}	84.6^{bcdef}	73.3^{gh}	92.3^{abc}	93.6^{ab}	93.0^{ab}	3.7
	IRWC	87.6^{abcde}	55.0^{i}	79.0^{efgh}	94.1^{ab}	94.8^{ab}	78.0^{efgh}	93.5^{ab}	95.8^{a}	94.9^{a}	
	PWC	90.1^{abcd}	69.5^{h}	78.8^{efgh}	nm	47.2^{ij}	46.8^{ij}	nm	55.8^{i}	42.3^{j}	
White clover	RGWC	0.3^{e}	0.2^{e}	2.5^{cde}	0.1^{e}	1.1^{cde}	4.0^{cde}	1.5^{cde}	0.6^{de}	3.3^{cde}	1.4
	IRWC	0.1^{e}	0.1^{e}	0.3^{e}	0.4^{e}	0.1^{e}	1.7^{cde}	1.4^{cde}	1.1^{cde}	2.9^{cde}	
	PWC	0.6^{de}	6.2^{c}	4.7^{cd}	nm	19.7^{a}	15.3^{a}	nm	2.2^{cde}	4.9^{c}	
Dead materials	RGWC	2.8^{ghij}	40.0^{b}	18.4^{cd}	5.7^{fgh}	5.7^{fg}	12.4^{e}	2.7^{ghij}	2.7^{ghij}	2.8^{ghij}	1.4
	IRWC	2.0^{ghij}	44.7^{a}	20.9^{c}	5.5^{fgh}	4.7^{fghi}	14.8^{de}	0.3^{j}	1.7^{hij}	2.1^{ghij}	
	PWC	7.9^{f}	21.3^{c}	5.8^{fg}	nm	1.2^{ij}	0.1^{j}	ns	0.8^{ij}	0.7^{j}	
Weeds	RGWC	16.4^{d}	5.5^{efgh}	4.5^{efgh}	11.8^{de}	8.7^{defg}	10.4^{def}	3.5^{fgh}	3.0^{fgh}	0.8^{gh}	2.9
	IRWC	10.4^{def}	0.3^{h}	0.1^{h}	0.1^{h}	0.4^{h}	5.5^{efgh}	4.8^{efgh}	1.4^{gh}	0.1^{h}	
	PWC	1.4^{gh}	3.1^{fgh}	4.0^{efgh}	nm	26.2^{c}	30.1^{c}	nm	41.6^{b}	52.1^{a}	

[a-j]Superscripts with different letters indicate that means are significantly different among forage treatments within each parameter across years ($P<0.05$); nm: not measured as PWC was not grazed in winter of Years 2 and late-autumn of Year 3. [1] Botanical composition of forages were determined only once per season.

5.4.2 Plasma, faecal and urinary nitrogen

The results of the main effect for urine, plasma and faecal N compositions are shown in Appendix 7. Overall, ewes grazing PWC and brassica treatments had lower ($P<0.05$) urine N, NH_3, urea N, and creatinine concentrations compared with ewes grazing RGWC. Urine N, NH_3 and urea N concentrations of ewes grazing IRWC were lower ($P<0.05$) than those grazing RGWC. The ewes in RGWC had 30 to 40% lower ($P<0.05$) estimated total urine volumes than those in other forages. Based on urine creatinine concentrations, ewes grazing brassica had lower ($P<0.05$) urinary N excretion than those grazing other forages. However, based on the plasma urea N concentration, the estimated N excretion of ewes grazing PWC and brassica were lower ($P<0.05$) than in RGWC. Across all forages, higher ($P<0.05$) urinary N composition and lower ($P<0.05$) daily urine output were observed in summer. However, greater ($P<0.05$) urinary N excretions were estimated in late-autumn and winter seasons than in other seasons.

Urine, plasma and faeces N characteristics of spot samples showed a significant ($P<0.05$) three-way interaction among year, treatment and season, whereas the three-way interaction for creatinine excretion factor and uric acid concentration were not significant ($P>0.05$; Table 5.7).

Table 5.7 P-value of main effects (forage treatment, year, season) and their three-way interaction (Treatment: T*Season: S*Year: Y) of spots samples of urine, plasma and faecal N compositions of breeding ewes grazing perennial ryegrass/white clover, Italian ryegrass/white clover, plantain/white clover and brassica in winter and spring of Year 1 (July to December 2019) and summer, late-autumn, winter and spring of Years 2 (January to December 2020), and 3 (January to December 2021).

	Forage treatment	Season	Year	T*S*Y
Urine				
Urine N concentration* (g N/L)	<.0001	<.0001	0.0100	0.0007
NH_3 (mmol/L)	<.0001	<.0001	0.0481	<.0001
Urea N* (mmol/L)	<.0001	<.0001	0.0308	<.0001
Creatinine (mmol/L)	<.0001	<.0001	0.0900	0.010
Creatinine excretion factor	0.94	0.3200	0.2600	0.0800
Estimated urine volume (L/ewe/d)	<.0001	<.0001	0.0005	<.0001
Urine N%	<.0001	<.0001	0.0100	0.0007
Estimated urinary N excretion (g/ewe/ d)	<.0001	<.0001	<.0001	<.0001
Purine derivatives (PD)				
Uric acid (mmol/L)	0.0017	<0.0001	0.0292	0.0687
Allantoin (mmol/L)	<0.0001	<0.0001	0.0220	0.0026
Total PD (mmol/L)	<0.0001	<0.0001	0.0054	0.0002
PD index	0.0446	<.0001	<.0001	<.0001
Plasma				
Plasma urea N (mmol/L)	<.0001	<.0001	<.0001	<.0001
Creatinine (μmol/L)	0.0028	<.0001	0.031	<.0001
Uric acid* (μmol/L)	0.0215	0.4568	<.0001	<.0001
Estimated urinary N excretion (g/ewe/d)	<.0001	<.0001	<.0001	<.0001
Faecal N[1] %	<.0001	<.0001	<.0001	<.0001

* P values for log-transformed data; [1]Data collected in winter and spring of Year 1 are not included.

Table 5.8 Composition of N in spot samples of urine sampled from breeding ewes grazing perennial ryegrass/white clover (RGWC), Italian ryegrass/white clover (IRWC), plantain/white clover (PWC) and brassica in winter and spring of Year 1 (July to December 2019) and summer, late-autumn, winter and spring of Years 2 (January to December 2020), and 3 (January to December 2021). Values are least-squares means.

		Year 1		Year 2				Year 3				SEM
		Winter	Spring	Summer	Late-Autumn	Winter	Spring	Summer	Late-Autumn	Winter	Spring	
NH_3 (mmol/L)	RGWC	1.8^{e}	0.9^{e}	10.5^{a}	nd	0.2^{e}	7.3^{b}	8.6^{ab}	4.3^{cd}	4.3^{cd}	2.1^{de}	0.79
	IRWC	0.5^{e}	0.6^{e}	6.4^{bc}	2.0^{de}	nd	2.1^{de}	8.6^{ab}	1.0^{e}	0.9^{e}	1.5^{e}	
	PWC	nm	0.8^{e}	2.6^{de}	2.0^{de}	nm	0.3^{e}	1.1^{e}	nm	0.3^{e}	1.3^{e}	
	Brassica	0.4^{e}	nm	nm	nm	nd	nm	nm	1.53^{e}	0.8^{e}	nm	
Urea N* (mmol/L)	RGWC	88.0^{efghij}	$71.8^{ghijklm}$	210.1^{ab}	$76.7^{fghijkl}$	57.5^{ijklmn}	153.5^{abcde}	148.1^{abcdef}	$113.3^{bcdefghi}$	250.7^{a}	172.5^{abc}	1.27
	IRWC	41.9^{lmnop}	44.6^{hijklm}	208.0^{ab}	$126.0^{bcdefgh}$	$45.6^{jklmnop}$	168.2^{abcd}	165.0^{abcd}	$82.2^{efghijk}$	$144.5^{abcdefg}$	44.1^{klmnop}	
	PWC	nm	66.3^{hijklm}	$116.0^{bcdefgh}$	37.6^{mnopq}	nm	$91.4^{cdefghij}$	$123.1^{bcdefgh}$	nm	$51.3^{ijklmno}$	30.9^{nopq}	
	Brassica	28.6^{opqr}	nm	nm	nm	17.4^{r}	nm	nm	20.3^{qr}	26.0^{pqr}	nm	
Creatinine (mmol/L)	RGWC	1.4^{fghi}	1.8^{efg}	4.4^{a}	0.6^{hij}	0.7^{hij}	1.6^{efgh}	2.6^{bcde}	0.6^{hij}	2.1^{cdef}	2.6^{bcd}	0.35
	IRWC	0.5^{ij}	0.7^{hij}	3.5^{ab}	1.3^{fghij}	0.4^{ij}	1.8^{cdefg}	3.4^{ab}	0.3^{j}	0.8^{hij}	1.1^{ghij}	
	PWC	nm	0.7^{hij}	2.9^{bc}	0.6^{hij}	nm	0.6^{hij}	1.6^{efgh}	nm	0.5^{ij}	0.4^{ij}	
	Brassica	0.4^{ij}	nm	nm	nm	1.1^{ghij}	nm	nm	0.52^{ij}	0.6^{hij}	nm	
Creatinine excretion factor (mg/kg/LW)[1]	RGWC	8.1^{ab}	8.1^{ab}	8.0^{b}	8.0^{b}	8.1^{ab}	8.1^{ab}	8.1^{ab}	8.2^{a}	8.0^{c}	8.1^{ab}	0.04
	IRWC	8.1^{ab}	8.1^{ab}	8.1^{ab}	8.1^{ab}	8.1^{ab}	8.2^{a}	8.1^{ab}	8.1^{ab}	8.1^{ab}	8.0^{b}	
	PWC	nm	8.1^{ab}	8.2^{a}	8.1^{ab}	nm	8.1^{ab}	8.1^{ab}	nm	8.1^{ab}	8.0^{b}	
	Brassica	8.1^{ab}	nm	nm	nm	8.1^{ab}	nm	nm	8.1^{ab}	8.0^{c}	nm	
Estimated urine N excretion (g/ewe/d)[2]	RGWC	$13.3^{efghijk}$	10.9^{ijkl}	6.0^{mn}	12.0^{ghijk}	15.7^{cdefgh}	15.5^{cdefgh}	9.7^{klm}	29.9^{a}	20.0^{bc}	16.9^{cdef}	1.58
	IRWC	$13.1^{efghijk}$	12.0^{hijk}	7.2^{mnl}	16.4^{cdefgh}	17.9^{bcd}	$14.6^{defghij}$	10.2^{jklm}	32.9^{a}	22.1^{b}	12.7^{fghijk}	
	PWC	nm	17.2^{cde}	6.8^{mnl}	10.3^{ijklm}	nm	9.1^{klm}	14.7^{defghi}	nm	17.1^{cdef}	15.9^{cdefgh}	
	Brassica	11.0^{ijkl}	nm	nm	nm	3.8^{n}	nm	nm	6.8^{mnl}	6.6^{mnl}	nm	

[a-q]Superscripts with different letters indicate that means are significantly different among forage treatments within each parameter across years ($P<0.05$); nm: not measured in all the seasons as ewes did not graze PWC in winter of experimental Years 1 and 2 and late-autumn of Year 3, and brassica was grazed only in winter of Years 1 and 2 and late-autumn and winter of Year 3; nd: not detected.

* Back transformed urinary N and urea N concentrations (mean and SEM); [1]Creatinine excretion factor (mg/kg LW) was calculated using Brody's equation: Mean daily creatinine excretion (mg) = ($12.7 \times LW^{0.896}$); LW: live weight (Brody 1945; David et al. 2015). [2]Urinary N excretion (g/day) = creatinine excretion factor (mg/kg) × live weight (kg) × $\left(\frac{1}{\text{urinary creatinine (mg/kg)}}\right)$ × urine N (g/kg), based on David et al. (2015).

Table 5.9 Nitrogen (N) composition of plasma collected from breeding ewes grazing perennial ryegrass/white clover (RGWC), Italian ryegrass/white clover (IRWC), plantain/white clover (PWC) and brassica in winter and spring of Year 1 (July to December 2019) and summer, late-autumn, winter and spring of Years 2 (January to December 2020), and 3 (January to December 2021). Values are least-squares means.

		Year 1		Year 2				Year 3				SEM
		Winter	Spring	Summer	Late-Autumn	Winter	Spring	Summer	Late-Autumn	Winter	Spring	
Urea N (mmol/L)	RGWC	6.9^{efghij}	6.2^{hijkl}	4.6^{lmno}	6.9^{efghij}	7.1^{defghi}	6.7^{efghijk}	8.0^{cdef}	10.2^{ab}	9.3^{bc}	6.7^{efghijk}	0.54
	IRWC	6.1^{hijklm}	6.4^{ghijk}	6.0^{ijklm}	4.6^{lmno}	8.5^{cd}	8.2^{cde}	6.6^{fghijk}	11.7^{a}	7.7^{cdefg}	5.1^{klmn}	
	PWC	nm	6.7^{efghijk}	4.6^{lmno}	6.5^{ghijk}	nm	7.5^{defgh}	7.1^{defghi}	nm	7.5^{defghi}	4.3^{nop}	
	Brassica	5.4^{jklmn}	nm	nm	nm	2.1^{q}	nm	nm	3.0^{pq}	3.5^{opq}	nm	
Creatinine (µmol/L)	RGWC	73.8^{cdefgh}	72.8^{cdefgh}	89.5^{a}	80.1^{abcd}	63.6^{hijkl}	63.3^{hijkl}	73.1^{cdefgh}	57.4^{jkl}	81.8^{abc}	74.1^{cdefgh}	3.69
	IRWC	76.6^{cdef}	74.8^{cdefg}	87.8^{ab}	73.9^{cdefgh}	70.0^{defghi}	64.4^{ijkl}	78.1^{bcde}	56.6^{l}	60.9^{ijkl}	76.4^{cdef}	
	PWC	nm	67.2^{fghijk}	77.5^{bcdef}	64.8^{ghijkl}	nm	60.0^{jkl}	72.5^{cdefgh}	nm	61.0^{ijkl}	71.3^{defghi}	
	Brassica	69.8^{defghi}	nm	nm	nm	78.0^{bcde}	nm	nm	68.7^{efghi}	73.9^{cdefgh}	nm	
Uric acid* µmol/L	RGWC	12.2^{b}	12.0^{bcd}	12.0^{bcd}	11.9^{bcde}	11.9^{bcde}	11.8^{cdef}	11.7^{cdefgi}	11.6^{efgh}	11.5^{fghi}	11.4^{i}	0.13
	IRWC	12.0^{bcd}	12.2^{b}	12.0^{bcd}	11.9^{bcde}	11.8^{cdef}	11.8^{cdef}	11.7^{cdefgi}	11.6^{efgh}	11.5^{fghi}	11.4^{i}	
	PWC	nm	12.2^{b}	12.0^{bcd}	11.9^{bcde}	nm	11.7^{cdefg}	11.6^{efghi}	nm	11.5^{fghi}	13.1^{a}	
	Brassica	12.0^{bcd}	nm	nm	nm	11.8^{cdef}	nm	nm	11.6^{efgh}	11.5^{fghi}	nm	
Estimated urine N excretion (g/ewe/d)[1]	RGWC	17.7^{efgh}	15.6^{fghij}	11.6^{klmn}	17.7^{efgh}	18.1^{defgh}	17.6^{efgh}	19.4^{defg}	25.8^{ab}	24.4^{bc}	17.4^{efghi}	1.41
	IRWC	15.5^{hijkl}	15.7^{fghij}	14.8^{hijkl}	11.9^{klmn}	21.7^{bcd}	20.0^{de}	16.7^{efghij}	29.5^{a}	20.2^{cde}	13.0^{jklm}	
	PWC	nm	16.7^{efghij}	11.9^{klmn}	16.5^{efghij}	nm	19.5^{def}	17.9^{defgh}	nm	18.8^{defg}	10.9^{mno}	
	Brassica	13.6^{hijklm}	nm	nm	nm	5.1^{p}	nm	nm	7.5^{op}	8.9^{nop}	nm	

[a-q]Superscripts with different letters indicate that means are significantly different among forage treatments within each parameter across years (P<0.05); nm: not measured in all the seasons as ewes did not graze PWC in winter of Years 1 and 2 and late-autumn of Year 3, and brassica was grazed only in winter of Years 1 and 2 and late-autumn and winter of Year 3.

* Back transformed plasma uric acid concentration (mean and SEM); [1]Urinary N excretion (g/day) = 1.2 X plasma urea concentration (g/L) × liveweight (kg), based on Kohn et al. (2005).

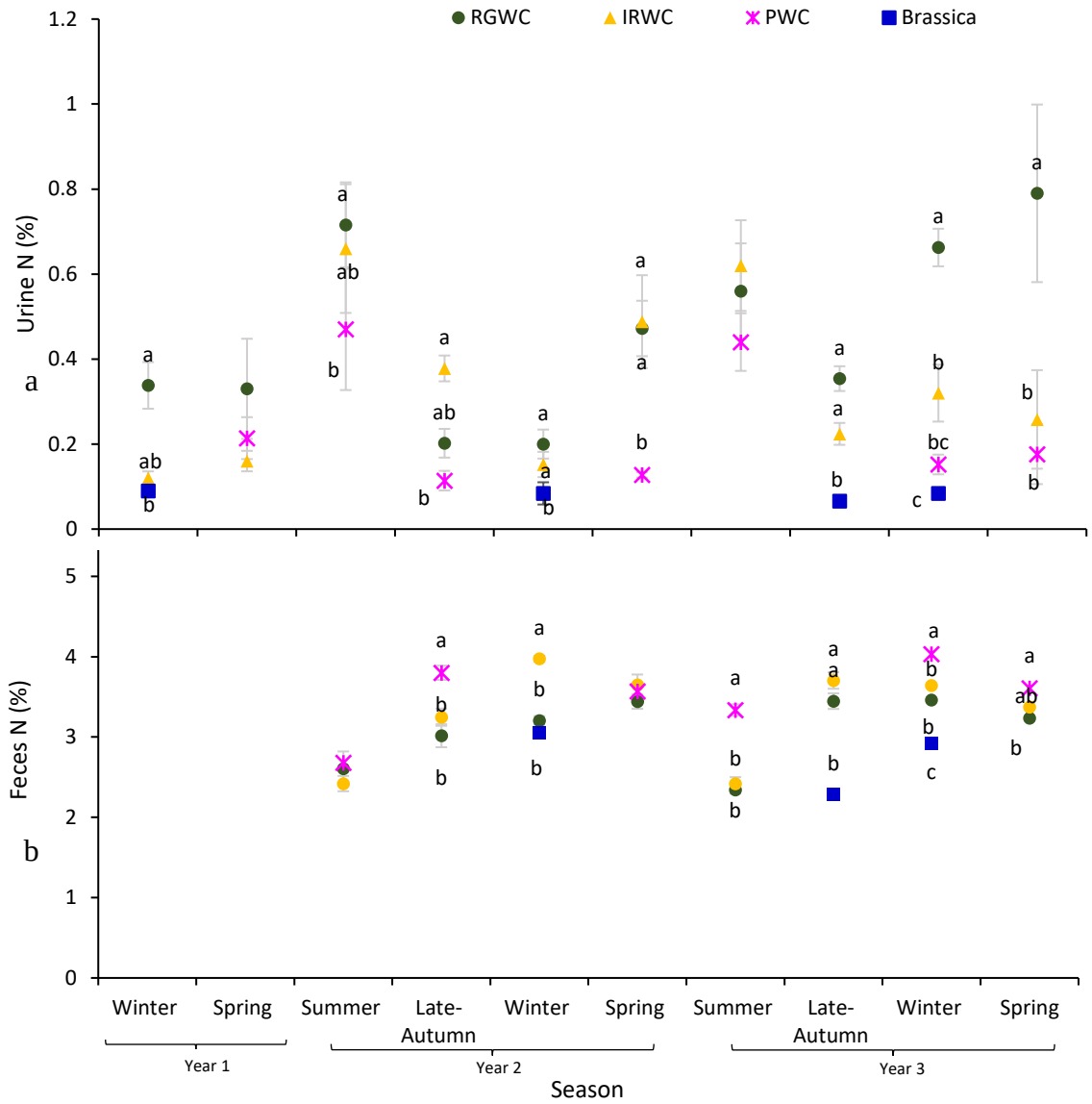

Figure 5.5 Nitrogen percentage (least-squares mean ± SE) of spot samples of urine (a) and faeces (b) collected from breeding ewes grazing perennial ryegrass/white clover (RGWC), Italian ryegrass/white clover (IRWC), plantain/white clover (PWC) and brassica in winter and spring of Year 1 (July to December 2019) and summer, late-autumn, winter and spring of Years 2 (January to December 2020), and 3 (January to December 2021). [abc]superscripts with different letters indicate that means are significantly different among forage treatment within seasons (P<0.05). Ewes grazing PWC and brassica were not sampled in all the seasons as brassica was only present in winter of Year 2 and late-autumn and winter of Year 3, and PWC was not grazed in winter of Year 2 and late-autumn of Year 3. The faeces samples were not freeze-dried in Year 1; therefore, the results from Years 2 and 3 are presented here.

The variation in chemical properties of spot urine and plasma samples split by season and forage treatment are shown in Tables 5.8 and 5.9. For all treatments, the mean individual urine N concentration of spot samples was 3.2 ± 0.2 g N/L and ranged between 0.7 and 8.0 g N/L. The urinary N concentration of spot samples fluctuated across the seasons and showed a general trend of being higher in the summer (Figure 5.5 a). There was no significant ($P>0.05$) difference in urine N concentration between ewes grazing RGWC and IRWC within season of a given year, except winter and spring of Year 3. Ewes grazing the PWC treatment had lower ($P<0.05$) urinary N concentrations than those ewes that grazed RGWC in summer and spring of Year 2 and winter and spring of Year 3 (Figure 5.5 a). In addition, ewes in the brassica treatment had lower ($P<0.05$) urinary N and urea concentrations compared to those on RGWC within all seasons it was measured (Table 5.8; Figure 5.5 a). Faecal N content ranged from 2 to 4% (Figure 5.5 b) with faecal N content of ewes grazing PWC being 0.4-0.7% higher ($P<0.05$) than those in RGWC in all seasons, except summer and spring of Year 2 (Figure 5.5 b).

The estimated mean daily urine volume of treatments ranged between 0.7 and 15.2 L/ewe/d (Figure 5.6). Estimated daily urine volume fluctuated across the seasons and showed a general trend of being lower ($P<0.05$) in the summer (Figure 5.6). Ewes grazing PWC had greater ($P<0.05$) estimated daily urine volume than those grazing RGWC within the seasons it was measured, except summer of Years 2 and 3 (Figure 5.6). Ewes grazing IRWC had greater ($P<0.05$) estimated urine volume in winter of Years 1 and 2 and late-autumn and spring of Year 3, compared to ewes grazing RGWC (Figure 5.6).

The differences between the estimated daily urinary N excretion based on urine creatinine and plasma urea concentrations were small (± 10 g: Tables 5.8 and 5.9). Overall, N excretion under sheep grazing forage treatments was low (<33 g N/ewe/d; Tables 5.8 and 5.9). The estimated

daily urinary N excretion of ewes grazing IRWC and RGWC did not differ (P>0.05) within seasons of a given year. Ewes grazing brassica excreted significantly less (P<0.05) urinary N than all other treatments in all seasons where brassica was grazed in Years 2 and 3 (Tables 5.8 and 5.9). Daily urinary N excretion of ewes grazing PWC was lower (P<0.05) than that on RGWC in spring of Year 2, whereas (based on plasma urea concentration and live weight), daily urinary N excretion of ewes grazing PWC was lower (P<0.05) in the winter and spring of Year 3 (Tables 5.8 and 5.9).

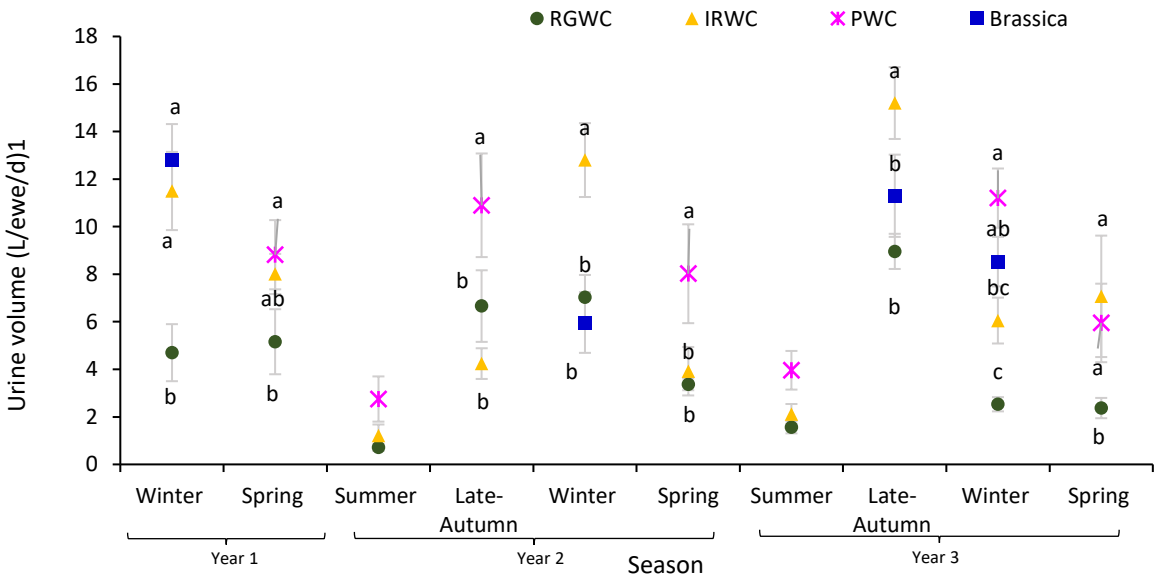

Figure 5.6 Estimated urine volume (L/ewe/d; least-squares mean ± SE) based on the urine creatinine concentrations of the spot urine samples collected from breeding ewes grazing perennial ryegrass/white clover (RGWC), Italian ryegrass/white clover (IRWC), plantain/white clover (PWC) and brassica in winter and spring of Year 1 (July to December 2019) and summer, late-autumn, winter and spring of Years 2 (January to December 2020), and 3 (January to December 2021). [abc]superscripts with different letters indicate that means are significantly different among forage treatments within seasons (P<0.05); [1]urine volume was calculated based on Pacheco et al. (2007) and David et al. (2015). Ewes grazing PWC and brassica were not sampled in all the seasons as brassica was only present in winter of Years 1 and 2 and late-autumn and winter of Year 3, and PWC was not grazed in winter of Years 1 and 2 and late-autumn of Year 3.

5.4.3 Urine purine derivatives

Purine derivatives (PD) and the PD index calculations associated with estimates of microbial N supply are given in Table 5.10. Uric acid concentrations of ewes grazing RGWC and IRWC did not differ ($P>0.05$) within the seasons of a given year, except ($P<0.05$) for the spring of experimental Years 2 and 3. Compared to ewes grazing RGWC, ewes grazing PWC had lower ($P<0.05$) uric acid concentrations in the summer and spring of Year 2 and the spring of Year 3 but did not differ ($P>0.05$) in any other season for a given year. The sum of PD ranged from 1.8 to 14.8 mmol/L. The PD index values ranged between 52 and 159 mmol/L: mmol/L. kg $W^{0.75}$. The PD index of ewes grazing RGWC and IRWC did not differ ($P>0.05$) within the seasons of a given year, except ($P<0.05$) for the winter and spring of Year 3. Similarly, PD indexes of ewes grazing PWC and RGWC did not differ ($P>0.05$) within the seasons of a given year it was measured except the summer of Year 3. Ewes grazing RGWC and ewes grazing brassica had similar ($P>0.05$) PD index in winter of Year 1 and late-autumn of Year 3.

Table 5.10 Urine purine derivative (PD) concentrations (mmol/L) of spots samples collected from breeding ewes grazing perennial ryegrass/white clover (RGWC), Italian ryegrass/white clover (IRWC), plantain/white clover (PWC) and brassica in winter and spring of Year 1 (July to December 2019) and summer, late-autumn, winter and spring of Years 2 (January to December 2020), and 3 (January to December 2021). Values are least-squares means.

		Year 1		Year 2				Year 3				SEM
		Winter	Spring	Summer	Late-Autumn	Winter	Spring	Summer	Late-Autumn	Winter	Spring	
Allantoin (mmol/L)	PRWC	4.9^{defg}	4.1^{efgh}	12.7^{a}	3.0^{gh}	2.4^{gh}	7.4^{bcde}	9.0^{bc}	2.8^{gh}	8.5^{bc}	9.9^{ab}	1.12
	IRWC	1.7^{h}	2.7^{gh}	9.3^{bc}	4.4^{defgh}	2.0^{gh}	7.5^{bcd}	8.9^{bc}	1.7^{gh}	4.1^{fgh}	2.6^{gh}	
	PWC	nm	2.6^{gh}	6.3^{cdef}	2.1^{gh}	nm	3.6^{fgh}	7.5^{bcd}	nm	1.5^{h}	2.7^{gh}	
	Brassica	1.8^{gh}	nm	nm	nm	2.4^{gh}	nm	nm	1.9^{gh}	1.4^{h}	nm	
Uric acid (mmol/L)	PRWC	0.5^{efg}	0.7^{cdef}	1.4^{ab}	0.4^{fg}	0.3^{fg}	1.0^{bcde}	1.1^{abcd}	0.4^{fg}	0.6^{defg}	1.3^{ab}	0.18
	IRWC	0.2^{g}	0.3^{fg}	1.3^{abc}	0.6^{efg}	0.3^{fg}	1.6^{a}	1.3^{ab}	0.2^{fg}	0.6^{defg}	0.7^{def}	
	PWC	nm	0.6^{defg}	0.7^{defg}	0.3^{fg}	nm	0.4^{fg}	1.1^{bcd}	nm	0.3^{fg}	0.6^{defg}	
	Brassica	0.3^{fg}	nm	nm	nm	0.4^{fg}	nm	nm	0.3^{fg}	0.3^{fg}	nm	
Total PD[1] (mmol/L)	PRWC	5.8^{cde}	5.0^{efgh}	14.8^{a}	3.6^{efgh}	2.9^{efgh}	8.9^{bcd}	10.6^{b}	3.3^{efgh}	8.9^{bcd}	11.7^{ab}	1.25
	IRWC	2.0^{fgh}	3.2^{efgh}	10.9^{b}	5.2^{defgh}	2.3^{efgh}	11.4^{ab}	10.8^{b}	2.1^{fgh}	5.6^{cdef}	3.2^{efgh}	
	PWC	nm	3.3^{efgh}	5.5^{cdefg}	2.6^{efgh}	nm	4.5^{efgh}	9.0^{bc}	nm	1.8^{h}	3.5^{efgh}	
	Brassica	2.2^{fgh}	nm	nm	nm	3.0^{efgh}	nm	nm	2.3^{fgh}	1.8^{gh}	nm	
PD index[2]	PRWC	$110.0^{defghijklm}$	$104.5^{fghijklmn}$	60.6^{op}	98.7^{ijklmn}	$108.0^{efghijklm}$	139.0^{abcd}	84.5^{lmno}	$132.4^{abcdfeg}$	$125.5^{cdefghij}$	135.07^{abcde}	10.75
	IRWC	$103.1^{ghijklmn}$	$106.3^{efghijklm}$	57.9^{op}	$101.5^{hijklmn}$	$117.9^{cdefghij}$	133.5^{abcdef}	81.4^{mnop}	148.1^{abc}	158.6^{ab}	$115.8^{defghijkl}$	
	PWC	nm	$128.0^{bcdefghi}$	52.4^{p}	$108.7^{efghijklm}$	nm	$117.1^{cdefghij}$	139.9^{abcd}	nm	95.6^{jklmn}	160.4^{a}	
	Brassica	$130.5^{abcdefgh}$	nm	nm	nm	75.6^{nop}	nm	nm	$116.9^{defghijk}$	93.6^{klmn}	nm	

[a-p]Superscripts with different letters indicate that means are significantly different among forage treatments within each parameter across years ($P<0.05$); nm: not measured in all the seasons as ewes did not graze PWC in winter of Years 1 and 2 and late-autumn of Year 3, and brassica was grazed only in winter of Years 1 and 2 and late-autumn and winter of Year 3.

[1]Total PD = allantoin (mmol/L) + uric acid (mmol/L) + *xanthine plus hypoxanthine (mmol/L) (Chen & Gomes 1992);* Xanthine plus hypoxanthine $= \frac{\text{allantoin concentration (mmol/L)} \times (5/100)}{(80/100)}$

[2]PD index $= \frac{\text{total PD (mmol/L)}}{\text{creatinine (mmol/L)}} \times \text{LW}^{0.75}$

5.5 Discussion

The aim of this study was to examine the effect of alternative forages on N partitioning and excretion in breeding ewes. In general, ewes grazing brassica forages showed reduced urinary N excretion compared to other forages. Although daily urinary N excretion of ewes grazing PWC and RGWC did not differ in all seasons studied, ewes grazing PWC had larger urinary volumes and lower N concentrations than ewes grazing RGWC. Several dairy studies have indicated that increased urine volume can result in a greater spread of urine-N at lower N-loading rates as the frequency of urination increases (Mangwe et al. 2019; Minnee et al. 2020). This suggests that PWC and brassica have the potential to reduce urinary N load per urine patch. However, both these species have some management limitations.

The estimated daily urinary N excretion levels across all seasons and forage types in this study were similar to those previously reported in indoor (9 to 27 g N/sheep/d; Jonker et al. (2015) and Marsden et al. (2020)) and outdoor studies (14 to 27 g N/sheep/d; Kohn et al. (2005) and Al-Marashdeh et al. (2020)).

Ewes grazing PWC had consistently lower urinary N concentration in spot samples compared to ewes grazing either RGWC or IRWC. The reduction in urinary N concentration from ewes grazing PWC compared with RGWC supported previous studies in sheep (Lindsay 2016) and dairy cows (Woodward et al. 2012; Box et al. 2016; Navarrete et al. 2016; Cheng et al. 2017; Gardiner et al. 2018; Bryant et al. 2019). This difference has been reported to be driven by (i) reduced rumen ammonia production, (ii) increased rumen microbial activity (Bryant et al. 2019; De Klein et al. 2019), (iii) shifting of N from urine to faeces, and (iv) increased urine volume as a result of the diuretic effect of plantain in sheep (Lindsay 2016; O'Connell et al. 2016; McGusty 2017). In the current study, daily urine volume of ewes grazing PWC was approximately 30% greater than that of RGWC. Urine volume is generally influenced by water

and/or minerals such as Na intake (O'Connell et al. 2016; Box et al. 2017) and/or secondary compounds (Box et al. 2016). Plantain has a lower DM% than RGWC (Box et al. 2017; Mangwe et al. 2019). While the apparent DMI did not differ between the ewes in RGWC and PWC (Table 5.3), the feed water intake from PWC would be 10 to 14% higher than the water intake from RGWC. High Na in plantain has been found to cause diuresis (Lindsay 2016; Box et al. 2017; Bryant et al. 2017; Cheng et al. 2017; McGusty 2017). In the current study, Na concentration was also higher in PWC than RGWC.

In-vitro studies indicate aucubin and acteoside (bioactive compounds found in plantain) may reduce rumen ammonia production (Navarrete et al. 2016). The aucubin and acteoside concentrations of PWC in the present study were similar to those reported by Navarrete et al. (2016). The presence of aucubin in plantain is known to stimulate the removal of uric acid from tissues and stimulate uric acid excretion from kidneys (Kato 1946; Tamura & Nishibe 2002). However, in the present study and in a dairy cow study (Box et al. 2017), uric acid concentrations of ewes grazing PWC were lower than those in ewes grazing RGWC. Plantain contains condensed tannin (CT), which may increase the ratio of N in faeces relative to urine (Carulla et al. 2005). Although CT was not measured in the present study, interestingly, greater faecal N% in relation to lower urinary N% was observed in ewes grazing PWC. The purine derivatives (PD) index sometimes explains lower urinary N concentrations (Chen et al. 1995); however, in this study, the PD index did not differ between ewes grazing RGWC and PWC. It is important to note that PWC was not grazed during the winter period, following best practice grazing guidelines (Cranston et al. 2015a). Consequently, other winter grazing options may be required for systems reliant on PWC pastures.

Ewes grazing brassica in the present study had lower N intake (~30 to 50%) compared to the ewes grazing RGWC, resulting in significantly lower N excretion, especially in Years 2 and 3.

Nitrogen intake has been identified as a major factor influencing urinary N excretion (Decandia et al. 2011; Totty et al. 2013). The positive relationship between CP content and N excretion is well recognised (Decandia et al. 2011; Totty et al. 2013). Similar observations have been made in dairy cows grazing leafy turnips (Kyamanywa et al. 2021). In this study, different varieties of brassica were sown in Years 1 (turnips), 2 (swedes) and 3 (kale). In Year 1, ewes in the brassica treatment had greater CP concentrations relative to other years. In the first year, forages had just been established by ploughing permanent pastures; this process can release significant amounts of mineral N in the soil (Cameron et al. 2013) as a result of organic matter decomposition, and this may have led to the higher CP of the brassica in Year 1, compared to later years. Moreover, ewes grazing brassica had a greater DM intake than ewes grazing RGWC in winter of Year 1. Given the higher daily intake and greater CP concentration of the forage, the daily N intake of ewes fed brassica was approximately 20% higher than RGWC, which probably explains why lower N excretion was not observed in ewes grazing brassica in Year 1. Edwards et al. (2007) indicated that a high ratio of WSC:CP in a diet improves the rumen energy balance, which is associated with reduced urinary N excretion.

In the present study, brassica consistently showed a higher soluble carbohydrate:CP ratio and reduced urinary N excretion. The daily urinary N excretion rate per day was calculated for an individual animal. However, brassica forages were grazed continuously for an extended time during the critical winter period. Therefore, the urine N load was likely to have been high in brassica plots. Based on urine N excretion during the winters of Years 2 and 3, the urinary N load under brassica (7.2 g urinary N excretion/ewe *35 days*46 ewes/0.4ha) and RGWC (22.8 g urinary N excretion/ewe*10 days*46 ewes/0.4ha) plots was estimated to be 26 and 29 kg N/ha, respectively. Since sheep urine patches are relatively small and dispersed, most of the N load under RGWC plots will be quickly taken up. However, due to the absence of plant cover

on brassica plots, most of the N deposited under brassica plots would potentially be leached during subsequent drainage events, as discussed in Chapter 6.

Under typical grazing conditions (i.e., brassica grazed in winter and PWC not grazed in winter), the ewes grazing PWC showed lower urinary N concentrations in late-autumn, summer and spring compared to ewes grazing RGWC or IRWC. In winter, ewes grazing brassica had lower urinary N concentrations compared to the ewes grazing RGWC. It is important to note that brassica yield was lower in Years 2 and 3 than expected for swedes (Year 2) and kale (Year 3): this may be due to less N in the soil being available (fertiliser application and release from cultivation). Moreover, in Year 2, ewes had not completely eaten all the bulbs before set stocking for lambing. As a result, there is a risk of greater urinary N excreted if the ewes had been able to graze for a longer duration. This suggests that PWC in late-autumn, summer and spring and brassica in winter have the potential to reduce urinary N load per animal if well managed. Nitrogen content in urine in spring may not have a significant impact on N leaching due to rapid pasture growth and associated N uptake at this time of the year (Shepherd & Lucci 2013) so it will be important to establish plant cover as soon as possible after the brassica is grazed.

It is important to note that, in New Zealand, rather than PWC, a mix of plantain, ryegrass, and clover is commonly used in sheep grazing systems (Cranston 2017). Although this study did not investigate urinary N excretion under a mixed grass-based sward, lower urinary N excretion has been reported under cows grazing swards of plantain and RGWC compared to cows grazing only RGWC (Box et al. 2017). Moreover, winter crops are generally grazed at high stocking rates during winter (Sprague et al. 2014; Agricom 2018). Following winter grazing, the land is left bare until conditions allow for re-sowing in spring. This process of grazing winter brassica species may return large amounts of N to the soil during winter, and subsequently may result

in very high rates of N leaching (Monaghan et al. 2013). High N leaching was observed under the brassica in Years 2 and 3 (Chapter 6). Therefore, the grazing of winter brassica with a higher stocking rate may in fact return large amounts of N to the soil during winter and lead to high N leaching, something future studies should consider.

While RGWC and IRWC had similar nutrient composition, particularly CP, SSS:CP and SSS:NDF, variables which in turn have a direct effect on urinary N concentration (Edwards et al. 2007; Jonker et al. 2015), IRWC showed a reduction in urinary N, NH_3 and urea N concentrations compared to RGWC. This may be explained by increased urine volume leading to lower urinary N concentrations in ewes grazing IRWC. Forage factors such as increased water intake (due to lower DM content), the presence of diuretic compounds and higher Na levels have been shown to increase urine volume previously in livestock grazing plantain (Bryant et al. 2019; De Klein et al. 2019). However, in the present study, there were no differences in forage DM % between RGWC and IRWC and it is unclear why a difference in urine volume was observed. Further grazing studies are required to determine of these results for IRWC can be reproduced and explained.

5.6 Conclusions

These results demonstrate the potential for using alternative forages in sheep grazing systems to reduce the environmental impacts. Overall, IRWC, PWC and brassica displayed lower urinary N concentrations compared to the RGWC. Urinary N excretion was smaller from ewes grazing PWC and brassica compared to ewes grazing RGWC. The diuretic effect of plantain contributed to the smaller excretion observed on PWC treatment. The smaller CP concentration of brassica seemed to be the major factor contributing to the smaller N excretion rate by sheep grazing the brassica crops. The urinary N concentration, urine volume and urinary N excretion results indicate that PWC and brassica have the potential to reduce the urinary N excretion per

animal. However, the timing and number of animals grazing brassica crops is also an important consideration. It is important to note that urinary N composition and faecal N concentrations were measured in spot samples and in order to provide a clear and unequivocal conclusion, it is suggested that total urine and faecal samples be collected in future studies.

CHAPTER 6: NITROGEN LEACHING UNDER ALTERNATIVE FORAGES GRAZED BY SHEEP

6.1 Abstract

Sheep farmers in New Zealand can increase production levels as a means to improve economic outcomes. However, this intensification can potentially result in more nitrogen (N) leaching which is at odds with the increasing pressure on farmers to reduce the quantity of N lost into the environment. Therefore, there is increasing interest in the use of alternative forages which could potentially allow farmers to increase production while decreasing N leaching. The objective of this study was to quantify and compare N leaching levels under sheep grazing four different forages: perennial ryegrass/white clover (RGWC), Italian ryegrass/WC (IRWC), plantain/WC (PWC) and a winter brassica within a farmlet system with an overall stocking rate of 14 ewes/ha. Five replicates of each forage were established on plots of 800 m^2 (40 m x 20 m) each of which were arranged in a randomised complete block design. Each plot had an independent mole-pipe drain system. Drainage volume and N concentrations (nitrate-N: NO_3^--N; ammonium-N: NH_4^+-N; total-N: TN) in drainage water collected from the drainage setup were used to estimate N losses. The forages were grazed according to the best grazing management for each forage type (i.e., different grazing days for different forages) over a two-and-a-half-year period (Year 1: July-December 2019; Year 2: January-December 2020; and Year 3: January-December 2021). Nitrate-N losses in drainage under RGWC, which ranged from 0.5 kg N/ha in Year 1 to 1.5 kg N/ha in Year 3, were very small and much lower than previously reported values. In the first two years of this study, the NO_3^--N losses from IRWC and PWC were also very small (ranging from 0.4 to 0.5 kg N/ha), and they were not different ($P>0.05$) from those under RGWC. However, in Year 3, the leaching losses under IRWC and PWC treatments were greater ($P<0.05$) than that under RGWC. This increased leaching was probably due to the need to re-establish these pastures, and as a consequence the IRWC and

PWC plots were fallow during the key late summer-autumn period. The poor persistence of IRWC and PWC on this fine-textured soil and the greater NO_3^- leaching associated with renovation of these forages in Year 3 undermined some of the advantages associated with the small leaching rate measured in Years 1 and 2. In contrast, NO_3^--N leaching losses under brassica forages in Year 2 (6.4 kg N/ha) and Year 3 (3.5 kg N/ha) were greater ($P<0.05$) than under RGWC. However, based on the assumption that the cropped area would occupy a relatively small portion of the farm, increases in whole farm NO_3^- leaching may be relatively small when sheep production systems practise fodder cropping. Overall, the leaching losses measured under sheep grazing forages were lower than those reported under dairy cattle studies.

Keywords: Modern sheep production systems, mole-pipe drain systems, nitrate leaching, water quality.

6.2 Introduction

In recent years, there has been an increased concern about the contamination of waterways with nutrients, in particular the N leached from pastoral farms (Ruz-Jerez et al. 1995; Silva et al. 1999; Houlbrooke et al. 2004; Ministry for the Environment 2020). Nitrate (NO_3^-) is the most common form of N contaminant in drainage water (Haynes & Williams 1993). At higher concentrations (>11.3 mg N/L), NO_3^- can be hazardous to human health and aquatic life (Di & Cameron 2002). More problematically, a relatively small concentration of NO_3^- can promote undesirable biological growth which, following eutrophication, results in a deterioration in water quality (Di & Cameron 2002). In pastoral systems, urine N deposited by livestock on pastures is the primary source of NO_3^- leaching (Williams & Haynes 1990). The surplus inorganic soil N that is not taken up by plants is subsequently susceptible to leaching when drainage occurs (Haynes & Williams 1993).

Nitrate leaching from sheep farming systems is typically considered to be considerably lower than that from dairy farming systems (Williams & Haynes 1994). However, in order to remain economically viable, sheep production systems in New Zealand have become more intensive over time, with greater use of fertiliser, improved 'per head' animal performance and/or higher stocking rates (Morris & Kenyon 2014). Consequently, it is possible that NO_3^- leaching losses are greater than historical figures indicate and, therefore, should be reassessed under modern intensive practices. In New Zealand, NO_3^- leaching from dairy cattle systems is relatively well characterised, and regional councils have begun to place limits on NO_3^- leaching rates from dairy farms (Ministry for the Environment 2020). Therefore, a number of mitigation technologies have been developed to reduce NO_3^- leaching under dairy systems (Houlbrooke 2005; Anastasiadis et al. 2012; Cameron et al. 2013; Christensen et al. 2019). However, due to differences between sheep and dairy cows and their management, many of the mitigation strategies that can be applied to dairy farms may not be feasible for sheep farms, such as housing, stand-off pads and duration-controlled grazing (Anastasiadis et al. 2012).

There is an increasing interest in the use of alternative forages in pastoral agriculture in New Zealand. Some of these forages are known to reduce NO_3^- leaching, i.e., improve environmental outcomes (e.g., plantain; *Plantago lanceolata* L. and Italian ryegrass; *Lolium multiflorum* Lam.), while others are used to increase animal performance (e.g., winter crops). Previous studies have shown that plantain on dairy farms can help to mitigate NO_3^- leaching losses (Box et al. 2016; Bryant et al. 2019). This is because plantain reduces the urine N concentration and amount of urinary N excreted as a result of an increase in urine production (Box et al. 2016; Bryant et al. 2019) and a decrease in ammonia production (Navarrete et al. 2016). In addition, the secondary metabolites produced by plantain may inhibit nitrification in the soil (Dietz et al. 2013; De Klein et al. 2019). Italian ryegrass, a rapidly growing pasture species, has been shown to exhibit greater N uptake during the winter months and, therefore,

reduce NO_3^- leaching compared to perennial ryegrass (*Lolium perenne* L.)/white clover (*Trifolium repens*) swards when grazed by dairy cows (Woods et al. 2018). In contrast, winter crops such as brassicas (e.g., swedes, turnips and kale) produce large amounts of forage (e.g., 8-15 t DM/ha) and are grazed with high stocking rates during winter when the grass growth rate is slow (Sprague et al. 2014; Agricom 2018). Following grazing, the land is left bare until conditions are favourable for re-sowing in spring. This process of grazing winter brassica species may result in large amounts of N being returned to the soil during winter, which can subsequently lead to high levels of NO_3^- leaching (Monaghan et al. 2013).

To date, most studies that have measured NO_3^- leaching under typical sheep grazing conditions have mostly used soil core samples, suction cups or lysimeter methods, and have reported NO_3^--N leaching rates ranging from 2 to 94 kg N/ha/year (Monaghan et al. 1989; Cuttle et al. 1992; Williams & Haynes 1994; Cuttle et al. 1996; Cuttle et al. 1998). A limited number of studies, which were conducted twenty years ago, have measured NO_3^- leaching using mole and pipe drains and have reported NO_3^--N leaching levels ranging from 8.6 to 50 kg N/ha/year (Heng et al. 1991; Magesan et al. 1996; White et al. 1998). At present, no studies have been conducted on leaching under current intensive sheep production systems, particularly when alternative forages are grazed. Therefore, the present study used larger plots on a Pallic soil with a mole-pipe drainage network which allows for the capture of nearly all of the drainage water and leached N. The aim of this study was to quantify and compare N leaching under four different forage types (perennial ryegrass/white clover (RGWC), plantain/white clover (PWC), Italian ryegrass/white clover (IRWC) and brassica) which were grazed intensively by sheep.

6.3 Materials and methods

6.3.1 Brief summary

This study was carried out at Massey University's 'Keeble' farm, which is located approximately 5 km southeast of Palmerston North, Manawatu, New Zealand (40°24'02.0"S 175°35'52.8"E). The site is located in a flat landscape (c. 3% slope) on the Tokomaru silt loam soil, a Fragic Perch-gley Pallic Soil (Hewitt 1998).

The details of the farmlet systems and ewe management are provided in Chapter 3. Briefly, the study area included four self-contained, contiguous farmlets (each approximately 3.32 ha), each of which consisted of a different combination of forages. The alternative forages or treatments were; IRWC, PWC, and brassica (turnips: *Brassica rapa* ssp. *rapa* in 2019, swedes: *Brassica napus* ssp. *Napobrassica* in 2020 and kale: *Brassica oleracea* var. *acephala* in 2021. On each farmlet, 0.81 ha was sown in the alternative forage and the remaining 2.51 ha were sown in a perennial ryegrass/white clover sward. The entire area of the fourth farmlet, which acted as the control, was sown in RGWC. A series of 20 drainage plots (each 40 m by 20 m) were established to measure N leaching losses. Each of the alternative forages, and the RGWC, were sown on five of the plots i.e., five replicates (the combined area of the treatments was 0.4 ha). That is, there were five drainage plots allocated to each farmlet. The area of the drainage plots accounted for approximately one-half of the area of the alternative forage area for each farmlet.

Each farmlet was stocked at 14 ewes/ha for the entirety of the experiment (46 ewes per treatment), which is typical of an intensive sheep-farming operation for this farm class and area (Beef + Lamb New Zealand 2021). Each mob of ewes was rotationally grazed within their farmlet. The duration of grazing and the interval between the grazing of paddocks were adjusted according to pasture growth rate and cover (see Chapter 3 for details of

measurements). When the ewes grazed the drainage plots, each mob was separated into five smaller mobs (i.e., into each replicate) to enable all five replicates to be grazed at the same time (approximately nine ewes/replicate). At any grazing event, all the replicate plots of a forage treatment were grazed for the same number of days and ewes were moved when the target post-grazing residual was reached in any replicate. Where appropriate, treatments on the drainage plots were grazed at the same time to minimise the effect of differing weather conditions on N leaching post grazing (Figure 6.1). However, the brassica crop was grazed continuously during winter, after the ewes were gradually adjusted to the diet using on-off grazing with increasing grazing durations of brassica each day (2, 4, 6, 8, hours on days one through four) before continuous grazing. Due to mild winter conditions in 2020, the brassica yield was considerably higher than had been predicted, and ewes were unable to graze all the bulb material before set-stocking (26 August 2020) in non-drainage plot areas, with an estimated 5,461 kg DM/ha remaining.

The data collected from drainage plots over two and a half consecutive production years (from June 2019 to December 2021) is presented in this chapter. The years will be referred as "Year 1 (July to December 2019)", "Year 2 (January to December 2020)", and "Year 3 (January to December 2021)".

6.3.2 Experimental design and treatments

The dimensions of each of the drainage plots were 40 m x 20 m and each plot was drained by a mole-pipe drainage system. Identical drainage systems and monitoring equipment have been used to successfully measure nutrient leaching in other studies (Houlbrooke 2005; Hanly et al. 2017; Christensen et al. 2019). The plots were sown with the treatment forages in the autumn of Year 1 (21 March 2019) with a roller drill and chain harrows. The PWC and IRWC plots were over-sown in Year 2 (13 April 2020). Furthermore, in the late summer to autumn of Year

3, the PWC plots developed a significant dock (*Rumex obtusifolius*) infestation, and the IRWC had reduced tiller numbers. Therefore, these forages were sprayed with herbicide (glyphosate: 6 L/ha; harmony: 30 g/ha), and the PWC and IRWC plots were resown on 15 March and 14 April 2021, respectively, using a direct drill. The brassica crops were sown by direct drilling; turnips on 21 March 2019 (Year 1 crop), swedes on 23 December 2019 (Year 2 crop) and kale on 15 December 2020 (Year 3 crop). Details of fertiliser applications are given in Table 6.1. The plots were grazed according to the accumulation of forage and using best grazing management practices for these crop species.

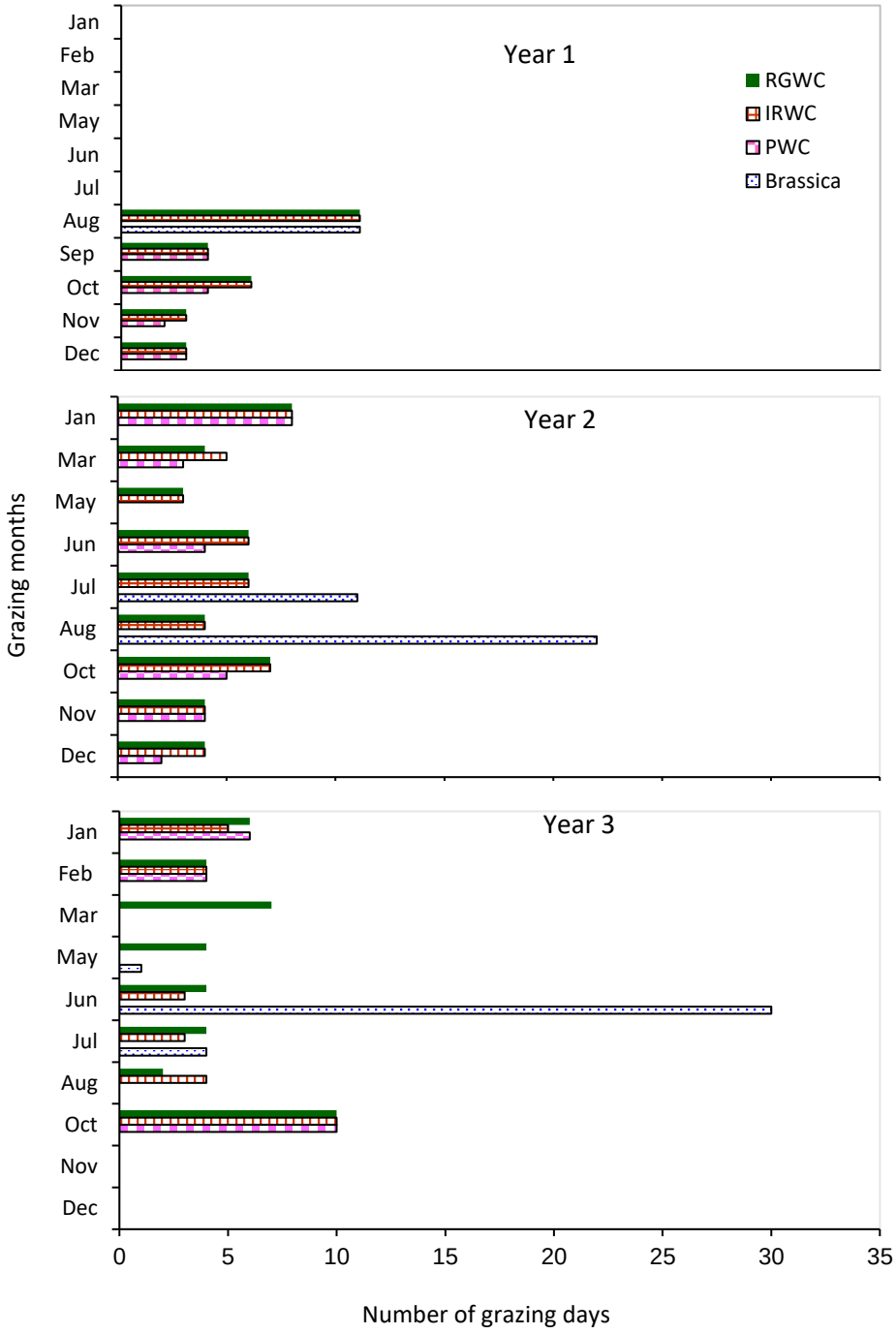

Figure 6.1 Number of days the sheep grazed each forage treatment (RGWC: perennial ryegrass/white clover; IRWC: Italian ryegrass/white clover; PWC: plantain/white clover; brassica) on the drainage plots area during Years 1 (July to December 2019), 2 (January to December 2020) and 3 (January to December 2021).

Table 6.1 Fertiliser applied (kg/ha) to the drainage plots in Year 1 (July to December 2019), Year 2 (January to December 2020), and Year 3 (January to December 2021).

Fertiliser	Year 1	Year 2	Year 3
Urea (46% N)	30 kg/ha for RGWC, IRWC, PWC in October	30 kg/ha for brassica in March	30 kg/ha for RGWC, IRWC in March
	30 kg/ha for PWC in December	30 kg/ha for RGWC, IRWC, PWC in October	50 kg/ha for brassica in March
Diammonium phosphate (DAP) (18% N and 46% P)	100 kg/ha for RGWC, IRWC, PWC, brassica in March		
Nitrophoska (12% N)		200 kg/ha for RGWC, IRWC, PWC in April	
Balance Super 10 (0% N and 9% P)		150 kg/ha for RGWC, IRWC, PWC in April	
Flexi N complete (25% N)			350 kg/ha for RGWC, IRWC, PWC in May
Cropmaster 15 (15.1% N and 10% of P)	250 kg/ha for Brassica in December	250 kg/ha for Brassica in December	

RGWC: Perennial ryegrass/white clover; IRWC: Italian ryegrass/white clover; PWC: Plantain/white clover

6.3.3 Drainage measurement and water analysis

Rainfall data were sourced from the National Institute of Water and Atmospheric Research (NIWA) site, located approximately 2 km from the research area. Drainage water from each plot was channelled through drainage pipes into individual tipping-bucket flow meters located in sampling pits nearby (Figure 6.2). The flow rate of drainage water was measured with individual tipping bucket (~5 L) for each plot (replicate). Water from the bucket was tipped over a pipe with a small hole in it which passed the sub-sample (~0.5 ml) to a larger container for storage until the completion of the drainage event. After the completion of each drainage event, water samples (~100 ml) were collected manually from the storage container for water quality analysis (Christensen et al. 2019).

In the laboratory, approximately 50 ml of the subsample was filtered through a 0.45 µm filter. The filtered and unfiltered (~50 ml) subsamples were then frozen until analysis. Filtered samples were analysed for nitrate-N (NO_3^--N) and ammonium-N (NH_4^+-N) using a Technicon Auto Analyser (Blakemore et al. 1987). The unfiltered samples were analysed for total N (TN) content using the persulphate digestion method of Hosomi & Sudo (1986). The various forms of N in the sample were converted to NO_3^--N. Total organic N (TON) was estimated from the differences between TN and inorganic N (NO_3^--N and NH_4^+-N). The amount of NO_3^--N, NH_4^+-N and TN losses were calculated as the product of the measured drainage volume and their concentrations (Christensen et al. 2019).

Figure 6.2 Individual tipping buckets and collection area for drainage water.

6.3.4 Statistical analysis

All data were tested for normality and outliers using Minitab 16 statistical software before the analysis in SAS version 9.4 (Statistical Analysis System, version 9.4; SAS Institute Inc., Cary, NC, USA). All data were subjected to analysis of variance using the MIXED or GLM procedure in SAS version 9.4 (SAS Institute 2015). The NO_3^--N and TN concentrations of drainage water samples were analysed separately for each year, using a model containing the fixed effects of treatment (RGWC, IRWC, PWC or brassica) and sampling date and their two-way interaction. The models also included replicates (plots) nested within treatment as a random effect.

Effect of forage treatment (RGWC, IRWC, PWC and brassica) on cumulative drainage (mm) and cumulative losses of TN and NO_3^--N were analysed using a mixed model that contained the fixed effect of year, forage treatment and the two-way interaction of treatment with year. The model included replicates (plots) nested within the treatment as a random effect. In Year

2, drainage (mm) from one of the PWC plots was five times greater than the average of the other plots and was deemed to be an outlier caused by a leak in a reticulated water pipe. Therefore, in the calculation of cumulative drainage and N losses, the data collected from this particular plot were omitted.

6.4 Results

6.4.1 Rainfall and drainage

The actual annual rainfall data at the meteorological station in 2019, 2020 and 2021 were 977, 824 and 1084 mm, respectively. Monthly rainfall for each month of the study period is shown in Figure 6.3.

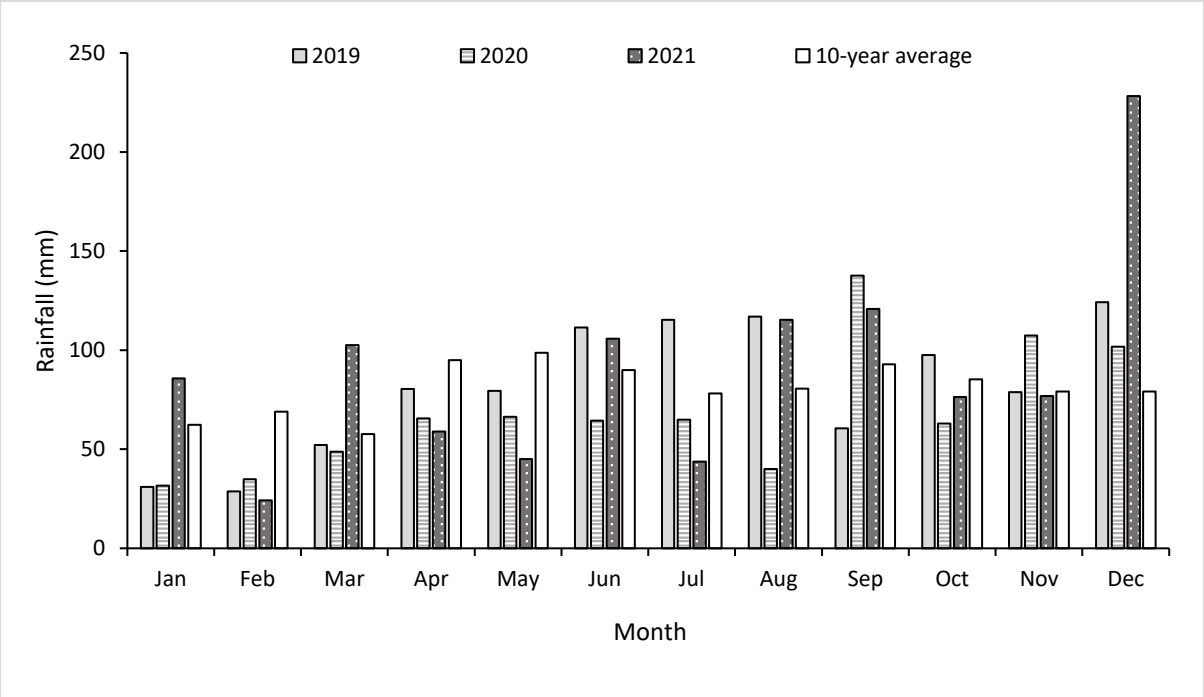

Figure 6.3 Ten-year average monthly rainfall and actual monthly rainfall for the trial site (sourced from NIWA/AgResearch, Palmerston North meteorological station, 2 km from study site) during 2019, 2020 and 2021.

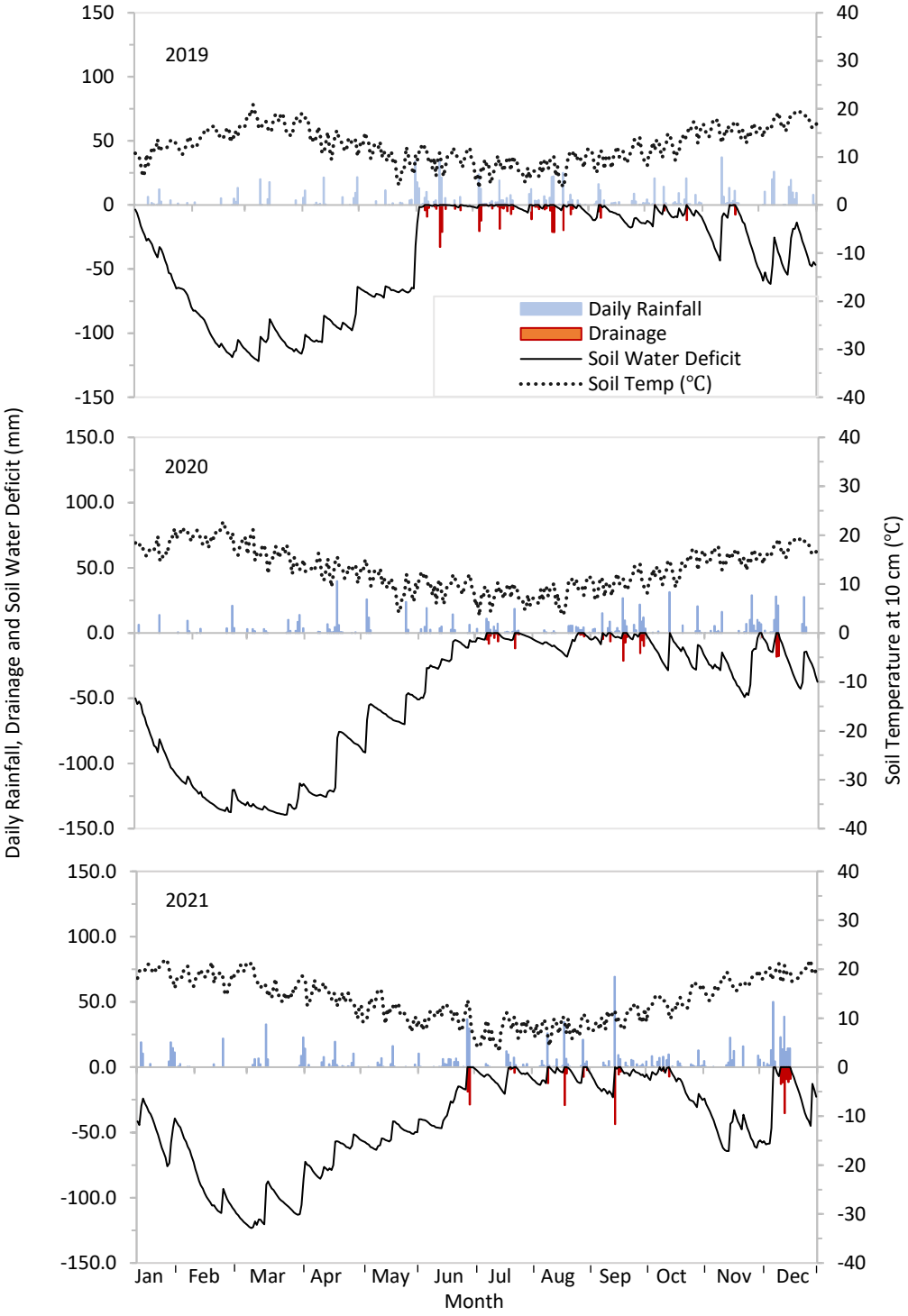

Figure 6.4 Mean drainage, rainfall, soil temperature and modelled soil water deficit for 2019-2021. Soil water deficit and drainage are absolute values but are displayed as negative values to add clarity to the figure.

The measured drainage events generally coincided with periods of soil water surplus, as predicted by the soil water balance of Scotter et al. (1979) (Figure 6.4). The average (across all plots) annual drainage (mm) did not differ ($P>0.05$) among treatments in Years 1 and 3;

however, PWC had less drainage ($P<0.05$) than the RGWC and brassica treatments in Year 2 (Figure 6.5). The variation in drainage observed across the plots was typical of that reported for field-scale studies of mole and pipe drainage (Houlbrooke et al. 2003). The commencement and spread of the drainage season varied from year to year (Figures 6.4 and 6.5). In Year 1, the water balance suggested that approximately 163 mm of drainage occurred before the commencement of monitoring in July. After monitoring started, the average quantity of drainage measured across all plots was 106 mm for the remainder of Year 1 (Figure 6.5). Therefore, the total quantity of drainage estimated for the entire Year 1 drainage season was about 269 mm.

The drainage season in Year 2 began in late April and ended in December (Figure 6.5). The spring was very wet compared to the winter, with a dry period in August followed by high rainfall (137 mm) in September (Figure 6.3). The average annual quantity of measured drainage across all plots in Year 2 was low, being only 80 mm (Figure 6.5). The size of drainage events from April to September was relatively consistent across the treatments but towards the end of the season some differences among treatments developed. In the last three months of the year, higher (76 mm; $P<0.05$) and lower (33 mm; $P<0.05$) drainage were observed for the brassica and PWC treatments, respectively.

The drainage season and sample collection in Year 3 began in early May and ended in October. A total of 113 mm of measured drainage occurred across all plots on average, during the sample collection period in Year 3 (Figure 6.5).

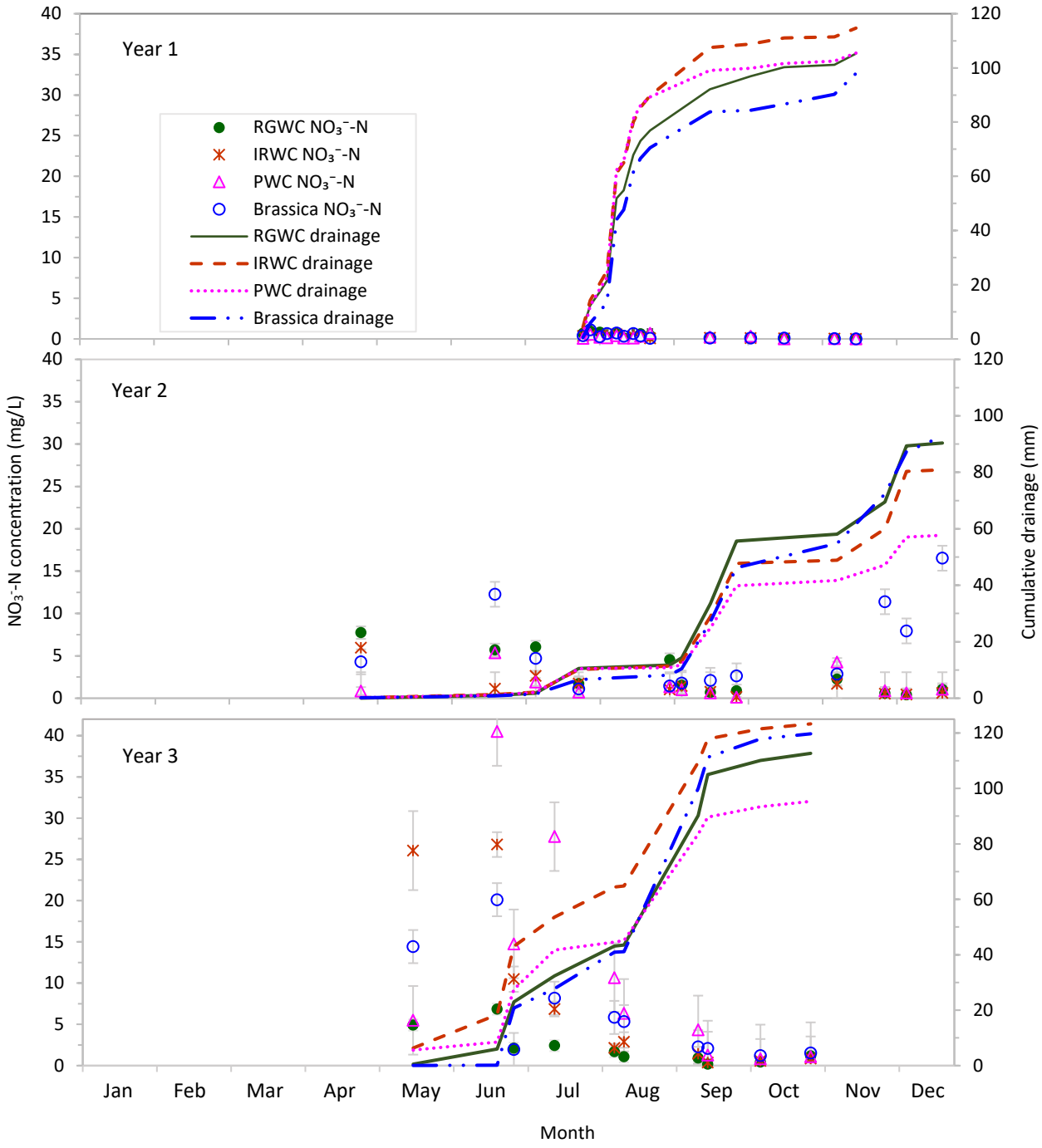

Figure 6.5 Cumulative measured drainage (mm) and concentrations of NO_3^--N (mg/L) in mole-pipe drainage from July 2019 to October 2021 (Year 1: July to December 2019; Year 2: January to December 2020; Year 3: January to December 2021) under the forage treatments (RGWC: perennial ryegrass/white clover; IRWC: Italian ryegrass/white clover; PWC: plantain/white clover and brassica) grazed by sheep. Values are least-squares means. Error bars represent standard error values at each sampling event.

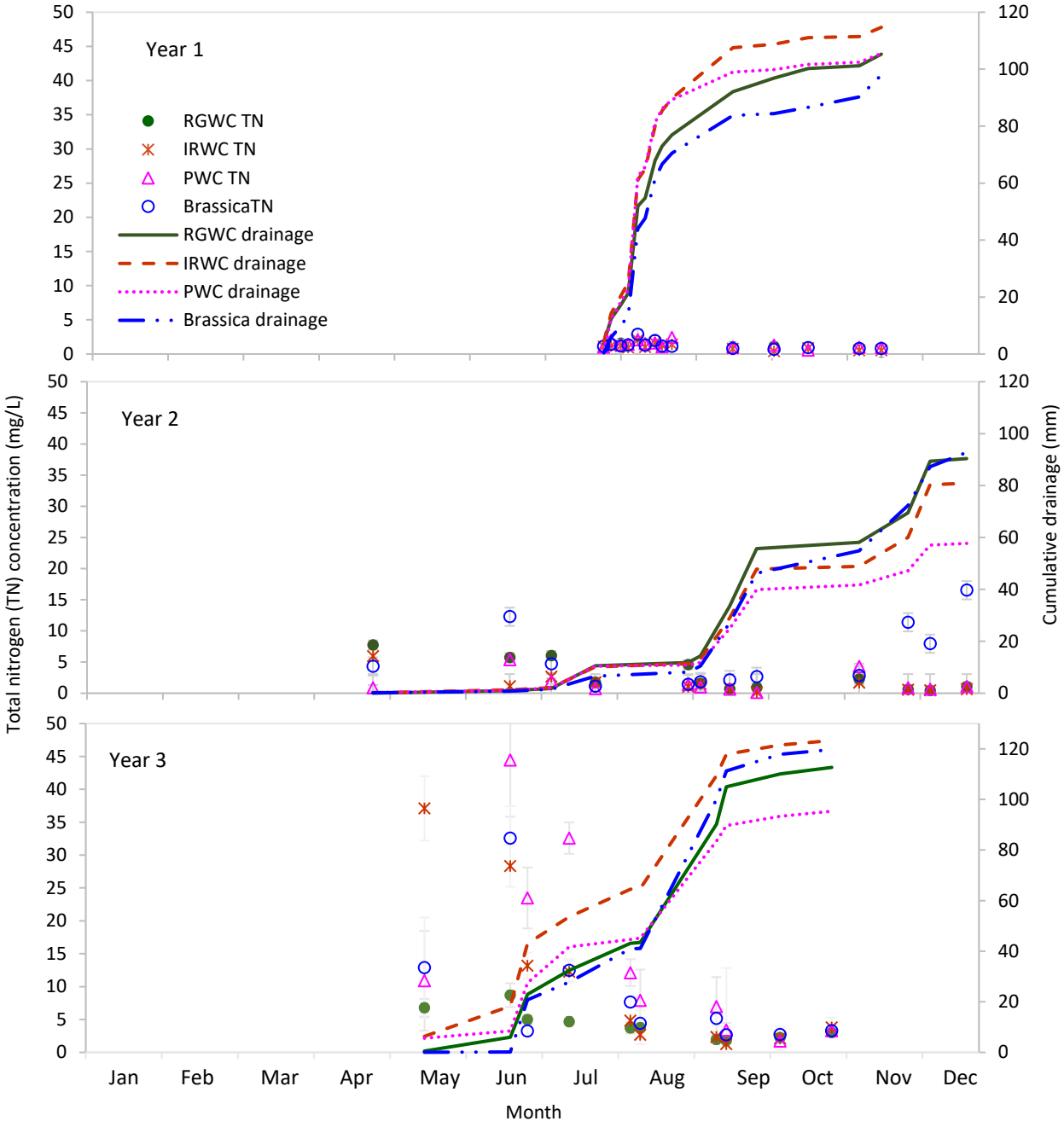

Figure 6.6 Cumulative measured drainage (mm) and total nitrogen (TN) concentrations (mg/L) in mole-pipe drainage from July 2019 to October 2021 (Year 1: July to December 2019; Year 2: January to December 2020; Year 3: January to December 2021) under the forage treatments (RGWC: perennial ryegrass/white clover; IRWC: Italian ryegrass/white clover; PWC: plantain/white clover and brassica) grazed by sheep. Values are least-squares means. Error bars represent standard error values at each sampling event.

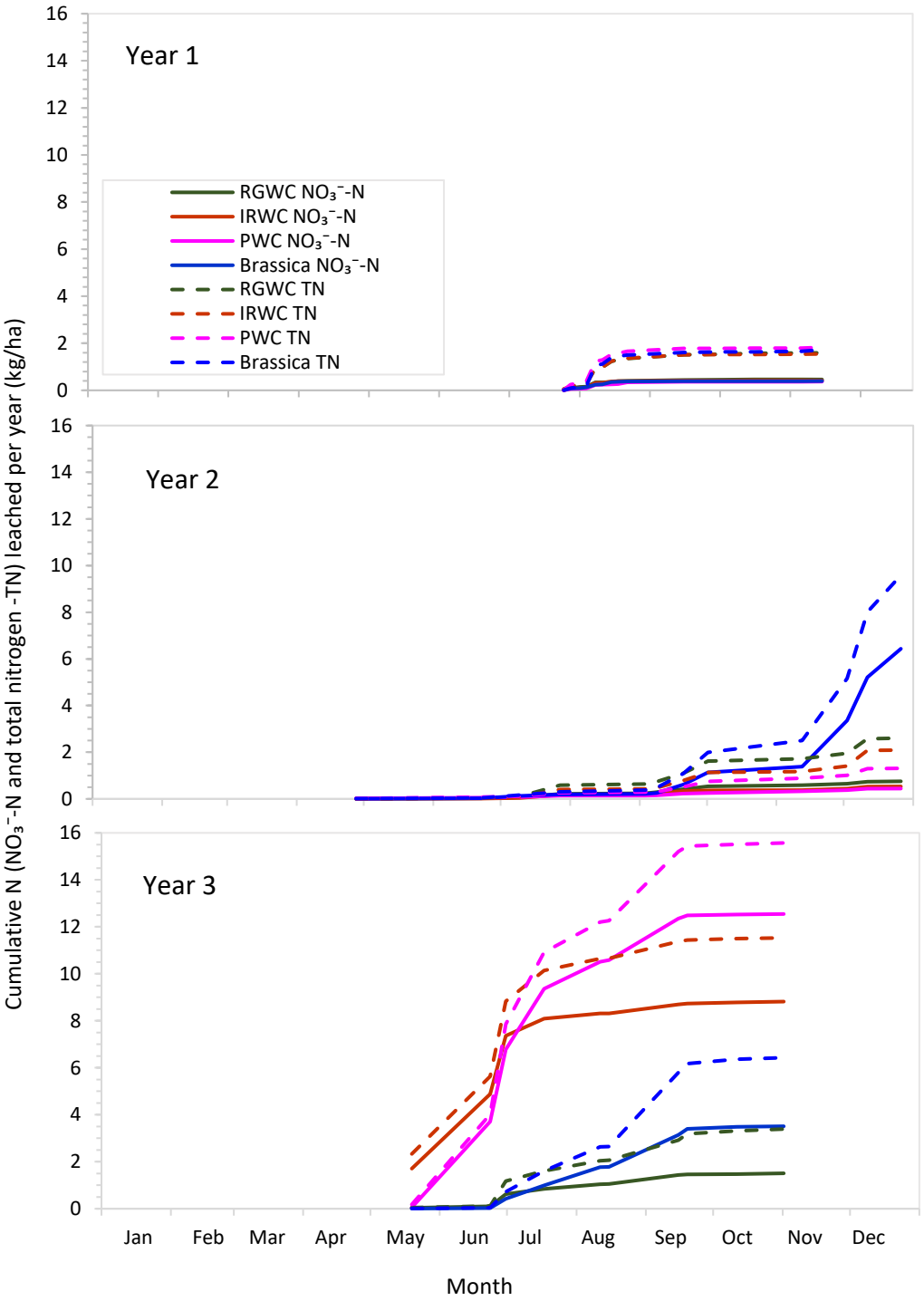

Figure 6.7 Cumulative leaching (kg/ha) of nitrate-nitrogen (NO_3^--N) and total nitrogen (TN) in mole-pipe drainage from July 2019 to October 2021 (Year 1: July to December 2019; Year 2: January to December 2020; Year 3: January to December 2021) under the forage treatments (RGWC: perennial ryegrass/white clover; IRWC: Italian ryegrass/white clover; PWC: plantain/white clover and brassica) grazed by sheep. Values are least-squares means.

6.4.2 Drainage water nitrogen

Inorganic N concentrations and cumulative losses

The NO_3^--N concentrations for all four treatments were <1 mg N/L ($P>0.05$) for drainage events monitored in the latter part of the Year 1, from July, drainage season (Figure 6.5). The majority of leaching losses under grazed pasture on this soil type tended to occur in the early drainage events (i.e., typically the first 100-150 mm), and drainage events in the later part of the season tended to have low N concentrations (Hanly et al. 2017; Christensen et al. 2019; Howes 2019).

In Year 2, the drainage NO_3^- concentration fluctuated among treatments over the year. The RGWC, IRWC and PWC treatments had higher concentrations of NO_3^--N (>5 mg N/L) in the initial drainage events i.e., the first 15 to 20 mm of drainage (Figure 6.5). In comparison, drainage under brassica had high NO_3^- concentrations in both the first three drainage events and the last three drainage events that occurred in December. The NO_3^--N concentration in December drainage under brassica was greater ($P<0.05$; >8 mg N/L) than concentrations in drainage from the other treatments. The peak concentrations of NO_3^--N in RGWC, IRWC, PWC and brassica were 8, 6, 5 and 17 mg N/L, respectively.

In Year 3, irrespective of forage treatments, larger concentrations (5 to 25 mg N/L) of NO_3^--N occurred in the initial drainage events i.e., the first 40 to 60 mm of drainage (Figure 6.5). The NO_3^--N concentrations in IRWC (range from 7 to 27 mg N/L) and PWC (range from 6 to 41 mg N/L) were greater ($P<0.05$) than those in RGWC during early drainage events that occurred from May to August. The larger concentrations in the initial drainage events were followed by a rapid decrease in the concentrations of NO_3^- in the subsequent drainage events. The peak NO_3^--N concentrations of RGWC, IRWC, PWC and brassica were 7, 27, 41 and 20 mg N/L, respectively.

The NH_4^+-N concentrations in the drainage events monitored over the study period were very low (<0.1 mg N/L), therefore, the data were not presented.

Table 6.2 Total amounts of nitrate-nitrogen (NO_3^--N) and Total-N leached (kg/ha) in mole-pipe drainage from July 2019 to October 2021 (Year 1: July to December 2019; Year 2: January to December 2020; Year 3: January to December 2021) under sheep grazing of the forage treatments (RGWC: perennial ryegrass/white clover; IRWC: Italian ryegrass/white clover; PWC: plantain/white clover).

		RGWC	IRWC	PWC	Brassica	P-value
Treatment						
NO_3^--N		0.90^{b}±0.38	3.24^{a}±0.38	3.50^{a}±0.38	3.41^{a}±0.38	0.0004
Total-N		2.49^{b}±0.64	4.98^{a}±0.64	4.92^{a}±0.64	5.81^{a}±0.64	0.0129
Year x Treatment						
NO_3^--N						<0.0001
	Year 1	0.46^{d}±0.59	0.42^{d}±0.59	0.37^{d}±0.59	0.37^{d}±0.59	
	Year 2	0.75^{d}±0.59	0.53^{d}±0.59	0.45^{d}±0.66	6.35^{b}±0.59	
	Year 3	1.49^{d}±0.59	8.77^{a}±0.59	9.68^{a}±0.59	3.51^{c}±0.59	
Total-N						<0.0001
	Year 1	1.49^{c}±0.93	1.26^{c}±0.93	1.79^{c}±0.93	1.52^{c}±0.93	
	Year 2	2.60^{c}±0.93	2.10^{c}±0.93	1.51^{c}±1.04	9.49^{a}±0.93	
	Year 3	3.37^{c}±0.93	11.6^{a}±0.93	11.5^{a}±0.93	6.43^{b}±0.93	

[a-c]Superscripts with different letters indicate that means are significantly different among forage treatments within each parameter (P<0.05).

Cumulative NO_3^--N losses from all treatments in Year 1 were less than 0.5 kg N/ha (Figure 6.7) and did not differ (P<0.05) across treatments. In Year 2, NO_3^--N losses from all treatments remained < 0.2 kg N/ha (P>0.05) until the beginning of September. In subsequent drainage events (Figure 6.7), the losses from the brassica treatment increased (P<0.05) from 0.2 to 6.3 kg N/ha, while the losses from other treatments remained relatively small (0.15 to 0.75 kg N/ha). The cumulative leaching of NO_3^--N from RGWC, IRWC and PWC were <1 kg N/ha and did not differ (P>0.05) among treatments, whereas the cumulative leaching of NO_3^- from brassica was greater (P<0.05) than in other treatments.

In Year 3, the cumulative leaching of NO_3^--N from RGWC was 1.5 kg N/ha, which was lower (P<0.05) than other treatments. The cumulative leaching of NO_3^--N from IRWC (8.8 kg N/ha)

and PWC (9.7 kg N/ha) were greater ($P<0.05$) than those from the RGWC and brassica treatments (Table 6.2), with large differences observed particularly during the early part of the drainage season (Figure 6.7). The cumulative leaching of NO_3^--N from brassica was 3.5 kg N/ha, which was greater ($P<0.05$) than RGWC and lower ($P<0.05$) than IRWC or PWC.

The annual amount of NH_4^+ that was leached in drainage was very small, with less than 0.12 kg NH_4^+-N/ha being leached from any treatment in any year. Given that NH_4^+ made only a minor contribution to TN losses in drainage, the data have not been presented.

Total N (TN) concentrations and cumulative losses

Concentrations of TN and the cumulative leaching in drainage water exhibited patterns similar to those observed for NO_3^- (Figures 6.6 and 6.7). In Year 1 (July onwards), the TN concentrations in drainage water were less than 3 mg N/L for all four treatments (Figure 6.6). In Year 2, the RGWC, IRWC and PWC treatments had peak TN concentrations of 14, 15 and 10 mg N/L in the initial drainage events, respectively, while the brassica treatment had a peak TN concentration of 21 mg N/L in the last drainage event of the Year. In Year 3, RGWC, IRWC, PWC and brassica treatments had peak TN concentrations of 9, 37, 44 and 33 mg N/L, respectively, in the first two to three drainage events.

The cumulative amount of TN leached in Year 1 was small (< 1.8 kg N/ha) for all treatments and did not differ ($P>0.05$) across treatments (Table 6.2). In Year 2, cumulative leaching loads of TN for the RGWC, IRWC and PWC treatments were < 2.6 kg N/ha and did not differ ($P>0.05$) among the treatments. While the cumulative leaching load of TN from the brassica treatment was 9.5 kg N/ha, which was three times greater ($P<0.05$) than the losses from the other three treatments (Table 6.2), with large differences observed, particularly in the latter part of the drainage season (Figure 6.7). In Year 3, the TN leached from PWC and IRWC treatments (~11.5 kg N/ha) were two to three times greater ($P<0.05$) than from the RGWC (3.4 kg N/ha)

and brassica (6.4 kg N/ha) treatments (Table 6.2). The leaching of TN from brassica treatment was greater ($P<0.05$) than RGWC and lower ($P<0.05$) than IRWC or PWC treatments.

While the annual NO_3^- load in drainage varied among treatments and years (loss of NH_4^+ was negligible), the remaining component of TN, referred to as total organic N (TON), was relatively uniform among treatments (average of 2, 2, 1 and 1 kg TON/ha for the RGWC, IRWC, PWC and brassica treatments, respectively) and years (1, 2 and 2 TON/ha for Years 1, 2 and 3, respectively).

6.4.3 Sheep performance and forage production

The effect of forage treatments on the performance of ewes and their lambs was described in Chapter 4. Overall, there was no significant difference ($P>0.05$) in animal performance among forage treatments (see Tables 4.8 to 4.10; Figures 4.5 and 4.6).

Sheep grazing days in drainage plots

The RGWC and IRWC treatments in the drainage plots were grazed five times for a total of 27 days in Year 1. The PWC treatment plots were grazed four times (13 days), while brassica plots were grazed once for a period of 11 days during the winter (Figure 6.1).

In Year 2, the RGWC and IRWC treatments in the drainage plots were grazed nine times each throughout the year (Figure 6.1) for a total of 46 and 47 days, respectively. The PWC treatment plots were grazed six times with a relatively small number of grazing days (26 days). The brassica treatment plots were grazed continuously for 33 days during winter.

During the sample collection period in Year 3, the RGWC and IRWC treatment plots were grazed seven times (Figure 6.1) for a total of 41 and 29 days, respectively. The PWC treatment plots were grazed four times, with a relatively small number of grazing days (20 days). The brassica treatment plots were continuously grazed for approximately 35 days during the winter.

Forage mass of treatments in drainage plots

The pre-and post-grazing DM mass of treatments plots are explained in Chapter 4 (see Figure 4.3). There was no difference (P>0.05) in total DM accumulation between the RGWC (9,909 and 10,438 kg DM/ha in Years 1 and 2, respectively), IRWC (11,479 and 12,345 kg DM/ha in Years 1 and 2, respectively) and PWC (9,753 and 9,162 kg DM/ha in Years 1 and 2, respectively) treatments in Years 1 and 2. However, in Year 3, total DM yield was greater (P<0.05) in the RGWC treatment (5,822 kg DM/ha) and was higher (P<0.05) than for the IRWC (3,203 kg DM/ha) and PWC (2,929 kg DM/ha; see Table 4.5) treatments. There was no significant difference (P>0.05) in total DM accumulation for the brassica treatment from year to year (5,815, 5,944 and 5,501 kg DM/ha in Years 1, 2 and 3, respectively).

Botanical composition of forage treatments

The botanical composition (grass, clover, weeds and dead material) for the RGWC, IRWC and PWC treatments is given in Table 6.3. In most seasons, RGWC and IRWC had high grass contents (>69%, except in the summer of Year 2). For the PWC treatment, the plantain content was high (>69%) initially (spring of Year 1 and summer and late-autumn of Year 2), but subsequently decreased to < 60% for the remainder of Year 2 and for all of Year 3. This decline in plantain content coincided with an increase in weed content, which was as high as 52.1% of the botanical composition by the spring of Year 3. Overall, the WC percentages of RGWC and IRWC were small and accounted for less than 4% of botanical composition. Although, PWC had around 2-6% of WC in summer and late-autumn of Year 2 and winter and spring of Year 3, a relatively greater (approximately 15-20%) percentage of WC was observed in the spring of Year 2 and summer of Year 3.

Table 6.3 Botanical composition[1] (% of total DM) of the forages (RGWC: perennial ryegrass/white clover; IRWC: Italian ryegrass/white clover; PWC: plantain/white clover) in spring of Year 1 (July to December 2019) and summer, late-autumn, winter and spring of Years 2 (January to December 2020), and 3 (January to December 2021). Values are least-squares means.

Species type		Year 1	Year 2				Year 3				SEM
		Spring	Summer	Late-Autumn	Winter	Spring	Summer	Late-Autumn	Winter	Spring	
Grass/herb	RGWC	80.5^{defg}	54.3^{i}	74.7^{fgh}	82.5^{bcdef}	84.6^{bcdef}	73.3^{gh}	92.3^{abc}	93.6^{ab}	93.0^{ab}	3.7
	IRWC	87.6^{abcde}	55.0^{i}	79.0^{efgh}	94.1^{ab}	94.8^{ab}	78.0^{efgh}	93.5^{ab}	95.8^{a}	94.9^{a}	
	PWC	90.1^{abcd}	69.5^{h}	78.8^{efgh}	nm	47.2^{ij}	46.8^{ij}	nm	55.8^{i}	42.3^{j}	
White clover	RGWC	0.3^{e}	0.2^{e}	2.5^{cde}	0.1^{e}	1.1^{cde}	4.0^{cde}	1.5^{cde}	0.6^{de}	3.3^{cde}	1.4
	IRWC	0.1^{e}	0.1^{e}	0.3^{e}	0.4^{e}	0.1^{e}	1.7^{cde}	1.4^{cde}	1.1^{cde}	2.9^{cde}	
	PWC	0.6^{de}	6.2^{c}	4.7^{cd}	nm	19.7^{a}	15.3^{a}	nm	2.2^{cde}	4.9^{c}	
Weeds	RGWC	16.4^{d}	5.5^{efgh}	4.5^{efgh}	11.8^{de}	8.7^{defg}	10.4^{def}	3.5^{fgh}	3.0^{fgh}	0.8^{gh}	2.9
	IRWC	10.4^{def}	0.3^{h}	0.1^{h}	0.1^{h}	0.4^{h}	5.5^{efgh}	4.8^{efgh}	1.4^{gh}	0.1^{h}	
	PWC	1.4^{gh}	3.1^{fgh}	4.0^{efgh}	nm	26.2^{c}	30.1^{c}	nm	41.6^{b}	52.1^{a}	
Dead material	RGWC	2.8^{ghij}	40.0^{b}	18.4^{cd}	5.7^{fgh}	5.7^{fg}	12.4^{e}	2.7^{ghij}	2.7^{ghij}	2.8^{ghij}	1.4
	IRWC	2.0^{ghij}	44.7^{a}	20.9^{c}	5.5^{fgh}	4.7^{fghi}	14.8^{de}	0.3^{j}	1.7^{hij}	2.1^{ghij}	
	PWC	7.9^{f}	21.3^{c}	5.8^{fg}	nm	1.2^{ij}	0.1^{j}	nm	0.8^{ij}	0.7^{j}	

[a-j]Superscripts with different letters indicate that means are significantly different among forage treatments within species type across years ($P<0.05$); nm: not measured as PWC was not grazed in winter of Year 2, and late-autumn of Year 3. [1]Botanical composition of forages were determined only once per season.

6.4.4 Sheep nitrogen intake and excretion

Nitrogen concentration of forages

In Chapter 5, crude protein (CP) concentration, apparent daily DM intake (DMI) and apparent N intake (NI) of the forage treatments are described in more detail (see Tables 5.3 and 5.4, and Figure 5.2). There was no difference (P>0.05) in N concentrations between the RGWC and IRWC treatments within seasons for a given year except in the winter of Year 1 and the late-autumn and spring of Year 3 (Table 6.4). The IRWC treatment had a higher (P<0.05) N concentration in the late-autumn of Year 3 than other treatments and/or seasons. In the summer of Years 2 and 3, PWC had greater (P<0.05) N concentrations compared to the RGWC treatment. The leaves of the brassica crop had higher (P<0.05) N concentrations than in either the bulb or stem.

Apparent N intake and urinary N excretion

More detailed information on apparent N intake and urinary N excretion are discussed in Chapter 5. No differences (P>0.05) were observed in apparent N intake between ewes grazing RGWC and IRWC within the seasons of a given year (see Figure 5.2). Ewes grazing PWC had greater (P<0.05) apparent N intake compared to ewes grazing RGWC in the spring of Year 2 (120 *vs.* 59 g/ewe/d, respectively) and summer of Year 3 (79 *vs.* 25 g/ewe/d, respectively). Ewes grazing brassica had greater (P<0.05) apparent N intakes compared to the ewes grazing RGWC in the winter of 2019 (155 *vs.* 74 g/ewe/d, respectively). In contrast, the apparent N intakes of ewes grazing brassica were lower (P<0.05) than those grazing RGWC in the winter of Year 2 (40 *vs.* 107 g/ewe/ d, respectively) and late-autumn of Year 3 (21 *vs.* 100 g/ewe d, respectively; see Figure 5.2).

Table 6.4 Nitrogen concentration (g/kg DM) of forages (RGWC: perennial ryegrass/white clover; IRWC: Italian ryegrass/white clover; PWC: plantain/white clover; brassica in winter and spring of Year 1 (July to December 2019) and summer, late-autumn, winter and spring of Years 2 (January to December 2020), and 3 (January to December 2021). Values are least-squares means.

	Year 1		Year 2				Year 3				MSE
	Winter	Spring	Summer	Late-Autumn	Winter	Spring	Summer	Late-Autumn	Winter	Spring	
RGWC	31.0^{ij}	33.6^{ghi}	18.1^{n}	34.1^{ghi}	45.4^{c}	39.0^{ef}	30.5^{ij}	48.5^{b}	44.8^{cd}	32.7^{hi}	1.10
IRWC	24.2^{klm}	32.5^{hi}	19.8^{n}	35.4^{gh}	46.1^{bc}	38.8^{ef}	29.1^{j}	54.6^{a}	44.3^{cd}	26.9^{kl}	
PWC	nm	35.0^{gh}	22.9^{m}	36.4^{fg}	nm	41.9^{de}	36.6^{fg}	nm	43.1^{cd}	29.2^{jk}	
Brassica	35.8^{fgh}	-	-	-	13.6^{p}	-	-	14.3^{op}	17.1^{no}	-	

[a-l]Superscripts with different letters indicate that means are significantly different ($P<0.05$) among forage treatments across the entire Table; nm: not measured as PWC was not grazed in winter of Years 1 and 2, and late-autumn of Year 3; -: no forage was present to sample as brassica was only present in winter of Years 1 and 2, and late-autumn and winter of Year 3.

Overall, N excretion was low under sheep grazing forage treatments (<33 g N/ewe/d; see Tables 5.8 and 5.9). The estimated daily urinary N excretion of ewes grazing IRWC and RGWC did not differ (P>0.05) within seasons of a given year. Daily urinary N excretion (based on creatinine concentration) of ewes grazing PWC was lower (P<0.05) than for the RGWC treatment in the spring of Year 2, whereas (based on plasma urea concentration and live weight), daily urinary N excretion of ewes grazing PWC was lower (P<0.05) in the winter and spring of Year 3 (see Tables 5.8 and 5.9). In Years 2 and 3, ewes grazing brassica had significantly lower (P<0.05) levels of urinary N than other treatments, but it was not significantly different (P>0.05) in Year 1 (see Tables 5.8 and 5.9).

The N intake, urinary N excretion and the NO_3^- leaching losses under sheep grazing different forages measured in Year 2 are presented in Table 6.5. In Year 2, lower (P<0.05) urinary N per sheep was excreted under sheep grazing the brassica treatment. In contrast, greater (P<0.05) NO_3^- leaching losses were observed under brassica compared to other treatments.

Table 6.5 Effect of forages on apparent nitrogen (N) intake and excretion in urine and faeces, and nitrate N losses under sheep grazing in Year 2 (2020). Values are least-squares means ±SE.

	RGWC	IRWC	PWC	Brassica
Apparent dietary nitrogen (N) intake (g/ewe/d)[1]	69.2^{b}±7	69.0^{b}±7	88.3^{a}±8	30.6^{c}±14
Urine N concentration (g N/L)	4.0^{a}±0.5	4.1^{a}±0.5	2.2^{b}±0.5	1.8^{b}±0.9
Estimated urine volume (L/ewe/d)[2]	4.6^{b}±0.7	5.4^{b}±0.7	7.7^{a}±0.9	4.5^{ab}±1.5
Estimated urinary N excretion (g/ewe/d)[3]	12.6^{a}±1.0	13.8^{a}±1.0	9.2^{b}±1.2	2.0^{c}±2.1
Estimated urinary N excretion (g/ewe/d)[4]	16.8^{a}±1.0	16.6^{a}±1.0	16.6^{a}±1.1	2.8^{b}±2.1
Faecal N[1]%	3.1^{b}±0.1	3.3^{ab}±0.1	3.5^{a}±0.1	2.7^{c}±0.1
NO_3^--N loss (kg/ha/year)	0.8^{b}±0.6	0.5^{b}±0.6	0.5^{b}±0.7	6.4^{a}±0.6
NO_3^--N loss (g/ewe/ha/year)[5]	53.6^{b}±43	37.9^{b}±43	32.1^{b}±50	453.6^{a}±43

[1]This was calculated based on Mathews et al., (1999) by dividing the difference between pre-and post-grazing DM mass for each grazing event by the number of grazing days and N concentration of the forages. [2]Urine volume (L/day) = $\frac{\text{urinary creatinine excretion (mg/kg daily of liveweight)} \times \text{live weight (kg)}}{\text{creatinine concentration (mg/deciliter)}}$, based on David et al. (2015). [3]Urinary N excretion (g/day) = creatinine excretion factor (mg/kg) × live weight (kg) × $\left(\frac{1}{\text{urinary creatinine (mg/kg)}}\right)$ × urine N (g/kg), based on David et al. (2015). [4]Urinary N excretion (g/day) = 1.2 X plasma urea concentration(g/L) × liveweight (kg), based on Kohn et al. (2005). [5] NO_3^--N loss (kg/ha) was divided by stocking rate of 14 ewes/ha.

6.5 Discussion

The impact of NO_3^- leaching under grazing systems, particularly dairy farms, on water quality is well known, and a number of mitigation practices have been developed to reduce NO_3^-

leaching (Houlbrooke 2005; Anastasiadis et al. 2012; Cameron et al. 2013; Christensen et al. 2019). However, many of the mitigation strategies suggested for dairy systems cannot be easily applied to sheep grazing systems due to the differences in the management systems and the relative profitability, e.g., housing (Anastasiadis et al. 2012). Fortunately, at least one of the mitigation options, that of alternative forages such as plantain and winter active Italian ryegrass, could be beneficial to sheep grazing systems in that their use may result in both an increase in production and a reduction in NO_3^- leaching. Therefore, this study was conducted to investigate how these forages influence NO_3^- leaching under modern, intensive sheep grazing systems.

The NO_3^- losses in drainage under RGWC were very small in all three years. The NO_3^--N leaching rates under RGWC ranged from 0.5 kg N/ha in Year 1, where only the latter part of the drainage season (July onwards) was monitored, to 1.5 kg N/ha in Year 3. The leaching rates of NO_3^--N measured under sheep grazing forages were very small when compared with losses of 12 to 23 kg N/ha measured during the same period at a very similar research site on a neighbouring dairy farm (approximately 500 m distance; Nguyen et al. (2022)). The amounts of NO_3^- leached per ha observed in this study were much lower than those previously reported (8 to 50 kg N/ha) for sheep grazing RGWC on the same soil type (Heng et al. 1991; Magesan et al. 1996; White et al. 1998). Differences in the stocking rate and grazing time may explain the differences among the sheep studies. For example, White et al. (1998) stocked 2,800 sheep grazing days/ha in May 1990 and 840 sheep grazing days/ha in July 1991 and reported losses of 35 and 43 kg N/ha, respectively, whereas in the current study, the stocking rate was kept constant (46 sheep in an area of 0.4 ha), and so there was an average of only 575 sheep grazing days/ha in May and July (Figure 6.1).

The small leaching losses measured under RGWC may be explained by the interaction between urine N load, plant uptake, and drainage (mm). Under a urine patch, the overall N load was estimated to be about 200 kg N/ha based on urine volume of 180 ml, urine N concentration of 4.5 g N/L, and patch size of 0.04 m^2. If the urine N load is small and spread throughout the plot, the potential for plant uptake is greater. In addition, the amount of drainage measured during the study period was low (< 150 mm/year). However, future studies are needed to confirm the effect of plant uptake, number and size of urine patches. It is also important to consider other soil factors, including the soil carbon to N ratio and the fertility of the soil. In addition, there is a possibility that some of the urine N might be oxidized and lost as nitrous oxide, but further research is required to confirm this. The Tokomaru silt loam soil is vulnerable to denitrification because of its fine texture (Saggar et al. 2004).

In terms of NO_3^- leaching, these data suggest that on this soil type (fine textured with mole and pipe drainage), sheep grazing of RGWC pastures has a very significant advantage over dairy cattle systems. Sheep farming, including dairy sheep, might be a mitigation option in cow dairying areas where it is difficult to achieve large reductions in NO_3^- leaching required to meet water quality objectives and/or N loss allocations imposed by regional councils. It is often argued that the relative profitability of sheep versus dairy farms mitigates against any such land use change. However, given the very small leaching losses measured in the current study, there would appear to be substantial scope to increase the livestock intensity within sheep farms which would allow for improved financial performance while maintaining 'acceptable' N leaching rates. However, stocking rates may be limited by forage production, and further research is required as to how such increases might be achieved.

For the first two years of this study, the NO_3^--N losses from IRWC and PWC were very small (ranging from 0.4 to 0.5 kg N/ha), and they were not different from losses under RGWC. As

noted above, there is evidence that NO_3^- leaching under IRWC (Moir et al. 2012; Malcolm et al. 2014; Malcolm et al. 2015) and PWC swards by dairy cows can be markedly smaller than losses under RGWC. However, no such reduction was observed in this study, but this is not surprising given the very small losses measured under all treatments in the first two years.

Interestingly, in Year 3 when there were differences in NO_3^- leaching losses between RGWC and IRWC or PWC treatments, the losses for the RGWC treatment were lower, not greater, than the other two treatments. However, this increase in leaching under the alternative pastures may have been less due to an intrinsic feature of the plants themselves, but rather the time that the plots with these treatments were fallow during the key late summer-autumn period. In the summer of Year 3, the PWC plots suffered from significant dock infestations, and the IRWC plots had reduced tiller numbers. Therefore, these plots were sprayed and resown in the autumn (14 April 2021) of Year 3, using a direct drill. As a consequence, after grazing in the late-summer of Year 3 (February 2021), there was limited forage cover in the PWC and IRWC plots until early May 2021. Urine N deposited in the latter part of February was likely leached in the drainage that occurred in May and June due to insufficient foliage cover to absorb the NO_3^-. Even with the resowing, PWC plots still comprised 55% of docks in the winter and spring of Year 3, which resulted in greater leaching from these plots in July. Additionally, the IRWC plots had patchy growth after resowing, so the plots did not have sufficient foliage cover to take up the deposited N. The poor establishment and growth of the IRWC sward (the amount of total DM harvested in Years 1 and 2 was ~12000 kg DM/year, whereas, in Year 3, it was ~3000 kg DM/year; see Chapter 4) could explain the high concentrations of NO_3^--N in drainage in May and June. If these alternative forages, such as IRWC and PWC fail to persist in certain soil and climate conditions and need be renewed every two to three years, with the attendant increase in NO_3^- leaching observed in this study, then this feature will erode the benefits of small leaching losses in the intervening years. If the focus is on achieving an environmental

benefit from the use of these forages, efficient and timely resowing is of vital importance. On a free draining soil, where N leaching rates are likely to be greater, these alternative forages may be well established, and so their benefits to NO_3^- leaching may be greater and more consistent.

It is important to consider the clover content of the sward mix because a high clover content is likely to cause a rise in the level of N in the biological system (Ruz-Jerez et al. 1995), which could then result in greater losses of NO_3^- (Rodriguez 2020). According to dairy cattle research, to reduce the leaching of NO_3^- from plantain-based pastoral systems, it should contain at least 30% plantain (Navarrete et al. 2022) and not more than 20% clover (Rodriguez 2020). However, in this study, clover content was less than 20% in all treatments throughout the study period, suggesting that clover would have had a limited influence on NO_3^- leaching.

Leaching of NO_3^- was significantly greater in Year 2 under the brassica treatment than under other treatments and leaching under the brassica was also greater than losses from RGWC in Year 3. The impact of fodder crops on NO_3^- leaching is well documented (Shepherd et al. 2012; Monaghan et al. 2013; Malcolm et al. 2016). Although leaching losses under the brassica crops grown in the current study were greater than those measured for the other treatments in Year 2, they were still smaller than rates reported in other dairy cattle studies (Shepherd et al. 2012; Monaghan et al. 2013; Malcolm et al. 2016). Furthermore, leaching of NO_3^- under brassica in Year 2 was likely greater than normal because the drainage season extended to December, which is not typical in most years.

A fodder crop (brassica) was selected as a treatment because of the role that these crops play in intensive sheep production systems. At key times in the year, these crops can provide the feed required to maintain greater stocking rates and improve animal performance (Sprague et al. 2014; Agricom 2018). The results presented here suggest that while more NO_3^- may leach

from areas of the farm growing a brassica crop, this increase will be small for the farm system. Given that the cropped area of a pastoral farm typically occupies a relatively small portion of the farm, any increases in whole farm NO_3^- leaching will be minor when sheep production systems practise fodder cropping. Further, while on this area at a higher stocking rate, the remainder of the farm, which is the greater area, will have fewer livestock.

Leaching losses of TN followed a similar pattern to that of NO_3^-, mostly because TN contains a large proportion of NO_3^- (Christensen et al. 2019). Studies conducted at the neighbouring dairy cattle farm site have reported that NO_3^- accounts for 54 to 82% of the amount of TN in drainage (Hanly et al. 2017; Christensen et al. 2019). Likewise, in this study, regardless of forage treatment, NO_3^- comprised between 55 and 84% of TN when losses exceeded 6 kg TN/ha, while NO_3^- comprised around 21-44% of TN when losses were small (<3 kg TN/ha). This reflects that relatively constant amount of TON leached from all treatments, which appeared not to be affected by forage treatments and years. Total ON is likely to be generated by the decomposition of plant litter and soil organic matter (Haynes & Williams 1993). The consistent TON losses suggest that 1-3 kg N ha^{-1} $year^{-1}$ of TON is leached regardless of the forage treatment and subsequent urine deposition.

The results reported here are best explained by the quantity of excess NO_3^- that accumulates in the soil profile and the timing of drainage events, particularly the first events of the season. In general terms, the NO_3^- concentration of drainage followed the pattern commonly observed in studies of leaching from this soil under this climate (Hanly et al. 2017; Christensen et al. 2019; Howes 2019). Most of the NO_3^- leaching under pastures in this study occurred in the first 40 mm or so of drainage, and drainage events in the latter part of the season tended to result in only marginal increases in leaching. This pattern in leaching can be explained by changes in the NO_3^- concentration in drainage. The mean NO_3^- concentration of drainage water collected

from RGWC, IRWC and PWC treatments in Years 2 and 3 was relatively large in the initial drainage events (e.g., 20 to 45 mg N/L for IRWC and PWC forage treatments in Year 3) and decreased with successive drainage events. The mean NO_3^- concentration in late season drainage was low. It is likely that the NO_3^- that had accumulated in the soil under urine patches prior to the onset of drainage events contributed to early NO_3^- leaching for the RGWC, IRWC and PWC treatments. A similar pattern has been observed in numerous studies of leaching from this soil under pasture grazed by sheep (Heng et al. 1991; Magesan et al. 1996) and dairy cattle (Hanly et al. 2017; Christensen et al. 2019; Howes 2019).

Other researchers have noted that differences in leaching rates among treatments imposed on pastoral plots on this soil manifest, or are most pronounced, in the initial drainage events and tend to be less apparent in later drainage events (Hanly et al. 2017; Christensen et al. 2019; Howes 2019). This point is nicely illustrated by the differences in NO_3^- leaching among RGWC and IRWC or PWC in Year 3, where the differences in leaching under these treatments are explained by the differences in NO_3^- concentration in drainage from these treatments between May and the end of June.

In Year 2, the brassica treatment had greater NO_3^- concentrations in drainage than other treatments in December, which was later than the normal end of the drainage season. The brassica plots were intensively grazed over the winter, after which time the land was left bare for 17 weeks before they were resown in late December. A lack of plant uptake likely resulted in the accumulation of NO_3^- in the soil and high concentrations of NO_3^- in subsequent drainage. The brassica treatment also had greater concentrations of NO_3^- in the initial drainage events of Years 2 and 3, which may have been due to the mineralised N that accumulated as a result of renewing the forage (spraying herbicide, fertiliser application and direct drilling; Fraser et al. (2013).

Nitrate leaching under the brassica treatment was greater than that under other treatments in Year 2 due, in the main, to the large concentrations of NO_3^- in the late season drainage. The length of the drainage season in Year 2 was longer than reported in previous studies conducted near the current research site (Magesan et al. 1996; Houlbrooke et al. 2003; Christensen et al. 2019; Howes 2019). In Year 2, at the end of the drainage season, the drainage measured under the brassica treatment was greater than that measured for other treatments, which may be due to the lower evapotranspiration rates associated with bare soil in spring (Hanly et al. 2017). It is likely that the combination of the increased NO_3^- concentrations and the larger drainage observed under brassica treatments led to a greater NO_3^- load at the end of the drainage season in Year 2. The occurrence of drainage after mid-October is unusual for this region, and in a soil-water balance model that was used to estimate the quantity of drainage occurring in mid-October, Hanly et al. (2017) reported that only 8 out of 30 years had greater than 50 mm of drainage after mid-October. Therefore, the drainage in Year 2 after mid-October was unusual. If no drainage had occurred after mid-October in the current study, then losses of NO_3^- are estimated to have been no more than about 2 kg N/ha under the brassica treatment.

Given that the ewes were in the brassica plots from late May to early July of Year 3, the N deposited during this period is likely to have contributed to the greater NO_3^- concentrations in the first drainage events of Year 3, as there was no plant uptake under bare soil during subsequent drainage events. A dairy cow study has also shown that N leaching losses from forage-crop grazing during winter can be more than twice that under grazed RGWC (Monaghan et al. 2013).

If the beginning of the drainage season was the most important period for NO_3^- leaching, and the initial drainage events of the Year 2 season were the most typical, then the results and trends discussed above can be explained by a consideration of urine-N dynamics (Table 6.5).

Although there are some small differences between the quantity of apparent dietary N intake on the forage treatments and dietary N excretion (according to one technique), these differences were small and were unlikely to affect leaching rates (Table 6.5). Hence the lack of differences in the quantity of NO_3^- leached from the forage treatments in Year 2. A comparison between the characteristics of sheep urine measured in this study and those of cows grazing the same soil (Appendix 8) helped explain the comparatively small values measured under sheep grazing pasture in the present study.

Interestingly, urine-N return by sheep grazing brassica is relatively small (Table 6.5). This would suggest that if these crops are grazed later in the season when the likelihood and quantity of drainage is decreasing then leaching losses may be small. Alternatively, if a catch crop (i.e., any fast-growing crop such as oats, triticale or rye corn is grown between successive plantings of the main crop with the primary objective of capturing excess N in soils that would otherwise be lost through leaching) is sown with the brassica or established immediately after the crop is grazed, it may mitigate the risk of leaching under bare soil. For example, had a catch crop been grown in Year 2, then it may will have reduced the NO_3^- losses measured in the December of that year.

6.6 Conclusion

This study shows that sheep grazing of traditional RGWC pastures resulted in very low NO_3^- leaching on fine-textured Pallic soil with mole and pipe drainage. Leaching losses under sheep grazing alternative forages such as IRWC and PWC were also very small in Years 1 and 2; however, these swards failed to persist in Year 3, so their benefits to NO_3^- leaching could not be observed in intervening years. The poor persistence of IRWC and PWC on this fine-textured soil and the greater NO_3^- leaching associated with the renovation of these pastures in Year 3

undermines some of the advantages associated with the small leaching rate measured in Years 1 and 2.

The grazing of a winter brassica is likely to result in a slightly greater NO_3^- leaching compared to RGWC; however, based on the assumption that the cropped area will occupy a relatively small portion of the farm, increases in whole farm NO_3^- leaching is expected to be relatively small when sheep production systems practise fodder cropping. Overall, the results indicated that current sheep grazing systems may offer mitigation options for dairying areas where it is difficult to achieve the large reductions in NO_3^- leaching required to meet water quality objectives.

CHAPTER 7: SUMMARY AND GENERAL DISCUSSION

7.1 Summary and justification of this study

In response to increasing costs of production, and in order to maintain economic viability, sheep farming systems in New Zealand are continuing to intensify through means such as increased fertiliser use, improved 'per head' animal performance, and/or higher stocking rates (Morris & Kenyon 2014). However, intensification may lead to a higher level of nitrogen (N) leaching into waterways. Historically, sheep production research in New Zealand has focused primarily on increasing productivity (Judson et al. 2009; Raeside et al. 2014; Cranston et al. 2015b), and little is known about the effects of sheep farming systems on N leaching.

New Zealand sheep farmers, like their dairy farming counterparts, sow a varying percentage of their farm area in alternative forages to minimise the time of seasonal pasture shortage (Beef + lamb 2018). Alternative forages such as plantain (Box et al. 2016; O'Connell et al. 2016; De Klein et al. 2019) and winter-active Italian ryegrass (Nichols & Crush 2007; Moir et al. 2013; Woods et al. 2016; Maxwell et al. 2019) have been reported to reduce N leaching under dairy cow grazing. Both these species match or exceed the growth rate of ryegrass at key times of the year, plantain in summer (Lee et al. 2015; DairyNZ 2022b), and Italian ryegrass in winter (Charlton & Stewart 1999; Ryan-Salter 2011), and therefore may improve sheep performance. Therefore, these forages could be useful for reducing N leaching under sheep grazing systems while increasing sheep performance. The present study was designed to evaluate N leaching losses under a range of forage types (perennial ryegrass/white clover: RGWC; Italian ryegrass/white clover: IRWC; plantain/white clover: PWC; a winter brassica) and their effect on sheep performance to identify the potential for forages to reduce N leaching while maintaining or improving sheep performance. A fodder crop, brassica, was used as one of the treatments, as these crops are typically high yielding which allows increased stocking rates

during winter. Following grazing, the land is left bare until conditions are favourable for re-sowing in spring. This grazing of brassica species in winter may result in large amounts of N being returned to the soil, which can subsequently lead to high levels of NO_3^- leaching (Monaghan et al. 2013). Different brassicas were used in these studies depending on the time of sowing (turnips, swedes and kale for autumn of 2019, late spring of 2019 and 2020, respectively) and the need to minimise the risk of clubroot (*Plasmodiophora brassicae*).

This study was undertaken on four self-contained contiguous farmlets (each approximately 3.32 ha) over two and a half years (Year 1: July to December 2019; Year 2: January to December 2020; Year 3: January to December 2021). An area of 0.81 ha (24% of grazing area) of the three farmlets was sown in one of three alternative forages (IRWC or PWC or brassica), and the remaining 2.51 ha were sown in a perennial ryegrass/white clover sward. The entire area (100% of grazing area) of the fourth farmlet, which acted as the control, was sown in RGWC. Approximately 0.4 ha of each farmlet was located in a paddock where a series of drainage plots were established to measure N leaching. There were 20 drainage plots (each 40 m by 20 m). Each of the alternative forages, and the RGWC, were sown on five of the drainage plots i.e., five replicates (combined area of 0.4 ha), which composed about one-half of each alternative forage area on each farmlet.

Not all forages were grazed year-round: RGWC and IRWC were grazed in all seasons, whereas PWC was only grazed in the spring of Years 1, 2 and 3, summer of Years 2 and 3, and autumn of Year 2 and winter of Year 3, while brassica crops were only grazed in the winter of Years 1, 2 and 3 and autumn of Year 3. The effects of the forages on DM production and subsequent ewe and lamb performances (Chapter 4), N excretion (Chapter 5), and N losses through mole and pipe drainage water (Chapter 6) were examined.

7.2 Main findings from this research

7.2.1 Ewe and lamb performance (Chapter 4)

The objective of this chapter was to examine the effects of the different alternative forages, within a perennial ryegrass/white clover-based system, on the performance of breeding ewes and lambs.

Pre-and post-grazing masses of the alternative forages were only measured on the drainage plots. The annual total DM (kg DM/ha) of IRWC and PWC harvested by sheep was similar to the quantity of RGWC harvested in Years 1 and 2. However, in Year 3, more RGWC was harvested than IRWC or PWC. This was due to the renewal and resowing of the IRWC and PWC plots. The pre-grazing masses of IRWC and PWC on the drainage plots were typically greater than 1500 kg DM/ha. Ewes were allocated 1.8 kg DM/day of brassica, with an average forage utilisation rate of 85%, indicating intake was not restricted when ewes were grazing the various brassica forages. The ME and CP contents of RGWC, IRWC, and PWC were in the range of 10-12 MJ ME/kg DM and 8-16%, respectively. Compared to RGWC, PWC had a higher CP content in the summer of Years 2 and 3 and a higher ME content in the summer of Year 3. The ME content of the brassica forages on the drainage plots remained above 12.0 MJ ME/kg DM over the study period. The results indicated that alternative forages performed equally as the RGWC, in terms of both quality and quantity.

The average forage covers of all treatments across the farmlets (in non-drainage plot areas) were above 1200 kg DM/ha for most of the study period. It is known that intakes are not limited when RGWC mass is greater than 1200 kg DM/ha (Morris & Kenyon 2004). Given that the RGWC area was at least 76% of the total grazed area in all treatments and that the average RGWC covers measured were consistently above 1200 kg DM/ha, the results indicate animal intakes were not restricted for extended periods. Ewe and lamb performances did not differ

among treatments. The live weight and BCS profiles of the ewes also indicated that ewes were well fed in all treatments. In addition, forages containing a ME content of 10-12 MJ ME/kg DM (Nichol 2007; Cranston et al. 2017) and CP content of 8-16% (National Research Council 2007; Hynd 2019) are adequate for sheep production. The CP and ME levels of the forages on the drainage plots were higher than, or equivalent to, the optimum level in most seasons. Given the similarity in both the quantity and quality of the diet across the farmlets, and the adequacy of this diet to high levels of animal performance, it is not surprising that the alternative forages had no impact on sheep performance per farmlet. However, it is unknown if the lack of treatment differences in animal performance would have occurred if greater stocking rates or greater areas of alternative forages had been used. Further research is needed to determine this.

7.2.2 Urinary nitrogen (N) excretion (Chapter 5)

The effects of the various forages (RGWC, IRWC, PWC and brassica) on N intake and its excretion in urine and faeces were examined in Chapter 5. There was no difference in estimates of urinary N excretion between ewes grazing RGWC or IRWC, whereas ewes grazing brassica and PWC excreted less urinary N, than ewes on RGWC. The reduction in urinary N concentration in dairy cows grazing plantain has been attributed to one of the following factors (i) increased urine volume, (ii) reduced rumen ammonia production, (iii) increased rumen microbial activity and (iv) shifting of N from urine to faeces (Bryant et al. 2019; De Klein et al. 2019). In this study, sheep grazing PWC had greater urine volumes, which resulted in smaller urine N concentrations than sheep grazing RGWC. In part, the larger urine volume observed in ewes grazing PWC may be attributed to lower forage DM% and greater Na concentration in plantain compared to RGWC. Moreover, compared with ewes grazing RGWC, ewes grazing PWC had higher faecal N concentrations, which also indicated that more N is being partitioned into faeces. Sheep grazing on brassica forages excreted significantly less urinary N than sheep grazing on other forages. This is not surprising given that ewes grazing

brassica had lower levels of CP and higher levels of WSC:CP intakes than those grazing other forages (Chapter 5; Edwards et al. (2007)). These results suggest that PWC and brassica have the potential to reduce urinary N load per animal to the soil. However, brassica forages are often grazed at high stocking rates or for longer days during the critical winter period. These management practices may result in higher leaching losses under brassica forages (Chapter 6 or see 7.2.3).

7.2.3 Nitrogen losses from alternative forages (Chapter 6).

This chapter describes the assessment of N leaching under sheep grazing of the different forages (RGWC, IRWC, PWC and brassica). Regardless of forage treatments, nitrate-N (NO_3^--N) concentrations in drainage water were high (5 to 25 mg N/L) at the beginning of all drainage seasons but decreased rapidly to very low levels within the first 20 to 60 mm of drainage. This suggests that the urine N deposited before the onset of the drainage season has largely contributed to the NO_3^- leaching. However, the brassica treatment in Year 2, had higher NO_3^- levels in December. It is likely that the combination of the increased NO_3^--N concentrations and the larger drainage observed under the brassica treatment led to a greater NO_3^- load at the end of the drainage season in Year 2. The greater drainage volume measured on the brassica plots may be due to the lower evapotranspiration rates associated with bare soil in spring. The occurrence of drainage after mid-October is rare. With the use of a soil-water balance model, Hanly et al. (2017) demonstrated that only 8 out of 30 years had greater than 50 mm of drainage after mid-October.

For the first two years of this study, the N losses from RGWC, IRWC and PWC were very small (<1 kg N/ha), and they did not differ among treatments. However, in Year 3, leaching losses under the IRWC and PWC were greater than losses under RGWC. This increase in leaching losses in Year 3 under IRWC and PWC is most likely due to the interventions that

were required to re-establish these swards. In Year 3, the plantain plots were identified as having a significant dock (*Rumex obtusifolius*) infestation, and the IRWC had lower tiller numbers; therefore, these forages were sprayed and re-sown. Hence, the plots with these treatments were fallow in the key late summer-autumn period and this resulted in a greater level of NO_3^- leaching under these two forages than those under RGWC. The poor persistence of IRWC and PWC on this fine textured soil and the greater NO_3^- leaching associated with renovation of these pastures in Year 3 undermines some of the advantages associated with the small leaching rate measured in Years 1 and 2.

In contrast, NO_3^- leaching losses under the brassica were greater than losses from RGWC in Years 2 and 3. As noted above, the late season drainage in Year 2 was atypical, and if no drainage had occurred after mid-October, the losses would likely have been 2 kg N/ha, which may not be much greater than the losses reported under RGWC. Although sheep grazing brassica forages excreted less urinary N per day (on individual animal basis), there was a greater leaching loss under the brassica treatment. This is likely because brassica plots were grazed for a more extended period during winter than other forages, and there was no crop cover until spring resowing; therefore, the urinary N accumulated during winter grazing might have been displaced by subsequent drainage. Nevertheless, losses measured under the brassica treatment are still smaller than rates reported in a similar study of fodder crops on the same soil grazed by dairy cows (Hanly et al. 2017). The results suggest that in winter when N leaching is likely to occur, sheep and beef cattle farmers may reduce N leaching by grazing their sheep on brassica and their cattle on pasture instead of the other way around, as is more common today.

Losses of total nitrogen (TN) followed a similar pattern to NO_3^-, primarily due to a high proportion of TN consisting of NO_3^-.

The leaching rates of NO_3^- measured under sheep grazing forages were very small compared with losses of 12 to 23 kg N/ha under dairy cattle grazing, measured during the same period at a very similar research site on a neighbouring farm (approximately 500 m distance) (Nguyen et al. 2022). This suggests that sheep grazing systems have a significant advantage over dairy farms in terms of NO_3^- leaching from this soil type (Tokomaru silt loam).

7.3 Practical Implications

7.3.1 Potential for sheep

This study indicates that brassica crops can produce high yields of supplementary feed for grazing, during the winter, when ryegrass growth is reduced, which is required to maintain higher stocking rates or to increase animal growth rates. Furthermore, given that the cropped area will generally occupy a relatively small portion of the farm area when used in sheep production systems, any increase in whole farm NO_3^- leaching, should be relatively small.

As noted above, sheep farming might be a mitigation option in dairying areas where it is difficult to achieve the large reductions in NO_3^- leaching required to meet water quality objectives and/or N loss allocations imposed by regional councils. However, it might be argued that the relative profitability of sheep farming compared to dairy farms will limit any such land-use change. Nevertheless, given the minimal leaching losses in this study, increasing the intensity of production within sheep farms may improve financial performance while maintaining 'acceptable' levels of NO_3^- loss. The annual income from milk solids (MS) produced (1000-1096 kg of MS/year/ha * $7.2/kg of MS) per unit of N applied (65 kg of urea/ha/year * $1.26/kg of urea) (data adapted from Rodriguez (2020)) may be only 1-5% greater than the annual income from lambs (502 kg live weight of lamb/ha/year * $6.5/kg of lamb) per unit of N (30 kg of urea/ha/year * $1.26/kg of urea). However, the N leached under the sheep grazing system (1.1 kg of N/ha, Chapter 6) would be 70% lower than that leached

under dairy systems (12 kg N/ha; Nguyen et al. (2022)). This suggests that, even if stocking rates were theoretically doubled in a sheep grazing system to compensate for the profitability of dairy systems, leaching losses would be of the order of 3 kg N/ha/year.

7.4 Limitation of this study

In this study, all replicates of a forage treatment were always grazed for the same number of days, and ewes were moved from all of the treatment plots when the target post-grazing residual was reached in any replicate. Notably, forage DM mass varied greatly within replicates of treatment; however, grazing length or stocking rate could not be adjusted since this would have confounded treatment comparisons of the leaching rate.

The length of the grazing period on each farmlet system was also adjusted in accordance with the availability of forage cover. The limited number of paddocks within each farmlet meant it was difficult to always match actual feed demand with pasture forage cover, and as a result, feed allocation was often in excess of feed demand. Furthermore, ewes were supplemented whenever necessary. This unrestricted feed intake resulted in no difference in sheep performance among treatments. Nevertheless, previous studies comparing the effects of forages on sheep performance demonstrated differences in performance when the forage intake was restricted during key production periods, such as pregnancy and lactation (Piaggio et al. 2018). Thus, in commercial farming operations where ewe flock sizes are larger and individual feed intake can be more controlled, and supplementary feed is used sparingly, it is likely that differences in sheep performance would be observed when different forages with different feed supply patterns or greater annual feed supply are utilised.

Continuous monitoring of urination events in sheep is challenging (David et al. 2015; Marsden et al. 2020); therefore, urine volume and urinary N excretion were estimated based on the N and creatinine concentrations of spot samples. However, as was observed in previous studies

(Betteridge et al. 2010; Hoogendoorn et al. 2010; Marsden et al. 2020), the urine N and creatinine concentrations of the animals within the treatment varied widely. Therefore, care must be taken when interpreting the present data for urinary N excretion and extrapolating it to 24 hours under grazing conditions.

Dairy studies have shown that cows grazing plantain-clover consume significantly more water from their feed (lower DM%) than those grazing ryegrass-clover, and therefore excrete more urine with smaller N concentrations (Box et al. 2017; Mangwe et al. 2019). In this study, however, rather than sheep grazing PWC having the largest urine volumes, greater urinary volumes were estimated for sheep grazing IRWC and brassica. In addition, some dairy studies (Box et al. 2017; Mangwe et al. 2019) have measured water intake from water troughs to estimate the relative influence of feed water on total urine volume, but water intake from water troughs was not measured in this study. Consequently, the effect of feed water intake on urine volume was not able to be evaluated.

The DM mass measured in the plots could not be used to calculate the forage growth rate due to the mowing, spraying, and over-sowing of the forages.

7.5 Opportunities for the future research

The performance of ewes and their lambs on different farmlet systems was evaluated based on their *ad libitum* intake levels. Therefore, this study could not compare the effects of incorporating a proportion of alternative forages within a farmlet, on the performance of sheep. Evaluation of sheep performance in a scenario where feed intakes are occasionally restricted or altering stocking rates (higher) according to the pasture cover, may be beneficial to the understanding of the potential effects of alternative forages on sheep performance and nutrient leaching, and therefore are recommended for future research, to help evaluate the differing herbages.

Spot samples were taken to estimate daily urine N excretion; however, there was large variation in urinary creatinine and, therefore, urine N concentrations among animals within the same treatment group. Metabolic studies that measure total urinary N excretion have reported smaller differences among animals. Therefore, to successfully predict total urine output (using spot urine samples), the relationship between creatinine excretion and urinary N excretion or daily urine volume must be clearly understood. Hence, further metabolic studies or total collection studies to investigate the relationship between total urine volume and creatinine excretion under different conditions, including animal weight, forage type, water intake, and the time of day, would likely be of benefit. Alternatively, sensors, such as a triaxial accelerometer (Marsden et al. 2021), can be used to collect urine samples continuously under grazing conditions, and is recommended for future research.

The winters of 2020 and 2021 were the warmest winters on record for the Manawatu region (NIWA 2022), and therefore, several years of studies with contrasting rainfall patterns and temperatures would be required to reliably determine NO_3^- losses under brassicas.

The majority of NO_3^- losses under brassica's were measured during the fallow period following intensive grazing during the winter period. Alternatively, a catch crop sown with the brassica or established immediately after the crop is grazed may mitigate the risk of leaching under bare soil and could be a future research option.

The amount of N fertiliser applied to all forages during the study period was minimal, and an increase in fertiliser level may increase dry matter production. In addition to increasing dry matter production, N uptake, and N recycling in urine, additional N fertiliser inputs also increase the likelihood of direct loss of N to the environment (Hatch et al. 2007). Therefore, there is an opportunity to conduct similar trials with different fertiliser levels to determine the relationship within increased plant production and sheep performance and leaching rate to

provide farmers with a general idea of maximum fertiliser rates for sustainable, profitable sheep farming.

Many dry stock farms in New Zealand have mixed flocks of sheep and cattle; therefore, it is required to conduct a number of studies to determine the impact of modern intensive cattle-sheep mix farming on nutrient leaching.

It would be beneficial to conduct a thorough economic analysis, considering all costs and returns associated with all treatments. The modelling of NO_3^- leached per unit of N would also be beneficial. This information could help farmers establish environmentally sustainable and profitable systems on a coarse textured free draining soil.

7.6 Overall Conclusions

The research presented in this thesis has provided information about the effect of a range of forage types on sheep performance and N leaching under intensive sheep grazing systems.

This study demonstrated that:

Overall, the average forage covers of all treatments were above 1200 kg DM/ha in the non-drainage plot areas. In Years 1 and 2 on the drainage plots, RGWC, IRWC and PWC showed similar total DM production; however, in Year 3, due to the need to re-establish the IRWC and PWC plots, greater total DM yield was harvested from RGWC compared to IRWC and PWC. Under the condition of the current study, the use of alternative forages did not positively or negatively influence animal performance. Therefore, the impacts of NO_3^- leaching under different forage types can be directly compared without confounding effects of different animal performance.

There were no differences in DMI among treatments, but ewes grazing brassica had a lower N intake compared with ewes grazing other treatments. Overall, the estimated daily urinary N

excretion levels across all seasons and forages ranged from 9 to 27 g N/sheep/d. The urinary N concentrations of ewes grazing on PWC, and brassica treatments were lower than those grazing on RGWC in all seasons where it was compared. The results suggest that PWC and brassica can reduce urinary N load per animal in the soil. Therefore, these forages could be advantageous under higher stocking rates on coarse-textured soils, where limiting or reducing N leaching rates might be a greater challenge.

In the first two years of this study, the NO_3^--N losses from IRWC and PWC forages were very small (<0.5 kg N/ha), and they were not different from those under RGWC. However, in Year 3 greater NO_3^- leaching associated with the renovation of these pastures undermines some of the advantages related to the small leaching rate measured in Years 1 and 2. Therefore, if using these forages for more than two years, swift pasture renewal is key to avoiding bare ground and minimum plant growth. In contrast, compared to RGWC, greater NO_3^- leaching losses were found under brassica treatments. However, under normal drainage patterns (Year 3) the leaching of NO_3^--N under brassica was relatively small (3.5 kg N/ha). Only in Year 2, where the drainage season extended into December, was a larger loss of NO_3^- measured under the brassica. Furthermore, based on the assumption that the cropped area occupies a relatively small portion of the farm, increases in whole farm NO_3^- leaching can be relatively small when sheep production systems practise fodder cropping. Nitrate leaching losses under sheep grazing were lower than those reported under dairy cow grazing, suggesting that sheep production may offer an alternative production option for dairying areas where it is difficult to achieve the large reductions in NO_3^- leaching required to meet water quality objectives.

REFERENCES

Agricom. 2018. Beet, brassica and forage cropping guide. agricom.co.nz. Accessed 30/9/2020 from https://www.agricom.co.nz/Files/Files/Public/Agricom/Guides/Cropping-Guide.pdf

Al-Marashdeh O, Cook GA, Anderson FC, Meyer JP, Logan CM, Edwards GR, Maxwell TM. 2020. Liveweight gain and urinary nitrogen excretion of lambs grazing diverse (plantain, italian ryegrass and red clover) or ryegrass-white clover pasture in autumn. In Proceedings of the New Zealand Society of Animal Production, Online, 70-75 p.

Al-Marashdeh O, Cameron K, Hodge S, Gregorini P, Edwards G. 2021. Integrating plantain (plantago lanceolata l.) and italian ryegrass (lolium multiflorum lam.) into New Zealand grazing dairy system: The effect on farm productivity, profitability, and nitrogen losses. Animals 11: 376. doi:10.3390/ani11020376.

Amer PR, McEwan JC, Dodds KG, Davis GH. 1999. Economic values for ewe prolificacy and lamb survival in New Zealand sheep. Livestock Production Science 58: 75-90. doi:10.1016/S0301-6226(98)00192-4.

Anastasiadis S, Kerr S, MacKay A, Roygard J, Shepherd M. 2012. The mitigation of nutrient loss from New Zealand agriculture: Separating the probable from the possible. Motu Economic and Public Policy Research, Wellington, New Zealand.

Anon. 2019. Beet, brassica and forage-cropping guide. *I*n: Agricom (ed.). Christchurch, New Zealand: Agricom.

AOAC. 2007. Official methods of analysis of aoac international, Gaithersburg, Maryland, AOAC International.

Appleton H. 2011. Summer lamb growth on pure swardss of ceres tonic plantain (plantago lanceolate) compared to perennial ryegrass (lolium perenne). A thesis completed in partial fulfilment of the requirements for the degree of Bachelor of Science, Massey University. Bachelor's dissertation.

Avondo M, Bonanno A, Pagano R, Valenti B, Grigoli A, Luigia Alicata M, Galofaro V, Pennisi P. 2008. Milk quality as affected by grazing time of day in mediterranean goats. Journal of Dairy Research 75: 48-54. doi:10.1017/S0022029907003019.

Bagg J. 2014. Italian ryegrass forage options. Ontario Ministry of Agriculture, Food and Rural Affairs. Accessed 23/10/2019 from https://fieldcropnews.com/2014/06/italian-ryegrass

Ball P, Keeney D. 1983. Nitrogen losses from urine-affected areas of a New Zealand pasture, under contrasting seasonal conditions [grazing ruminants, lolium perenne, trifolium repens]. *I*n: Smith, Hays (eds.) XIV International Grassland Congress. 1 ed. Lexington: CRC Press.

Barham D, Trinder P. 1972. Enzymatic determination of uric acid. Analyst 97: 142-145.

Barraclough D, Jarvis S, Davies G, Williams J. 1992. The relation between fertilizer nitrogen applications and nitrate leaching from grazed grassland. Soil Use and Management 8: 51-56. doi:10.1111/j.1475-2743.1992.tb00894.x.

Barrell L, Burke J, Waghorn G, Attwood G, Brookes I. 2000. Preparation of fresh forages for incubation and prediction of nutritive value. In Proceedings of the New Zealand Society of Animal Production, Hamilton, New Zealand, 5-8 p.

Barry T, Drew K. 1978. Responses of young sheep grazing swedes, turnips, and kale to intraperitoneal and oral supplementation with dl-methionine. New Zealand Journal of Agricultural Research 21: 395-399. doi:10.1080/00288233.1978.10427425.

Barry TN. 1981. Protein metabolism in growing lambs fed on fresh ryegrass (lolium perenne) -clover (trifolium repens) pasture ad lib. 1. Protein and energy deposition in response to abomasal infusion of casein and methionine. British Journal of Nutrition 46: 521-32. doi:10.1079/bjn19810061.

Barry TN. 2013. The feeding value of forage brassica plants for grazing ruminant livestock. Animal Feed Science and Technology 181: 15-25. doi:10.1016/j.anifeedsci.2013.01.012.

Bartels H, Bohmer M. 1972. Kinetic determination of creatinine concentration. Clinical Chimica Acta 37: 193-197.

Beef + lamb. 2018. A guide to feed planning for sheep farmers. Beef and lamb New Zealand, New Zealand. Accessed 30/09/2019 from https://beeflambnz.com/knowledge-hub/PDF/guide-feed-planning-sheep-farmers.pdf

Beef + Lamb New Zealand. 1992. The New Zealand sheep and beef farm survey 1990-1991. New Zealand. 72 p.

Beef + Lamb New Zealand. 2021. Economic service sheep & beef farm survey taranaki / rangitikei / manawatu 2021- 22 forecast and quintile analysis of final 2019 20 survey data | p21018. Beef & Lamb New Zealand. Accessed 28/4/2022 from https://beeflambnz.com/data-tools/benchmark-your-farm

Beef + Lamb New Zealand Economic Service. 2021. New season outlook 2021-22. Beef + Lamb New Zealand Economic Service, New Zealand. Accessed 10/7/2022 from

https://beeflambnz.com/sites/default/files/data/files/New-Season-Outlook-2021-22.pdf

Beef + Lamb New Zealand Economic Service. 2022a. Compendium of New Zealand farm facts 2022. Beef + Lamb New Zealand Economic Service, New Zealand. Accessed 7/3/2023 from https://beeflambnz.com/sites/default/files/data/files/Compendium-22.pdf

Beef + Lamb New Zealand Economic Service. 2022b. Data-tools -sheep-beef-farm-survey. Beef + Lamb New Zealand Economic Service, New Zealaamd. Accessed 6/3/2023/2023 from https://beeflambnz.com/data-tools/sheep-beef-farm-survey

Ben Salem H, Makkar H. 2009. Defatted moringa oleifera seed meal as a feed additive for sheep. Animal Feed Science and Technology 150: 27-33. doi:10.1016/j.anifeedsci.2008.07.007.

Benech A, Cal-Pereyra L, Da Silva S, Acosta-Dibarrat J, Gonzalez-Montana J. 2015. Transient apnoea in sheep: An alternative method for serial urine sample collection. Veterinarski Arhiv 85: 293-307.

Betteridge K, Costall D, Balladur S, Upsdell M, Umemura K. 2010. Urine distribution and grazing behaviour of female sheep and cattle grazing a steep New Zealand hill pasture. Animal Production Science 50: 624-629. doi:10.1071/An09201.

Blakemore L, Searle P, Daly B. 1987. Methods for chemical analysis of soils. New Zealand Society of Soil Science, Lower Hutt, New Zealand. New Zealand soil Bureau scientific report: 80. 72-103 p.

Bowatte S, Hoogendoorn C, Newton P, Liu Y, Brock S, Theobald P. 2018. Grassland plant species and cultivar effects on nitrous oxide emissions after urine application. Geoderma 323: 74-82. doi:10.1016/j.geoderma.2018.03.001.

Box L, Edwards G, Bryant R. 2016. Milk production and urinary nitrogen excretion of dairy cows grazing perennial ryegrass-white clover and pure plantain pastures. In Proceedings of the New Zealand Society of Animal Production, 18-21 p.

Box LA, Edwards GR, Bryant RH. 2017. Milk production and urinary nitrogen excretion of dairy cows grazing plantain in early and late lactation. New Zealand Journal of Agricultural Research 60: 470-482. doi:10.1080/00288233.2017.1366924.

Bristow AW, Whitehead DC, Cockburn JE. 1992. Nitrogenous constituents in the urine of cattle, sheep and goats. Journal of the Science of Food and Agriculture 59: 387-394. doi:10.1002/jsfa.2740590316.

Brock J. 1973. Growth and nitrogen fixation of pure stands of three pasture legumes with high/low phosphate. New Zealand Journal of Agricultural Research 16: 483-491. doi:10.1080/00288233.1973.10421093.

Brock J, Caradus J, Hay M. 1989. Fifty years of white clover research in New Zealand. In Proceedings of the New Zealand Grassland Association, 25-39 p.

Brock J, Hay M. 2001. White clover performance in sown pastures: A biological/ecological perspective. In Proceedings of the New Zealand Grassland Association, Hamilton, New Zealand, 73-83 p.

Brody S. 1945. Bioenergetics and growth, with special reference to the efficiency complex in domestic animals, Reinhold Publishing Corporation, New York.

Brougham R. 1959. The effects of frequency and intensity of grazing on the productivity of a pasture of short-rotation ryegrass and red and white clover. New Zealand Journal of Agricultural Research 2: 1232-1248.

Bryant RH, Miller ME, Greenwood SL, Edwards GR. 2017. Milk yield and nitrogen excretion of dairy cows grazing binary and multispecies pastures. Grass and Forage Science 72: 806-817. doi:10.1111/gfs.12274.

Bryant RH, Welten BG, Costall D, Shorten PR, Edwards GR. 2018. Milk yield and urinary-nitrogen excretion of dairy cows grazing forb pasture mixtures designed to reduce nitrogen leaching. Livestock Science 209: 46-53. doi:10.1016/j.livsci.2018.01.009.

Bryant RH, Snow VO, Shorten PR, Welten BG. 2019. Can alternative forages substantially reduce n leaching? Findings from a review and associated modelling. New Zealand Journal of Agricultural Research 63: 3-28. doi:10.1080/00288233.2019.1680395.

Burke J, Waghorn G, Brookes I, Attwood G, Kolver E. 2000. Formulating total mixed rations from forages-defining the digestion kinetics of contrasting species. In Proceedings of the New Zealand Society of Animal Production, Hamilton, New Zealand., 26-29 June; 9-14 p.

Burke J, Waghorn G, Brookes I. 2002. An evaluation of sulla (hedysarum coronarium) with pasture, white clover and lucerne for lambs. In Proceedings of the New Zealand Society of Animal Production, Palmerston North, New Zealand, 152-156 p.

Burns GA, O'Kiely P, Grogan D, Watson S, Gilliland TJ. 2015. Comparison of herbage yield, nutritive value and ensilability traits of three ryegrass species evaluated for the irish recommended list. Irish Journal of Agricultural and Food Research 54: 31-40. doi:10.1515/ijafr-2015-0003.

Cameron KC, Di HJ, Moir JL. 2013. Nitrogen losses from the soil/plant system: A review. Annals of Applied Biology 162: 145-173. doi:10.1111/aab.12014.

Caradus J, Woodfield D, Stewart A. 1996. Overview and vision for white clover, Palmerston North, New Zeasland, Grasslands Association.

Carlton AJ, Cameron KC, Di HJ, Edwards GR, Clough TJ. 2019. Nitrate leaching losses are lower from ryegrass/white clover forages containing plantain than from ryegrass/white clover forages under different irrigation. New Zealand Journal of Agricultural Research 62: 150-172. doi:10.1080/00288233.2018.1461659.

Carran R. 1978. Nitrogen fixation in pasture x. Southland, gore. New Zealand Journal of Experimental Agriculture 7: 39-41. doi:10.1080/03015521.1979.10426160.

Carulla JE, Kreuzer M, Machmuller A, Hess HD. 2005. Supplementation of acacia mearnsii tannins decreases methanogenesis and urinary nitrogen in forage-fed sheep. Australian Journal of Agricultural Research 56: 961-970. doi:10.1071/Ar05022.

Cave L, Kenyon P, Morris S, Lopez-Villalobos N, Kemp P. 2014. Ewe lamb diet selection on plantain (plantago lanceolata) and on a herb and legume mix, including plantain, chicory (cichorium intybus), red clover (trifolium pratense) and white clover (trifolium repens). Animal Production Science 55: 515-525. doi:10.1071/AN13379.

Charlton J, Stewart A. 1999. Pasture species and cultivars used in New Zealand-a list. In Proceedings of the New Zealand Grassland Association, Hawkes Bay, New Zealand, 147-166 p.

Chen X, Ørskov E. 2004. Research on urinary excretion of purine derivatives in ruminants: Past, present and future. *I*n: Makkar, Chen (eds.) Estimation of microbial protein supply in ruminants using urinary purine derivatives. The Netherlands: Kluwer Academic Publishers.

Chen XB, Gomes MJ. 1992. Estimation of microbial protein supply to sheep and cattle based on urinary excretion of purine derivatives: An overview of the technical details. Rowett Research Institute, Aberdeen, UK. 23 p.

Chen XB, Mejia AT, Kyle DJ, Orskov ER. 1995. Evaluation of the use of the purine derivative-creatinine ratio in spot urine and plasma samples as an index of microbial protein supply in ruminants - studies in sheep. Journal of Agricultural Science 125: 137-143. doi:10.1017/S002185960007458x.

Cheng L, Nicol AM, Dewhurst RJ, Edwards GR. 2013. The effects of dietary nitrogen to water-soluble carbohydrate ratio on isotopic fractionation and partitioning of nitrogen in non-lactating sheep. Animal 7: 1274-1279. doi:10.1017/S1751731113000311.

Cheng L, Mccormick J, Hussein AN, Logan C, Pacheco D, Hodge MC, Edwards GR. 2017. Live weight gain, urinary nitrogen excretion and urination behaviour of dairy heifers grazing pasture, chicory and plantain. Journal of Agricultural Science 155: 669-678. doi:10.1017/S0021859616001076.

Christensen CL, Hedley MJ, Hanly JA, Horne DJ. 2019. Duration-controlled grazing of dairy cows. 2: Nitrogen losses in sub-surface drainage water and surface runoff. New Zealand Journal of Agricultural Research 62: 48-68. doi:10.1080/00288233.2017.1418396.

Corson D, Waghorn G, Ulyatt M, Lee J. 1999. Nirs: Forage analysis and livestock feeding. In Proceedings of the New Zealand Grassland Association, Hawkes Bay, New Zealand, 127-132 p.

Cranston L, Kenyon P, Morris S, Kemp P. 2015a. A review of the use of chicory, plantain, red clover and white clover in a sward mix for increased sheep and beef production. Journal of New Zealand Grasslands 77: 89-94. doi:10.33584/jnzg.2015.77.475.

Cranston L, Corner-Thomas R, Kenyon P, Morris S. 2016. Growth of early weaned lambs on a plantain-clover mix compared with lambs suckling their dam on a plantain-clover mix or a grass based sward. In Proceedings of the New Zealand Society of Animal Production, Adelaide, New Zealand, 65-68 p.

Cranston L. 2017. Herb and clover mixes for New Zealand sheep and cattle grazing systems. Livestock 22: 86-89. doi:10.12968/live.2017.22.2.86.

Cranston L, Ridler A, Kenyon P. 2017. Sheep production. In: Livestock production in New Zealand: The complete guide to dairy cattle, beef cattle, sheep, deer, goats, pigs and poultry. Stafford (ed.). Massey University Press, Auckland, New Zealand.86-122 p.

Cranston LM. 2014. Chicory (cichorium intybus) and plantain (plantago lanceolata); physiological and morphological responses to water stress, defoliation, and grazing preference with implications for the management of the herb and legume mix. A thesis presented in partial fulfilment of the requirements for the degree of Doctor of Philosophy in Plant Science, Massey University. Doctoral dissertation.

Cranston LM, Kenyon PR, Morris ST, Lopez-Villalobos N, Kemp PD. 2015b. Effect of post-grazing height on the productivity, population and morphology of a herb and legume mix. New Zealand Journal of Agricultural Research 58: 397-411. doi:10.1080/00288233.2015.1044014.

Cranston LM, Kemp PD, Morris ST, Wood BA. 2018. An on-farm study of the herbage quality and sward characteristics of plantain-clover mixes during late summer and autumn and

resulting lamb growth rates. Journal of New Zealand Grasslands 80: 107-112. doi:10.33584/jnzg.2018.80.315.

Crush JR, Cosgrove GP, Brougham RW. 1983. Nitrogen-fixation during 1979-81 in 2 pastures on the manawatu plains. New Zealand Journal of Experimental Agriculture 11: 17-20. doi:10.1080/03015521.1983.10427720.

Cuttle SP, Hallard M, Daniel G, Scurlock RV. 1992. Nitrate leaching from sheep-grazed grass clover and fertilized grass pastures. Journal of Agricultural Science 119: 335-343. doi:10.1017/S002185960001217x.

Cuttle SP, Bourne PC. 1993. Uptake and leaching of nitrogen from artificial urine applied to grassland on different dates during the growing-season. Plant and Soil 150: 77-86. doi:10.1007/Bf00779178.

Cuttle SP, Scholefield D. 1995. Management options to limit nitrate leaching from grassland. Journal of Contaminant Hydrology 20: 299-312. doi:10.1016/0169-7722(95)00075-5.

Cuttle SP, Hallard M, Gill EK, Scurlock RV. 1996. Nitrate leaching from sheep-grazed upland pastures in wales. The Journal of Agricultural Science 127: 365-375. doi:10.1017/S0021859600078539.

Cuttle SP, Scurlock RV, Davies BMS. 1998. A 6-year comparison of nitrate leaching from grass/clover and n-fertilized grass pastures grazed by sheep. Journal of Agricultural Science 131: 39-50. doi:10.1017/S0021859698005565.

DairyNZ. 2022a. Annual and italian ryegrass. DairyNZ, New Zealand. Accessed 27/07/2020 from https://www.dairynz.co.nz/feed/pasture/pasture-renewal/select-pasture-species/ryegrass

DairyNZ. 2022b. Plantain. DairyNZ, New Zealand. Accessed 3/10/2021 from https://www.dairynz.co.nz/feed/crops/plantain/#:~:text=Growth%20rates%20of%20Tonic%20plantain,DM%2Fha%2Fday.

DairyNZ. 2022c. Kale. DairyNZ, New Zealand. Accessed 28/7/2022 from https://www.dairynz.co.nz/feed/crops/kale

DairyNZ. 2022d. Swedes. DairyNZ, New Zealand. Accessed 27/7/2022 from https://www.dairynz.co.nz/feed/crops/swedes

Dalley DE, Malcolm BJ, Chakwizira E, de Ruiter JM. 2017. Range of quality characteristics of New Zealand forages and implications for reducing the nitrogen leaching risk from grazing dairy cows. New Zealand Journal of Agricultural Research 60: 319-332. doi:10.1080/00288233.2017.1345762.

David DB, Poli CHEC, Savian JV, Amaral GA, Azevedo EB, Jochims F. 2015. Urinary creatinine as a nutritional and urinary volume marker in sheep fed with tropical or temperate forages. Arquivo Brasileiro de Medicina Veterinária e Zootecnia 67: 1009-1015. doi:10.1590/1678-4162-7759.

De Klein CAM, van der Weerden TJ, Luo J, Cameron KC, Di HJ. 2019. A review of plant options for mitigating nitrous oxide emissions from pasture-based systems. New Zealand Journal of Agricultural Research 63: 29-43. doi:10.1080/00288233.2019.1614073.

De Ruiter J, Dalley D, Hughes T, Fraser T, Dewhurst R. 2007. Types of supplements: Their nutritive value and use, Christchurch, New Zealand, New Zealand Society of Animal Production.

De Ruiter J, Wilson D, Maley S, Fletcher A, Fraser T, Scott W, Berryman S, Dumbleton A, Nichol W. 2009. Management practices for forage brassicas. Christchurch, New Zealand: Forage Brassica Development Group.

Deaker J. 1994. Carcass, liver and kidney characteristics of lambs grazing plantain (plantago lanceolata), chicory (cichorium intybus), white clover (trifolium repens) or perennial ryegrass (lolium perenne). In Proceedings of the New Zealand Society of Animal Production, 197-200 p.

Decandia M, Atzori A, Acciaro M, Cabiddu A, Giovanetti V, Molina Alcaide E, Carro M, Ranilla M, Molle G, Cannas A. 2011. Nutritional and animal factors affecting nitrogen excretion in sheep and goats. Challenging strategies to promote the sheep and goat sector in the current global context. 99: 201-209.

Di HJ, Cameron KC. 2002. Nitrate leaching in temperate agroecosystems: Sources, factors and mitigating strategies. Nutrient Cycling in Agroecosystems 64: 237-256. doi:10.1023/A:1021471531188.

Di HJ, Cameron KC. 2007. Nitrate leaching losses and pasture yields as affected by different rates of animal urine nitrogen returns and application of a nitrification inhibitor - a lysimeter study. Nutrient Cycling in Agroecosystems 79: 281-290. doi:10.1007/s10705-007-9115-5.

Dietz M, Machill S, Hoffmann H, Schmidtke K. 2013. Inhibitory effects of plantago lanceolata l. On soil n mineralization. Plant and Soil 368: 445-458. doi:10.1007/s11104-012-1524-9.

Dineen M, McCarthy B, Ross D, Ortega A, Dillon P, Van Amburgh M. 2021. Characterization of the nutritive value of perennial ryegrass (lolium perenne l.) dominated pastures using

updated chemical methods with application for the cornell net carbohydrate and protein system. Animal Feed Science and Technology 272: 114752. doi:10.1016/j.anifeedsci.2020.114752.

Doak BW. 1952. Some chemical changes in the nitrogenous constituents of urine when voided on pasture. Journal of Agricultural Science 42: 162-171. doi:10.1017/S0021859600058767.

Drew K, Stephen R, Barry T. 1974. The composition and productive features of some forage crops for sheep. In Proceedings of the Agronomy Society of New Zealand, Lincoln, Canterbury, New Zealand, 53-56 p.

Easton MA, StJohn DH. 2001. A model of grain refinement incorporating alloy constitution and potency of heterogeneous nucleant particles. Acta Materialia 49: 1867-1878. doi:10.1016/S1359-6454(00)00368-2.

Edwards G, Bryant R, Smith N, Hague H, Taylor S, Ferris A, Farrell L. 2015. Milk production and urination behaviour of dairy cows grazing diverse and simple pastures. New Zealand Society of Animal Production. Dunedin, New Zealand.

Edwards GR, Parsons A, Rasmussen S, Bryant RH. 2007. High sugar ryegrasses for livestock systems in New Zealand. In Proceedings of the New Zealand Grassland Association, Wairakei, New Zealand, 161-171 p.

Eerens J, Ryan D. 2000. Managing white clover for productive and profitable sheep farming in southland. In Proceedings of the New Zealand Grassland Association, Invercargill, New Zealand, 137-140 p.

Farrell LJ, Kenyon PR, Tozer PR, Ramilan T, Cranston LM. 2020. Quantifying sheep enterprise profitability with varying flock replacement rates, lambing rates, and breeding strategies in New Zealand. Agricultural Systems 184: 102888. doi:10.1016/j.agsy.2020.102888.

Field A, Sykes A, Gunn R. 1974. Effects of age and state of incisor dentition on faecal output of dry matter and on faecal and urinary output of nitrogen and minerals, of sheep grazing hill pastures. Journal of Agricultural Science 83: 151-160. doi:10.1017/S0021859600047110.

Field T, Ball PR, Theobald P. 1985. Leaching of nitrate from sheep-grazed pastures. In Proceedings of the New Zealand Grassland Association, Timaru,New Zealand, 209-214 p.

Fossati P, Prencipe L, Berti G. 1980. Use of 3,5-dichloro-2-hydroxybenzenesulfonic acid-4-aminophenazone chromogenic system in direct enzymic assay of uric-acid in serum and urine. Clinical Chemistry 26: 227-231. doi:10.1093/clinchem/26.2.227.

Frame J. 1993. Herbage mass. In: Sward measurement handbook. Hodgson, Baker, Davies, Laidlaw, Leaver (eds.). British Grassland Society, Reading, UK.39-69 p.

Fraser PM, Curtin D, Harrison-Kirk T, Meenken ED, Beare MH, Tabley F, Gillespie RN, Francis GS. 2013. Winter nitrate leaching under different tillage and winter cover crop management practices. Soil Science Society of America Journal 77: 1391-1401. doi:10.2136/sssaj2012.0256.

Fraser T, Rowarth J. 1996. Legumes, herbs or grass for lamb performance?. In Proceedings of the New Zealand Grassland Association, Oamaru, New Zealand, 49-52 p.

Fulkerson WJ, Neal JS, Clark CF, Horadagoda A, Nandra KS, Barchia I. 2007. Nutritive value of forage species grown in the warm temperate climate of australia for dairy cows: Grasses and legumes. Livestock Science 107: 253-264. doi:10.1016/j.livsci.2006.09.029.

Gardiner CA, Clough TJ, Cameron KC, Di HJ, Edwards GR, de Klein CAM. 2018. Potential inhibition of urine patch nitrous oxide emissions by plantago lanceolata and its metabolite aucubin. New Zealand Journal of Agricultural Research 61: 495-503. doi:10.1080/00288233.2017.1411953.

Garrett K, Watson C, Jordan C, Steen R, Smith R. 1992. Nitrogen economy of grazed grassland. In Proceedings of the the Fertiliser Society, Belfast, UK., 32 p.

Garwood E, Ryden J. 1986. Nitrate loss through leaching and surface runoff from grassland: Effects of water supply, soil type and management. In: Nitrogen fluxes in intensive grassland systems. Ryden, Ennik (eds.). Springer, Dordrechtthe.99-113 p.

Gillingham A, During C. 1973. Pasture production and transfer of fertility within a long-established hill pasture. New Zealand Journal of Experimental Agriculture 1: 227-232.

Goh KM, Mansur I, Mead DJ, Sweet GB. 1996. Biological nitrogen fixing capacity and biomass production of different understorey pastures in a pinus radiata pasture agroforestry system in New Zealand. Agroforestry Systems 34: 33-49. doi:10.1007/Bf00129631.

Golding KP, Wilson ED, Kemp PD, Pain SJ, Kenyon PR, Morris ST, Hutton PG. 2011. Mixed herb and legume pasture improves the growth of lambs post-weaning. Animal Production Science 51: 717-723. doi:10.1071/An11027.

Grace N. 1983. Quantities of zinc, copper, manganese and iron associated with body weight gain and wool growth of young sheep. In Proceedings of the New Zealand Society of Animal Production, Jan; 127-130 p.

Hanly JA, Hedley MJ, Home DJ. 2017. Effects of summer turnip forage cropping and pasture renewal on nitrogen and phosphorus losses in dairy farm drainage waters: A three-year field study. Agricultural Water Management 181: 10-17. doi:10.1016/j.agwat.2016.11.009.

Harrington K, Thatcher A, Kemp P. 2006. Mineral composition and nutritive value of some common pasture weeds. New Zealand Plant Protection 59: 261-265. doi:https://doi.org/10.30843/nzpp.2006.59.4414.

Harris S, Clark D, Auldist M, Waugh C, Laboyrie P. 1997. Optimum white clover content for dairy pastures. In Proceedings of the New Zealand Grassland Association, Auckland, New Zealand, 29-34 p.

Harris SL, Auldist MJ, Clark DA, Jansen EB. 1998. Effects of white clover content in the diet on herbage intake, milk production and milk composition of New Zealand dairy cows housed indoors. Journal of Dairy Research 65: 389-400. doi:10.1017/s0022029998002969.

Hatch DJ, Goodlass G, Joynes A, Shepherd MA. 2007. The effect of cutting, mulching and applications of farmyard manure on nitrogen fixation in a red clover/grass sward. Bioresource Technology 98: 3243-8. doi:10.1016/j.biortech.2006.07.017.

Haynes RJ, Williams PH. 1993. Nutrient cycling and soil fertility in the grazed pasture ecosystem. Advances in Agronomy 49: 119-199. doi:10.1016/S0065-2113(08)60794-4.

Heng LK, White RE, Bolan NS, Scotter DR. 1991. Leaching losses of major nutrients from a mole-drained soil under pasture. New Zealand Journal of Agricultural Research 34: 325-334. doi:10.1080/00288233.1991.10417672.

Henry R, Cannon D, Winkleman J. 1974. Automated determination of serum and urine creatinine. In: Clinical chemistry principles and techniques. 2nd ed.: Harper and Row Publishers, New York.525-555 p.

Hewitt A. 1998. New Zealand soil classification, Lincoln, New Zealand, Manaaki Whenua-Landcare Research New Zealand Ltd Press.

Hodgson J, Brookes I. 1999. Nutrition of grazing animals. In: New Zealand pasture and crop science. White, Hodgson (eds.). Oxford University Press, Oxford.117-132 p.

Hoglund J, Brock J. 1987. Nitrogen fixation in managed grasslands. In: Managed grasslands. R.W. (ed.). Elsevier; Ecosystems of the World.187-196 p.

Hoogendoorn CJ, Betteridge K, Costall DA, Ledgard SF. 2010. Nitrogen concentration in the urine of cattle, sheep and deer grazing a common ryegrass/cocksfoot/white clover pasture. New Zealand Journal of Agricultural Research 53: 235-243. doi:10.1080/00288233.2010.499899.

Hoogendoorn CJ, Betteridge K, Ledgard SF, Costall DA, Park ZA, Theobald PW. 2011. Nitrogen leaching from sheep-, cattle- and deer-grazed pastures in the lake taupo catchment in New Zealand. Animal Production Science 51: 416-425. doi:10.1071/An10179.

Hoogendoorn CJ, Lambert MG, Devantier BP, Theobald PW, Park ZA. 2017. Nitrogen fertiliser application rates and nitrogen leaching in intensively managed sheep grazed hill country pastures in New Zealand. New Zealand Journal of Agricultural Research 60: 154-172. doi:10.1080/00288233.2017.1287100.

Hopkins C. 2003. The nutritive value of italian ryegrass (lolium multiflorum) selected for high dry matter and nonstructural carbohydrate contents. A thesis submitted in partial fulfilment of the requirements for the degree of Doctor of Philosophy, University of Kwazulu. Doctoral Dissertation.

Hosomi M, Sudo R. 1986. Simultaneous determination of total nitrogen and total phosphorus in freshwater samples using persulfate digestion. International Journal of Environmental Studies 27: 267-275. doi:10.1080/00207238608710296.

Houlbrooke D, Horne D, Hedley M, Hanly J, Snow V. 2003. The impact of intensive dairy farming on the leaching losses of nitrogen and phosphorus from a mole and pipe drained soil. In Proceedings of the New Zealand Grassland Association, Palmerston North, New Zealand, 1/1/2003; 179-184 p.

Houlbrooke DJ, Horne DJ, Hedley MJ, Hanly JA, Snow VO. 2004. A review of literature on the land treatment of farm-dairy effluent in New Zealand and its impact on water quality. New Zealand Journal of Agricultural Research 47: 499-511. doi:10.1080/00288233.2004.9513617.

Houlbrooke DJ. 2005. A study of the quality of artificial drainage under intensive dairy farming and the improved management of farm dairy effluent using'deferred irrigation. A thesis presented in partial fulfilment of the requirements for the degree of Doctor of Philosophy in Soil Science, Massey University. Doctoral dissertation.

Howarth RW. 1988. Nutrient limitation of net primary production in marine ecosystems. Annual Review of Ecology and Systematics 19: 89-110. doi:10.1146/annurev.es.19.110188.000513.

Howes J. 2019. Targeted duration controlled grazing-the effects of timing of grazing on nitrate leaching and treading damage. A thesis presented in partial fulfilment of the requirements for the degree of Doctor of Philosophy in Soil Science, Massey University. Doctoral dissertation.

Humphrey MD, Istok JD, Lee JY, Hevesi JA, Flint AL. 1997. A new method for automated dynamic calibration of tipping-bucket rain gauges. Journal of Atmospheric and Oceanic Technology 14: 1513-1519. doi:10.1175/1520-0426(1997)014<1513:Anmfad>2.0.Co;2.

Hutton PG, Kenyon PR, Bedi MK, Kemp PD, Stafford KJ, West DM, Morris ST. 2011. A herb and legume sward mix increased ewe milk production and ewe and lamb live weight gain to weaning compared to a ryegrass dominant sward. Animal Feed Science and Technology 164: 1-7. doi:10.1016/j.anifeedsci.2010.11.014.

Hynd P. 2019. Animal nutrition: From theory to practice, Melbourne, VIC, Australia, CABI Publishing.

Jefferies B. 1961. Body condition scoring and its use in management. Tasmanian journal of agriculture 32: 19-21.

Jonker A, Cheng L, Edwards G, Molano G, Taylor P, Sandoval E, Cosgrove G. 2015. Nitrogen partitioning in sheep offered three perennial ryegrass cultivars at two allowances in spring and autumn. In Proceedings of the New Zealand Society of Animal Production, Dunedin, New Zealand, 74-78 p.

Jonker A, Cheng L, Sun X. 2021. Using urinary creatinine to predict daily urine output in sheep fed fresh forages. Animal-science proceedings 12: 24. doi:10.1016/j.anscip.2021.03.035.

Judson H, McAnulty RW, Sedcole R. 2009. Evaluation of ‘ceres tonic’plantain (plantago lanceolata) as a lactation feed for twin-bearing ewes. In Proceedings of the New Zealand Grassland Association, Waitangi, New Zealand, 201-205 p.

Judson H, Moorhead AJE. 2014. Benefits and uses of ceres tonic plantain (plantago lanceolata) in sheep and beef systems. In Proceedings of the Sheep and Beef Cattle Veterinarians of the New Zealand Veterinary Association, New Zealand, 1-6 p.

Kato Y. 1946. Mechanism of uric acid excretion stimulation by aucubin. Folia Pharmacologica Japonica 42: 27-40. doi:10.1254/fpj.42.4_123.

Keim JP, Gandarillas M, Benavides D, Cabanilla J, Pulido RG, Balocchi OA, Bertrand A. 2020. Nutrient concentrations and profile of non-structural carbohydrates vary among different brassica forages. Animal Production Science 60: 1503-1513. doi:10.1071/An19472.

Kemp P, Matthew C, Lucas R. 1999. Pasture species and cultivars. In: New Zealand pasture and crop science. White, Hodgson (eds.). Oxford University Press, Oxford, UK.83-99 p.

Kemp PD, Kenyon PR, Morris ST. 2010. The use of legume and herb forage species to create high performance pastures for sheep and cattle grazing systems. Revista Brasileira de Zootecnia 39: 169-174. doi:10.1590/S1516-35982010001300019.

Kenyon P, Webby R. 2007. Pastures and supplements in sheep production systems. In: Pasture and supplements for grazing animals. Rattray, Brooks, Nicol (eds.). Hamilton, New Zealand.255-274 p.

Kenyon P, Kemp P, Stafford K, West D, Morris S. 2010. Can a herb and white clover mix improve the performance of multiple-bearing ewes and their lambs to weaning?. Animal Production Science 50: 513-521.

Kenyon PR, Maloney SK, Blache D. 2014. Review of sheep body condition score in relation to production characteristics. New Zealand Journal of Agricultural Research 57: 38-64. doi:10.1080/00288233.2013.857698.

Kenyon PR, Morel PCH, Corner-Thomas RA, Perez HL, Somasiri SC, Kemp PD, Morris ST. 2017. Improved per hectare production in a lamb finishing system using mixtures of red and white clover with plantain and chicory compared to ryegrass and white clover. Small Ruminant Research 151: 90-97. doi:10.1016/j.smallrumres.2017.04.019.

Kenyon PR, Fraga FJR, Blumer S, Thompson AN. 2019. Triplet lambs and their dams - a review of current knowledge and management systems. New Zealand Journal of Agricultural Research 62: 399-437. doi:10.1080/00288233.2019.1616568.

Keogh B, French P, McGrath T, Storey T, Mulligan FJ. 2009. Comparison of the performance of dairy cows offered kale, swedes and perennial ryegrass herbage in situ and perennial ryegrass silage fed indoors in late pregnancy during winter in ireland. Grass and Forage Science 64: 49-56. doi:10.1111/j.1365-2494.2008.00667.x.

Kobayashi H, Takahashi Y, Matsumoto K, Nishiguchi Y. 2008. Changes in nutritive value of italian ryegrass (lolium multiflorum lam.) during overwintering period. Plant production science 11: 228-231. doi:10.1626/pps.11.228.

Koch DW, Kercher C, Jones R. 2002. Fall and winter grazing of brassicas-a value-added opportunity for lamb producers. Sheep Goat Research Journal 17: 1-13.

Kohn RA, Dinneen MM, Russek-Cohen E. 2005. Using blood urea nitrogen to predict nitrogen excretion and efficiency of nitrogen utilization in cattle, sheep, goats, horses, pigs, and rats. Journal of Animal Science 83: 879-889.

Kumar K, Goh KM. 2000. Biological nitrogen fixation, accumulation of soil nitrogen and nitrogen balance for white clover (trifolium repens l.) and field pea (pisum sativum l.) grown for seed. Field Crops Research 68: 49-59. doi:10.1016/S0378-4290(00)00109-X.

Kyamanywa N, Tait IM, Mitchell CM, Hedley MJ, Pacheco D, Bishop P. 2021. Effect of a late summer diet change from pasture to brassica crop and silages on dairy cow milk production and urinary nitrogen excretion. New Zealand Journal of Agricultural Research 64: 36-55. doi:10.1080/00288233.2020.1713176.

Lambert M, Litherland A, McLaren P. 1999. Effects of herbage mass and ewe condition score at lambing on lamb survival and liveweight gain. In Proceedings of the New Zealand Society of Animal Production, Jan; 104-107 p.

Langlands J. 1966. Creatinine as an index substance for estimating the urinary excretion of nitrogen and potassium by grazing sheep. Australian Journal of Agricultural Research 17: 757-763. doi:10.1071/AR9660757.

Ledgard SF, Menneer JC, Dexter MM, Kear MJ, Lindsey S, Peters JS, Pacheco D. 2008. A novel concept to reduce nitrogen losses from grazed pastures by administering soil nitrogen process inhibitors to ruminant animals: A study with sheep. Agriculture Ecosystems & Environment 125: 148-158. doi:10.1016/j.agee.2007.12.006.

Lee JM, Hemmingson NR, Minnee EMK, Clark CEF. 2015. Management strategies for chicory (cichorium intybus) and plantain (plantago lanceolata): Impact on dry matter yield, nutritive characteristics and plant density. Crop & Pasture Science 66: 168-183. doi:10.1071/Cp14181.

Li FY, Betteridge K, Cichota R, Hoogendoorn CJ, Jolly B. 2012. Effects of nitrogen load variation in animal urination events on nitrogen leaching from grazed pasture. Agriculture Ecosystems & Environment 159: 81-89. doi:10.1016/j.agee.2012.07.003.

Lindsay C, Kemp P, Kenyon P, Morris S. 2007. Summer lamb finishing on forage crops. In Proceedings of the New Zealand Society of Animal Production, Wanaka, New Zealand, 121-125 p.

Lindsay G. 2016. An investigation of the effects of plantain (plantago lanceolata) ingestion on kidney function in sheep. A dissertation submitted in partial fulfilment of the requirements for the Degree of Bachelor of Agricultural Science, Lincoln University. Bachelor's Dissertation.

Litherland A, Lambert M. 2007. Factors affecting the quality of pastures and supplements produced on farms, Hamilton, New Zealand Society of Animal Production.

Lucci G, Shepherd M, Carlson B, Lane M, Berry I. 2013. Establishment of winter forage crops on pumice soils–experimental results and farmers' perspectives. *I*n: LD Currie and C L. Christensen (ed.) Accurate and efficient use of nutrients on farms. Massey University, Palmerston North, New Zealand: Fertilizer and Lime Research Centre.

Luo J, Balvert SF, Wise B, Welten B, Ledgard SF, de Klein CAM, Lindsey S, Judge A. 2018. Using alternative forage species to reduce emissions of the greenhouse gas nitrous oxide from cattle urine deposited onto soil. Science of the Total Environment 610-611: 1271-1280. doi:10.1016/j.scitotenv.2017.08.186.

Lush L, Wilson RP, Holton MD, Hopkins P, Marsden KA, Chadwick DR, King AJ. 2018. Classification of sheep urination events using accelerometers to aid improved measurements of livestock contributions to nitrous oxide emissions. Computers and Electronics in Agriculture 150: 170-177. doi:10.1016/j.compag.2018.04.018.

Lynch JJ, Marjoram AR, Mottershead BE. 1973. Measurement of urine flow from grazing sheep. Medical and Biological Engineering 11: 621-7. doi:10.1007/BF02477409.

Magesan G, White R, Scotter D. 1996. Nitrate leaching from a drained, sheep-grazed pasture. I. Experimental results and environmental implications. Soil Research. 34: 55-67.

Maheswaran S, Cranston L, Millner J, Horne D, Hanly J, Kenyon P, Kemp P. 2021. Effects of alternative forages on nitrate leaching under intensive sheep grazing. New Zealand Journal of Animal Science and Production 81: 146-152.

Maheswaran S, Cranston LM, Millner JP, Horne DJ, Hanly JA, Kenyon PR, Kemp PD. 2022a. Nutrient leaching under sheep grazing systems. (Unpublished work). School of Agriculture and Environment, Private Bag 11-222, Massey University.

Maheswaran S, Cranston LM, Millner JP, Horne DJ, Hanly JA, Kenyon PR, Kemp PD. 2022b. Effects of sheep grazing systems on water quality with a focus on nitrate leaching. Agriculture 12: 758. doi:ARTN 758 10.3390/agriculture12060758.

Malcolm BJ, Cameron KC, Di HJ, Edwards GR, Moir JL. 2014. The effect of four different pasture species compositions on nitrate leaching losses under high n loading. Soil Use and Management 30: 58-68. doi:10.1111/sum.12101.

Malcolm BJ, Moir JL, Cameron KC, Di HJ, Edwards GR. 2015. Influence of plant growth and root architecture of italian ryegrass (lolium multiflorum) and tall fescue (festuca arundinacea) on n recovery during winter. Grass and Forage Science 70: 600-610. doi:10.1111/gfs.12157.

Malcolm BJ, Cameron KC, Edwards GR, Di HJ, de Ruiter JM, Dalley DE. 2016. Nitrate leaching losses from lysimeters simulating winter grazing of fodder beet by dairy cows. New Zealand Journal of Agricultural Research 59: 194-203. doi:10.1080/00288233.2016.1150307.

Mangwe MG, Bryant RH, Beck MR, Beale N, Bunt C, Gregorini P. 2019. Forage herbs as an alternative to ryegrass-white clover to alter urination patterns in grazing dairy systems. Animal Feed Science and Technology 252: 11-22. doi:10.1016/j.anifeedsci.2019.04.001.

Marsden KA, Lush L, Holmberg JA, Whelan MJ, King AJ, Wilson RP, Charteris AF, Cardenas LM, Jones DL, Chadwick DR. 2020. Sheep urination frequency, volume, n excretion and chemical composition: Implications for subsequent agricultural n losses. Agriculture Ecosystems & Environment 302: 107073. doi:10.1016/j.agee.2020.107073.

Marsden KA, Lush L, Holmberg JA, Harris IM, Whelan MJ, Webb S, King AJ, Wilson RP, Jones DL, Charteris AF, Cardenas LM, Chadwick DR. 2021. Quantifying the frequency and volume of urine deposition by grazing sheep using tri-axial accelerometers. Animal 15: 100234. doi:10.1016/j.animal.2021.100234.

Marshall CJ, Beck MR, Garrett K, Barrell GK, Al-Marashdeh O, Gregorini P. 2020. Grazing dairy cows with low milk urea nitrogen breeding values excrete less urinary urea nitrogen. Science of the Total Environment 739: 139994. doi:10.1016/j.scitotenv.2020.139994.

Mathisen O, Raeder M, Kiil F. 1981. Mechanism of osmotic diuresis. Kidney International 19: 431-7. doi:10.1038/ki.1981.36.

Matthews P, Harrington K, Hampton J. 1999a. Management of grazing systems. In: New Zealand pasture and crop science. White, Hodgso (eds.). Oxford University Press, Auckland, New Zealand.153-174 p.

Matthews P, Hodgson J, White J. 1999b. Livestock farming systems in New Zealand. In: New Zealand pasture crop science. Hodgson, White (eds.). Oxford University Press, Auckland, New Zealand.101-116 p.

Maxwell TMR, McLenaghen RD, Edwards GR, Di HJ, Cameron KC. 2019. Italian ryegrass swards reduce n leaching via greater n uptake and lower drainage over perennial ryegrass cultivars varying in cool season growth rates. New Zealand Journal of Agricultural Research 62: 69-82. doi:10.1080/00288233.2018.1426615.

McGusty A. 2017. An investigation into the effect of sodium content in plantain (plantago lanceolata) on urine production in sheep. A Dissertation submitted in partial fulfilment of the requirements for the Degree of Bachelor of Agricultural Science, Lincoln University. Bachelor's Dissertation.

Melland AR, Mc Caskill MR, White RE, Chapman DF. 2008. Loss of phosphorus and nitrogen in runoff and subsurface drainage from high and low input pastures grazed by sheep in southern australia. Australian Journal of Soil Research 46: 161-172. doi:10.1071/Sr07084.

Miller LA, Moorby JM, Davies DR, Humphreys MO, Scollan ND, MacRae JC, Theodorou MK. 2001. Increased concentration of water-soluble carbohydrate in perennial ryegrass (lolium perenne l.): Milk production from late-lactation dairy cows. Grass and Forage Science 56: 383-394. doi:10.1046/j.1365-2494.2001.00288.x.

Ministry for the Environment. 2020. National policy statement for freshwater management 2020. Ministry for the Environment, New Zealand. Accessed 10/5/2021 from https://environment.govt.nz/publications/national-policy-statement-for-freshwater-management-2020

Minnee EMK, Leach CMT, Dalley DE. 2020. Substituting a pasture-based diet with plantain (plantago lanceolata) reduces nitrogen excreted in urine from dairy cows in late lactation. Livestock Science 239: 104093. doi:10.1016/j.livsci.2020.104093.

Misselbrook TH, Powell JM, Broderick GA, Grabber JH. 2005. Dietary manipulation in dairy cattle: Laboratory experiments to assess the influence on ammonia emissions. Journal of Dairy Science 88: 1765-77. doi:10.3168/jds.S0022-0302(05)72851-4.

Mitchell K. 1956. Growth of pasture species under controlled environment. 1. Growth at various levels of constant temperature. New Zealand Journal of Science and Technology 38: 203-15.

Moir JL, Malcolm BJ, Cameron KC, Di HJ. 2012. The effect of dicyandiamide on pasture nitrate concentration, yield and n offtake under high n loading in winter and spring. Grass and Forage Science 67: 391-402. doi:10.1111/j.1365-2494.2012.00857.x.

Moir JL, Edwards GR, Berry LN. 2013. Nitrogen uptake and leaching loss of thirteen temperate grass species under high n loading. Grass and Forage Science 68: 313-325. doi:10.1111/j.1365-2494.2012.00905.x.

Monaghan R, Hedley M, Di H, McDowell R, Cameron K, Ledgard S. 2007. Nutrient management in New Zealand pastures—recent developments and future issues. New Zealand Journal of Agricultural Research 50: 181-201. doi:10.1080/00288230709510290.

Monaghan R, Semadeni-Davies A, Muirhead R, Elliott S, Shankar U. 2010. Land use and land management risks to water quality in southland. Agresearch, Southland, New Zealand. Available: https://envirolink.govt.nz/assets/Envirolink/885-ESRC230-Land-use-and-land-management-risks-to-water-quality-in-Southland.pdf. 1-85 p.

Monaghan RM, Cameron KC, Mclay CDA. 1989. Leaching losses of nitrogen from sheep urine patches. New Zealand Journal of Agricultural Research 32: 237-244. doi:10.1080/00288233.1989.10423459.

Monaghan RM, Paton RJ, Drewry JJ. 2002. Nitrogen and phosphorus losses in mole and tile drainage from a cattle-grazed pasture in eastern southland. New Zealand Journal of Agricultural Research 45: 197-205. doi:10.1080/00288233.2002.9513510.

Monaghan RM, Smith LC, de Klein CAM. 2013. The effectiveness of the nitrification inhibitor dicyandiamide (dcd) in reducing nitrate leaching and nitrous oxide emissions from a grazed winter forage crop in southern New Zealand. Agriculture Ecosystems & Environment 175: 29-38. doi:10.1016/j.agee.2013.04.019.

Moorby JM, Fraser MD, Theobald VJ, Wood JD, Haresign W. 2004. The effect of red clover formononetin content on live-weight gain, carcass characteristics and muscle equol content of finishing lambs. Animal Science 79: 303-313. doi:10.1017/S1357729800090160.

Moorhead A, Piggot G. 2009. The performance of pasture mixes containing 'ceres tonic'plantain (plantago lanceolata) in northland. In Proceedings of the New Zealand Grassland Association, Waitangi, New Zealand, 195-199 p.

Moot D, Matthew C, Kemp P, Scott W. 2007. Husbandry and role of pastures and forage crops in grazing systems, Hamilton, New Zealand, New Zealand Society of Animal Production.

Morris ST, Kenyon PR. 2004. The effect of litter size and sward height on ewe and lamb performance. New Zealand Journal of Agricultural Research 47: 275-286. doi:10.1080/00288233.2004.9513596.

Morris ST, Kenyon PR. 2014. Intensive sheep and beef production from pasture -a New Zealand perspective of concerns, opportunities and challenges. meat science 98: 330-5. doi:10.1016/j.meatsci.2014.06.011.

National Research Council 2007. Nutrient requirements of small ruminants: Sheep, goats, cervids, and new world camelids, Washington, DC, The National Academies Press.

Navarrete QSDC. 2015. Evaluation of herb pastures for New Zealand dairy systems. A thesis presented in partial fulfilment of the requirements for the degree of Doctor of Philosophy, Massey University. Doctoral dissertation.

Navarrete S, Kemp PD, Pain SJ, Back P. 2016. Bioactive compounds, aucubin and acteoside, in plantain (*plantago lanceolata* l.) and their effect on in vitro rumen fermentation. Animal Feed Science and Technology 222: 158-167. doi:10.1016/j.anifeedsci.2016.10.008.

Navarrete S, Rodriguez M, Horne D, Hanly J, Hedley M, Kemp P. 2022. Nitrogen excretion by dairy cows grazing plantain (plantago lanceolata) based pastures during the lactating season. Animals (Basel) 12: 469. doi:10.3390/ani12040469.

Neeley WE, Phillipson J. 1988. Automated enzymatic method for determining ammonia in plasma, with 14-day reagent stability. Clinical Chemistry 34: 1868-1869. doi:10.1093/clinchem/34.9.1866.

New Zealand Plant Breeding and Research Association. 2021. Forage trial results. New Zealand Plant Breeders Association, Christchurch, New Zealand. Accessed 1/2/2022 from https://www.nzpbra.org/wp-content/uploads/Italian-Ryegrass-Summary-2021.pdf

Nguyen TT, Navarrete S, Horne DJ, Donaghy DJ, Kemp PD. 2022. Effect of plantain content in ryegrass-based dairy pastures on nitrate leaching and key components of the nitrogen cycle. *I*n: Christensen, Horne , Singh (eds.) Adaptive Strategies for Future Farming. Palmerston North, New Zealand: Farmed Landscapes Research Centre, Massey University.

Nichol W. 2007. Nutritional disorders of ruminants caused by consumption of pasture and fodder crops. In: Pasture supplements for grazing animals. Rattray, Brookes, Nicol (eds.). New Zealand Society of Animal Production, Hamilton, NewZealand.133 p.

Nichols S, Crush J. 2007. Selecting forage grasses for improved nitrate retention-a progress report. In Proceedings of the New Zealand Grassland Association, Wairakei, New Zealand, 207 p.

Nicol A, Brookes I. 2007. The metabolisable energy requirements of grazing livestock. In: Pasture and supplements for grazing animals. Rattray, Brooks, Nicol (eds.). Hamilton, New Zealand.151-172 p.

NIWA. 2021. Annual climate summary 2020. NIWA (National Institute of Water and Atmospheric Research) National Climate Centre, New Zealand. Accessed 8/4/2021 from https://niwa.co.nz/climate/summaries/annual-climate-summary-2020

NIWA. 2022. Annual climate summary 2021, New Zealand. Accessed 3/6/2022 from https://niwa.co.nz/climate/summaries/annual-climate-summary-2021#:~:text=Annual%20temperatures%20were%20above%20average,of%20Plenty%2C%20Tasman%20and%20Fiordland.

O'Connell C, Judson H, Barrell GK. 2016. Sustained diuretic effect of plantain when ingested by sheep. In Proceedings of the New Zealand Society of Animal Production, Adelaide, New Zealand, 14-17 p.

Pacheco D, Burke J, Death A, Cosgrove G. 2007. Comparison of models for estimation of urinary nitrogen excretion from dairy cows fed fresh forages. In Proceedings of the Meeting the Challenges for Pasture-based Dairying. Proceedings of the 5th Australasian Dairy Science Symposium, University of Melbourne, Victoria, Melbourne, Australia, 18-20 p.

Pain SJ, Corkran JR, Kenyon PR, Morris ST, Kemp PD. 2015. The influence of season on lambs' feeding preference for plantain, chicory and red clover. Animal Production Science 55: 1241-1249. doi:10.1071/An14440.

Parfitt R, Mackay A, Ross D, Budding P. 2009. Effects of soil fertility on leaching losses of n, p and c in hill country. New Zealand Journal of Agricultural Research 52: 69-80. doi:10.1080/00288230909510490.

Pelletier G, Darisse J, Donefer E. 1976. Effects of dates of seeding and levels of n fertilization on yields, chemical composition and in vitro digestibility of forage kale. Canadian Journal of Plant Science 56: 63-70. doi:10.4141/cjps76-011.

PGG Wrightson Seeds. 2017. Choosing the right kale. Accessed 1/10/2020/2020 from https://www.pggwrightson.co.nz/news/choosing-right-kale

Piaggio L, Quintans G, San Julian R, Ferreira G, Ithurralde J, Fierro S, Pereira ASC, Baldi F, Banchero GE. 2018. Growth, meat and feed efficiency traits of lambs born to ewes submitted to energy restriction during mid-gestation. Animal 12: 256-264. doi:10.1017/S1751731117001550.

Powell A, Kemp P, Jaya I, Osborne M. 2007. Establishment, growth and development of plantain and chicory under grazing. In Proceedings of the New Zealand Grassland Association, Wairakei, 41 p.

Rae A, Brougham R, Barton R. 1964. A note on liveweight gains of sheep grazing different ryegrass pastures. New Zealand Journal of Agricultural Research 7: 491-495. doi:10.1080/00288233.1964.10416376.

Raeside MC, Nie ZN, Robertson M, Partington DL, Behrendt R. 2014. Plantain (plantago lanceolata l.) and perennial ryegrass (lolium perenne l.) as pregnancy and lactation feed for ewes joined at 8 months of age. Animal Production Science 54: 1023-1029. doi:10.1071/An13205.

Ramirez-Restrepo CA, Barry TN. 2005. Alternative temperate forages containing secondary compounds for improving sustainable productivity in grazing ruminants. Animal Feed Science and Technology 120: 179-201. doi:10.1016/j.anifeedsci.2005.01.015.

Rodriguez MJ. 2020. Plantain (plantago lanceolata l.) as a natural mitigation strategy to reduce nitrogen losses from pasture-based dairy systems. A thesis presented in partial fulfilment of the requirements for the degree of Doctor of Philosophy in Agricultural Sciences Massey University. Doctoral dissertation.

Rodriguez MJ, Kemp P, Navarrete S, Hanly J, Horne D, Bishop P. 2020. Nitrogen losses from plantain: What can we say?. *I*n: Christensen, Horne , Singh (eds.) Nutrient management in farmed landscapes. Palmerston North, New Zealand: Fertilizer and Lime Research Centre, Massey University.

Rodriguez MJ, Navarrete S, Horne DJ, Hanly JA, Bishop P, Kemp PD. 2021. Can secondary metabolites of plantain reduce n losses from urine patches? New Zealand Journal of Agricultural Research: 1-18. doi:10.1080/00288233.2021.1991388.

Roughan PG, Holland R. 1977. Predicting in-vivo digestibilities of herbages by exhaustive enzymic hydrolysis of cell walls. Journal of the Science of Food and Agriculture 28: 1057-1064. doi:10.1002/jsfa.2740281204.

Rubio C, Lucas JR, Gutierrez AJ, Glez-Weller D, Perez Marrero B, Caballero JM, Revert C, Hardisson A. 2012. Evaluation of metal concentrations in mentha herbal teas (mentha piperita, mentha pulegium and mentha species) by inductively coupled plasma spectrometry. Journal of Pharmaceutical and Biomedical Analysis 71: 11-7. doi:10.1016/j.jpba.2012.07.015.

Rumball W, Keogh RG, Lane GE, Miller JE, Claydon RB. 1997. 'Grasslands lancelot' plantain (plantago lanceolata l.). New Zealand Journal of Agricultural Research 40: 373-377. doi:10.1080/00288233.1997.9513258.

Ruz-Jerez B, White R, Ball P. 1995. A comparison of nitrate leaching under clover-based pastures and nitrogen-fertilized grass grazed by sheep. The Journal of Agricultural Science 125: 361-369. doi:10.1017/S0021859600084860.

Ryan-Salter T. 2011. Yield and composition of short-term pasture mixtures containing italian ryegrass, red clover and balansa clover. A dissertation submitted in partial fulfilment of the requirements for the Degree of Bachelor of Agricultural Science with Honours, Lincoln University. Bachelor's dissertation.

Ryan-Salter T, Black A. 2012. Yield of italian ryegrass mixed with red clover and balansa clover. In Proceedings of the New Zealand Grassland Association, Gore, New Zealand, 201-208 p.

Saggar S, Andrew R, Tate K, Hedley C, Rodda N, Townsend J. 2004. Modelling nitrous oxide emissions from dairy-grazed pastures. Nutrient Cycling in Agroecosystems 68: 243-255.

Sanderson MA, Rotz CA, Fultz SW, Rayburn EB. 2001. Estimating forage mass with a commercial capacitance meter, rising plate meter, and pasture ruler. Agronomy Journal 93: 1281-1286. doi:10.2134/agronj2001.1281.

Sanderson MA, Labreveux M, Hall MH, Elwinger GF. 2003. Nutritive value of chicory and english plantain forage. Crop Science 43: 1797-1804. doi:10.2135/cropsci2003.1797.

SAS Institute. 2015. Base sas 9.4 procedures guide, Cary, NC, USA, SAS Institute.

Scotter D, Clothier B, Turner M. 1979. The soil water balance in a gragiaqualf and its effect on pasture growth in central New Zealand. Australian Journal of Soil Research 17: 455-465. doi:10.1071/SR9790455.

Selbie DR, Buckthought LE, Shepherd MA. 2015. The challenge of the urine patch for managing nitrogen in grazed pasture systems. Advances in Agronomy, Vol 129 129: 229-292. doi:10.1016/bs.agron.2014.09.004.

Shepherd M, Stafford A, Smeaton D. 2012. The use of a nitrification inhibitor (dcn™) to reduce nitrate leaching under a winter-grazed forage crop in the central plateau. In Proceedings of the New Zealand Grassland Association, Gore, New Zealand, 1 January 2012; 103-103 p.

Shepherd M, Lucci G. 2013. A review of the effect of autumn nitrogen fertiliser on pasture nitrogen concentration and an assessment of the potential effects on nitrate leaching risk. In Proceedings of the New Zealand Grassland Association, Tauranga, 197-202 p.

Silva RG, Cameron KC, Di HJ, Hendry T. 1999. A lysimeter study of the impact of cow urine, dairy shed effluent, and nitrogen fertiliser on nitrate leaching. Australian Journal of Soil Research 37: 357-369. doi:10.1071/S98010.

Sinhadipathige S, Kenyon P, Kemp P, Morris S, Morel P. 2012. Can herb-clover mixes increase lamb liveweight gains in spring. In Proceedings of the New Zealand Grassland Association, 137-142 p.

Smith SJ, Schepers JS, Porter LK. 1990. Assessing and managing agricultural nitrogen losses to the environment. In: Soil restoration. Stewart (ed.). Springer New York, New York, NY.1-43 p.

Smith VH, Schindler DW. 2009. Eutrophication science: Where do we go from here? Trends in Ecology and Evolution 24: 201-207. doi:10.1016/j.tree.2008.11.009.

Somasiri SC. 2014. Effect of herb-clover mixes on weaned lamb growth. A thesis presented in partial fulfilment of the requirements for the degree of Doctor of Philosophy in Animal Science, Massey University. Doctoral dissertation.

Somasiri SC, Kenyon PR, Kemp PD, Morel PCH, Morris ST. 2015. Growth performance and carcass characteristics of lambs grazing forage mixes inclusive of plantain (plantago lanceolata l.) and chicory (cichorium intybus l.). Small Ruminant Research 127: 20-27. doi:10.1016/j.smallrumres.2015.04.005.

Somasiri SC, Kenyon PR, Kemp PD, Morel PCH, Morris ST. 2016. Mixtures of clovers with plantain and chicory improve lamb production performance compared to a ryegrass-white clover sward in the late spring and early summer period. Grass and Forage Science 71: 270-280. doi:10.1111/gfs.12173.

Somasiri SC, Kenyon PR, Morel PCH, Morris ST, Kemp PD. 2020. Selection by lambs grazing plantain (plantago lanceolata l.), chicory (cichorium intybus l.), white clover (trifolium repens l.), red clover (trifolium pratense l.) and perennial ryegrass (lolium perenne l.) across seasons. Animals (Basel) 10: 2292. doi:10.3390/ani10122292.

Sprague SJ, Kirkegaard JA, Graham JM, Dove H, Kelman WM. 2014. Crop and livestock production for dual-purpose winter canola (brassica napus) in the high-rainfall zone of south-eastern australia. Field Crops Research 156: 30-39. doi:10.1016/j.fcr.2013.10.010.

Stevens D, Turner J, Gowers S, Baxter G, Armstrong S. 1994. Improving hogget performance through efficient management of the spring surplus. In Proceedings of the New Zealand Grassland Association, Hanmer Springs, New Zealand, 85-90 p.

Stevens D. 2016. Influence of methodology on the potential ranking of ryegrass types when assessing their relative value under grazing. *I*n: Alan Iwaasa, HA, Schellenberg, Willms, Larson (eds.) 10th International Rangeland Congress. Saskatoon, SK, TCU Place: 10th International Rangeland Congress.

Stevens D, Canton M, Harpham S. 2021. Farming with reduced winter cropping in southern New Zealand: The risks and practicalities. Journal of New Zealand Grasslands: 123-134. doi:10.33584/jnzg.2021.83.3500.

Stewart A. 1996. Plantain (plantago lanceolata)-a potential pasture species. In Proceedings of the New Zealand Grassland Association, 77-86 p.

Stewart A, Kerr G, Lissaman W, Rowarth J. 2014. Chapter 8 − endophyte in ryegrass and fescue. In 'pasture and forage plants for New Zealand'. In Proceedings of the Grassland Research and Practice Series no. 8, Dunedin, NZ, 66-77 p.

Sun XZ, Waghorn GC, Hoskin SO, Harrison SJ, Muetzel S, Pacheco D. 2012. Methane emissions from sheep fed fresh brassicas (brassica spp.) compared to perennial ryegrass (lolium perenne). Animal Feed Science and Technology 176: 107-116. doi:10.1016/j.anifeedsci.2012.07.013.

Tamura Y, Nishibe S. 2002. Changes in the concentrations of bioactive compounds in plantain leaves. Journal of Agricultural and Food Chemistry 50: 2514-8. doi:10.1021/jf011490x.

Thant A, Millner J, McGill C, Tsimba R. 2020. Productivity and quality of kale, swede, fodder beet and maize in manawatu. Forage crop productivity and quality in Manawatu. Agronomy New Zealand.

Thomas RJ, Logan KAB, Ironside AD, Bolton GR. 1988. Transformations and fate of sheep urine-n applied to an upland uk pasture at different times during the growing-season. Plant and Soil 107: 173-181. doi:10.1007/Bf02370544.

Thompson DJ, Stout DG, Moore T, Mir Z. 1992. Yield and quality of forage from intercrops of barley and annual ryegrass. Canadian Journal of Plant Science 72: 163-172. doi:10.4141/cjps92-016.

Tietz NW. 1976. Fundamentals of clinical chemistry Philadephia, WB Saunders Co.

Totty VK, Greenwood SL, Bryant RH, Edwards GR. 2013. Nitrogen partitioning and milk production of dairy cows grazing simple and diverse pastures. Journal of Dairy Science 96: 141-149. doi:10.3168/jds.2012-5504.

Tozer K, BourdÃ G, Edwards G. 2011a. What factors lead to poor pasture persistence and weed ingress? In: Pasture persistence-grassland research and practice series.129-138 p.

Tozer K, Cameron C, Thom E. 2011b. Pasture persistence: Farmer observations and field measurements, Hamilton, New Zealand, New Zealand Grassland Association.

Ulrich BH. 1965. Methods of enzymatic analysis, London, Verlag Chemie, Academic Press.

Ulyatt M, Barclay P, Lancashire J, Armstrong C. 1974. The feeding value to sheep of 'grasslands tama'westerwolds ryegrass,'grasslands paroa'italian ryegrass, and 'grasslands ruanui'perennial ryegrass under rotational grazing management. New Zealand Journal of Experimental Agriculture 2: 231-236. doi:10.1080/03015521.1974.10427681.

Ulyatt M. 1981. The feeding value of temperate pastures. World Animal Science: 125-141.

Valentine I, Kemp P. 2007. Pasture and supplement resources. In: Pasture and supplements for grazing animals. Rattray, Brooks, Nicol (eds.). New Zealand Society of Animal Production, Hamilton, New Zealand.3-11 p.

Wachendorf C, Taube F, Wachendorf M. 2005. Nitrogen leaching from n-15 labelled cow urine and dung applied to grassland on a sandy soil. Nutrient Cycling in Agroecosystems 73: 89-100. doi:10.1007/s10705-005-8313-2.

Waghorn G, Burke J, Kolver E. 2007. Principles of feeding value. In: Pasture and supplements for grazing animals. Rattray, Brooks, Nicol (eds.). New Zealand Society of Animal Production.35-59 p.

Westwood C, Mulcock H. 2012. Nutritional evaluation of five species of forage brassica. In Proceedings of the New Zealand Grassland Association, Gore, New Zealand, 31-38 p.

White J, Hodgson JG. 1999. New Zealand pasture and crop science, Australia, Oxford University Press.

White RE, Magesan GN. 1991. A stochastic-empirical approach to modeling nitrate leaching. Soil Use and Management 7: 85-94. doi:10.1111/j.1475-2743.1991.tb00855.x.

White RE, Heng LK, Magesan GN. 1998. Nitrate leaching from a drained, sheep-grazed pasture. Ii. Modelling nitrate leaching losses. Australian Journal of Soil Research 36: 963-977. doi:10.1071/S98012.

Whitehead D, Bristow A. 1990. Transformations of nitrogen following the application of 15 n-labelled cattle urine to an established grass sward. Journal of Applied Ecology 27: 667-678. doi:10.2307/2404310.

Williams PH, Haynes RJ. 1990. Influence of improved pastures and grazing animals on nutrient cycling within new-zealand soils. New Zealand Journal of Ecology 14: 49-57.

Williams PH, Haynes RJ. 1994. Comparison of initial wetting pattern, nutrient concentrations in soil solution and the fate of15n-labelled urine in sheep and cattle urine patch areas of pasture soil. Plant and Soil 162: 49-59. doi:10.1007/BF01416089.

Winkler ORT. 2017. Agronomic studies of forage brassicas as full-season and cover crops for grazing in north dakota. A thesis presented in partial fulfilment of the requirements for the degree of Doctor of Philosophy in Animal Science, Graduate Faculty of the North Dakota State University of Agriculture and Applied Science, Fargo, North Dakota. Doctoral Dissertation.

Woods RR, Cameron KC, Edwards GR, Di HJ, Clough TJ. 2016. Effects of forage type and gibberellic acid on nitrate leaching losses. Soil Use and Management 32: 565-572. doi:10.1111/sum.12297.

Woods RR. 2017. The effect of alternative forage species and gibberellic acid on nitrate leaching. A thesis submitted in partial fulfilment of the requirements for the Degree of Doctor of Philosophy, Lincoln University. Doctoral dissertation.

Woods RR, Cameron KC, Edwards GR, Di HJ, Clough TJ. 2018. Reducing nitrogen leaching losses in grazed dairy systems using an italian ryegrass-plantain-white clover forage mix. Grass and Forage Science 73: 878-887. doi:10.1111/gfs.12386.

Woodward S, Waghorn G, Bryant M, Benton A. 2012. Can diverse pasture mixtures reduce nitrogen losses? In Proceedings of the 5th Australasian Dairy Science Symposium, Melbourne, Australia, 13-15 November 2012; 463-464 p.

Young JM, Thompson AN, Curnow M, Oldham CM. 2011. Whole-farm profit and the optimum maternal liveweight profile of merino ewe flocks lambing in winter and spring are influenced by the effects of ewe nutrition on the progeny's survival and lifetime wool production. Animal Production Science 51: 821-833. doi:10.1071/An10078.

APPENDICES

Appendix 1 Effects of alternative forages on nitrate leaching under intensive sheep grazing.

Publications

Maheswaran S, Cranston L, Millner J, Horne D, Hanly J, Kenyon P, Kemp P. 2021. Effects of alternative forages on nitrate leaching under intensive sheep grazing. New Zealand Journal of Animal Science and Production 81: 146-152.

Abstract

There is increasing interest in the use of alternative forages to improve animal performance and reduce nitrogen (N) leaching on farms. A system-scale field trial, which included 20 hydrologically isolated drainage plots (800 m^2/plot) was carried out at Massey University's 'Keeble' farm to investigate the effects of forage type on ewe and lamb performance and the quantity of N lost in drainage. Four forage types (treatments) were studied: perennial ryegrass/white clover (RGWC), plantain/WC (PWC), Italian ryegrass/WC (IRWC) and swedes (Brassica). The forage types were included as ~24% area of a self-contained farmlet, with the remaining area (~76%) being RGWC. Ewes were weighed at mating, pre-lambing and weaning, and lambs were weighed at weaning. Drainage (mm) from each plot was measured using a tipping-bucket flow meter and sub-samples of water were analysed for nitrate-N, ammonium-N and total-N losses. The forage types had no effect ($P>0.05$) on ewe or lamb live weight at any occasion. The average nitrate-N losses for RGWC (0.77 kg N ha^{-1}), PWC (0.45 kg N ha^{-1}) and IRWC (0.56 kg N ha^{-1}) treatments did not differ ($P>0.05$) and were lower than the brassica treatment (6.36 kg N ha^{-1}).

Keywords: drainage water; forage species; live weight; nitrogen loss; sheep grazing

Introduction

Globally, there is increasing concern about the contamination of waterways with nutrients, in particular, the nitrogen (N) leached from agricultural land (Houlbrooke et al. 2004). Nitrate (NO_3^-) is the most common form of N contaminant in drainage water (Haynes & Williams 1993). Urine N deposited by sheep on the pasture is the main source of NO_3^- leaching in the sheep grazing pasture system (Williams & Haynes 1990). Surplus N (not required for plant growth) in urine patches is susceptible to leaching when drainage occurs (Williams & Haynes 1994. Nitrate leaching is widely believed to be significantly lower from sheep farming systems compared to dairy cow systems (Williams & Haynes 1994). However, in order to remain economically viable, sheep production systems in New Zealand have intensified over time with greater use of N fertiliser and higher stocking rates (Morris & Kenyon 2014). Therefore, it is possible that N leaching losses are greater than historical figures would indicate and should be reassessed under modern intensive practices.

There is increasing interest in the use of alternative forages in pastoral agriculture in New Zealand. Some of these forages are used to reduce N leaching, i.e., improve environmental outcomes (e.g., plantain; *Plantago lanceolata* L. and Italian ryegrass; *Lolium multiflorum* Lam.), while others are employed to increase animal performance (e.g., winter crops). There is evidence that the use of plantain on dairy farms can help to mitigate N losses as a result of a decrease in the concentration and amount of urinary N excreted, due to an increase in urine volume and decrease in ammonia production in the rumen (Navarrete et al. 2016). It is known that rapidly growing pasture species, such as Italian ryegrass, display greater N uptake during winter and, therefore, reduce N leaching compared to a perennial ryegrass (*Lolium perenne* L.)/white clover (*Trifolium repens*) sward when grazed by dairy cows (Woods et al. 2016). In contrast, winter crops such as brassicas (e.g., swedes and turnips) produce large quantities of forage (e.g., 8-15 t DM/ha) and are grazed with high stocking rates during winter when grass

growth rate is low (Lucci et al. 2013). Following grazing, the land is left bare until conditions allow for re-sowing in spring. This process of grazing winter brassica species may return large amounts of N to the soil during winter, and subsequently may result in very high rates of N leaching (Monaghan et al. 2013).

Currently, there are no studies of leaching under modern, intensive sheep production systems, particularly when alternative forages are grazed. Further, the limited previous studies carried out estimated N leaching indirectly through animal data (e.g., based on grazing study data) or the use of lysimeters or suction cups. The present study utilises hydrologically isolated plots, which allow for the complete capture of all leached nutrients and drainage water. The aim of this study was to quantify and compare N leaching under sheep grazing on four different forage types (perennial ryegrass (RGWC), plantain/white clover (PWC), Italian ryegrass/white clover (IRWC) and brassica), and to compare the sheep performance in farm systems that incorporated alternative forages. These forages were selected for their ability to reduce N leaching and/or improve animal performance.

Materials and methods

This study was carried out at Massey University's Keeble farm, 5 km southeast of Palmerston North, Manawatu, New Zealand (40°24'02.0"S 175°35'52.8"E) with the approval of the Massey University Animal Ethics Committee (MUAEC 19/20). The site is located in a flat to easy rolling landscape (c. 3% slope) on the Tokomaru silt loam soil, a Fragic Perch-gley Pallic Soil (Hewitt 1998). This paper presents the data collected during 2020.

Experimental design and treatments

The design included four farmlets (each approximately 3.32 ha). Three of the farmlets had 0.81 ha (24% of the area) sown in one of three alternative forages, i.e., IRWC or PWC or brassica (swedes; *Brassica napus* ssp. *Napobrassica*). The remaining area of each farmlet was in

RGWC. The fourth farmlet was entirely RGWC (3.31 ha). Each farmlet was stocked at 14 ewes/ha for the entirety of the experiment (46 ewes per farmlet) and was reflective of an intensive sheep-farming operation for this farm class and area (Beef + Lamb New Zealand Economic Service 2022a).

The research site included an area with 20 drainage plots (five plots/treatment). Each plot was 40 m x 20 m and contained a hydrologically isolated mole pipe drainage system. The drainage plots were sown with the pasture species reflective of their treatment. These pastures were sown in the autumn of 2019 (21 March 2019) with a roller drill and chain harrows. The plantain/WC and IRWC plots were over-sown in April 2020. The brassica was sown by direct drilling in the spring of 2019. Nitrogen fertiliser, in the form of urea (46% N), was applied to the brassica plots at the rate of 30 kg N ha^{-1}in March. Nitrophoska (12% N) and urea were applied to the other three treatment plots at the rate of 30 kg N ha^{-1} in April and October, respectively. The plots were grazed according to the cumulation of forage and best grazing management practices for these species.

Ewe management and measurements

A total of 184 pregnant mixed-age Romney ewes were selected from Massey University's "Keeble" flock at the start of the study (27 June 2019). The ewes were stratified by live weight (LW) and number of foetuses carried (single/twin/triplet) and allocated to the four forage treatments (n=46 at start) to ensure that treatments did not differ in LW, or number of foetuses carried. Ewes that died or were culled were replaced in February 2020, with ewes of a similar LW. Romney rams were introduced on 9 April 2020 for a period of 34 days (two rams per farmlet of 46 ewes). The rams were rotated between mobs on Day 17, to avoid the impact of ram performance on pregnancy rate. All ewes were pregnancy diagnosed using trans-abdominal ultrasound on 1 July 2020 and diagnosed as non-pregnant, single, twin- or triplet-

bearing. Three ewes diagnosed as non-pregnant were replaced by pregnant ewes of similar stage of gestation.

Each mob of ewes was rotationally grazed within their farmlet. The duration of grazing and the interval between paddocks was adjusted based on pasture growth rate and cover, which were assessed visually. When the ewes grazed the drainage plots, each mob was separated into five mobs to enable all five replicates to be grazed at the same time (approximately nine ewes/replicate). All replicates within a forage treatment were always grazed for the same number of days and ewes were moved when the target post-grazing residual was reached in any replicate. Where appropriate, treatments on the drainage plots were grazed at once to minimise the effect of differing weather conditions (see Figure 1). However, the brassica crop was grazed continuously during winter, after the ewes were gradually adjusted to the diet using on-off grazing with increasing grazing durations of brassica each day (2, 4, 6, 8, hours on days one through four) before continuous grazing. Due to mild winter conditions in 2020, the brassica yield was considerably higher than had been estimated, and ewes were unable to graze all the bulb material before set-stocking, with an estimated 5,461 kg DM/ha remaining.

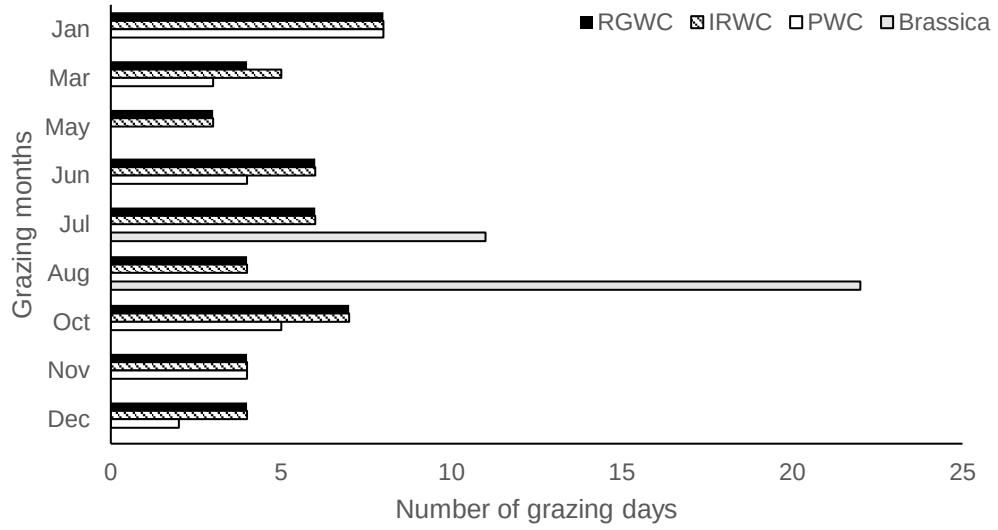

Figure 1 Number of days sheep grazed each of the forage treatments (RGWC: perennial ryegrass/white clover; IRWC: Italian ryegrass/ white clover; PWC: plantain/ white clover; brassica) on the hydrologically isolated drainage plots during 2020.

Approximately one week before the planned start of lambing (26 August 2020), ewes were set stocked on the RGWC area of each farmlet at a stocking rate of 18 ha^{-1}, until the end of lambing (15 October 2020). Ewes and lambs within each treatment were then rotationally grazed as one mob until weaning (12 December 2020). Ewes were weighed at mating, pre-lambing, and weaning. Lambs were also weighed at weaning.

Drainage water volume measurements and water analysis

Rainfall data were sourced from the National Institute of Water and Atmospheric Research (NIWA) site which was located approximately 2 km from the research area. Drainage water from each isolated plot was channelled through drainage pipes into individual tipping-bucket flow meters located in sampling pits nearby. The flow rate of drainage water was measured with an individual tipping bucket (~5 L) for each plot. Each tipping bucket was calibrated dynamically to account for large tip volumes at high flow rates (Humphrey et al. 1997). All tipping buckets were instrumented with data loggers to provide continuous flow rate measurements. Water from the bucket was tipped over a pipe with small hole on it which passed the sub-sample (~0.5 ml) to a larger container for storage until the completion of the drainage event. After the completion of each drainage event, water samples (~100 ml) were collected manually from the storage container for water quality analysis.

Approximately 50 ml of the subsample was filtered through a 0.45 µm filter. Filtered samples were analysed for NO_3^--N and ammonium-N (NH_4^+-N), using a Technicon Auto Analyser (Blakemore et al. 1987). The unfiltered samples were analysed for total N (TN) content using the persulphate digestion method of Hosomi & Sudo (1986). The amount of NO_3^--N, NH_4^+-N and TN losses (kg ha^{-1}) were calculated as the product of the measured drainage quantity and their concentrations.

Statistical analysis

All data were analysed in SAS 9.4 (Statistical Analysis System, version 9.4; SAS Institute Inc., Cary, NC, USA). Live weight of ewes was subjected to an analysis of variance using the MIXED procedure. The model included the fixed effects of measurement time, forage treatment and the interaction between measurement time and forage treatment. Lamb LW, cumulative drainage (mm), NO_3^--N concentrations and cumulative losses of TN, NO_3^--N and NH_4^+-N were analysed using proc GLM with forage treatment as fixed effect and means were separated using LSD procedure. Drainage (mm) from one of the PWC plots was five times greater than the average of the other plots and was deemed to be an outlier caused by a leak in a water pipe. Therefore, in the calculation of cumulative drainage and N losses, the data collected from this particular plot were omitted.

Results

Rainfall and drainage

In 2020, the spring was very wet compared to winter, with a particularly high monthly rainfall (136.7 mm) being recorded in September (Figure 2a). The drainage season in 2020 began in late April and ended in December. The average annual quantity of water that drained across all plots was 83 mm. Throughout the season, there were 13 drainage events from the mole-pipe drainage system, ranging between 0.13 to 19.0 mm (average across all plots). Large drainage events were measured in September and December (Figure 2a), and the majority of drainage occurred in December (average of 33 mm).

The drainage (mm) of all the treatments remained consistent during autumn (April to June) (average of 1 mm) and winter (July to September) (average of 28 mm); however, during spring (October to December), differences in drainage (mm) was observed among treatments. In the last three months of the year, high (76.2 mm; $P<0.05$) and low (32.9 mm $P<0.05$) drainage (mm) were observed from the brassica and PWC treatments, respectively. Overall, the annual

cumulative drainage of the brassica (103 mm) was 7 to 20% greater than that of the other treatments (Figure 2b).

Cumulative NH_4^+-N and total N losses

Ammonium made a minor contribution to the total N leached, and across all treatments represented 7% of inorganic N. Total N losses for RGWC (2.6 kg N ha^{-1}), PWC (1.5 kg N ha^{-1}) and IRWC (2.1 kg N ha^{-1}) treatments were similar ($P>0.05$), but 73 to 84% lower ($P<0.05$) than that of the brassica treatment (16.8 kg N ha^{-1}).

Sheep performance and number of grazing days on alternative forages

Forage treatments had no effect ($P>0.05$) on ewe or lamb LW on any occasion (Table 1). The RGWC and IRWC plots were grazed nine times throughout the year (Figure 1) for a total of 46 and 47 days, respectively. The PWC plots were grazed six times and the number of grazing days was relatively low (26 days). The brassica plots were grazed only once continuously during winter (33 days).

Table 1 Effect of forage treatment (RGWC: perennial ryegrass/white clover; IRWC: Italian ryegrass/ white clover; PWC: plantain/ white clover; brassica) on live weight (LW; kg) of ewes at mating, pre-lambing and weaning, and lambs at weaning.

Treatment	Ewes					Lambs		
	No of ewes	LW (kg)			SEM	No of lambs	LW (kg)	SEM
		Mating	Pre-lambing	Weaning			Weaning	
RGWC	40	72.3	95.1	75.6	1.40	62	27.3	0.49
IRWC	39	70.5	94.8	76.2	1.44	56	26.6	0.49
PWC	42	74.8	94.2	73.0	1.40	69	25.4	0.45
Brassica	43	69.7	89.3	74.6	1.42	62	26.6	0.46

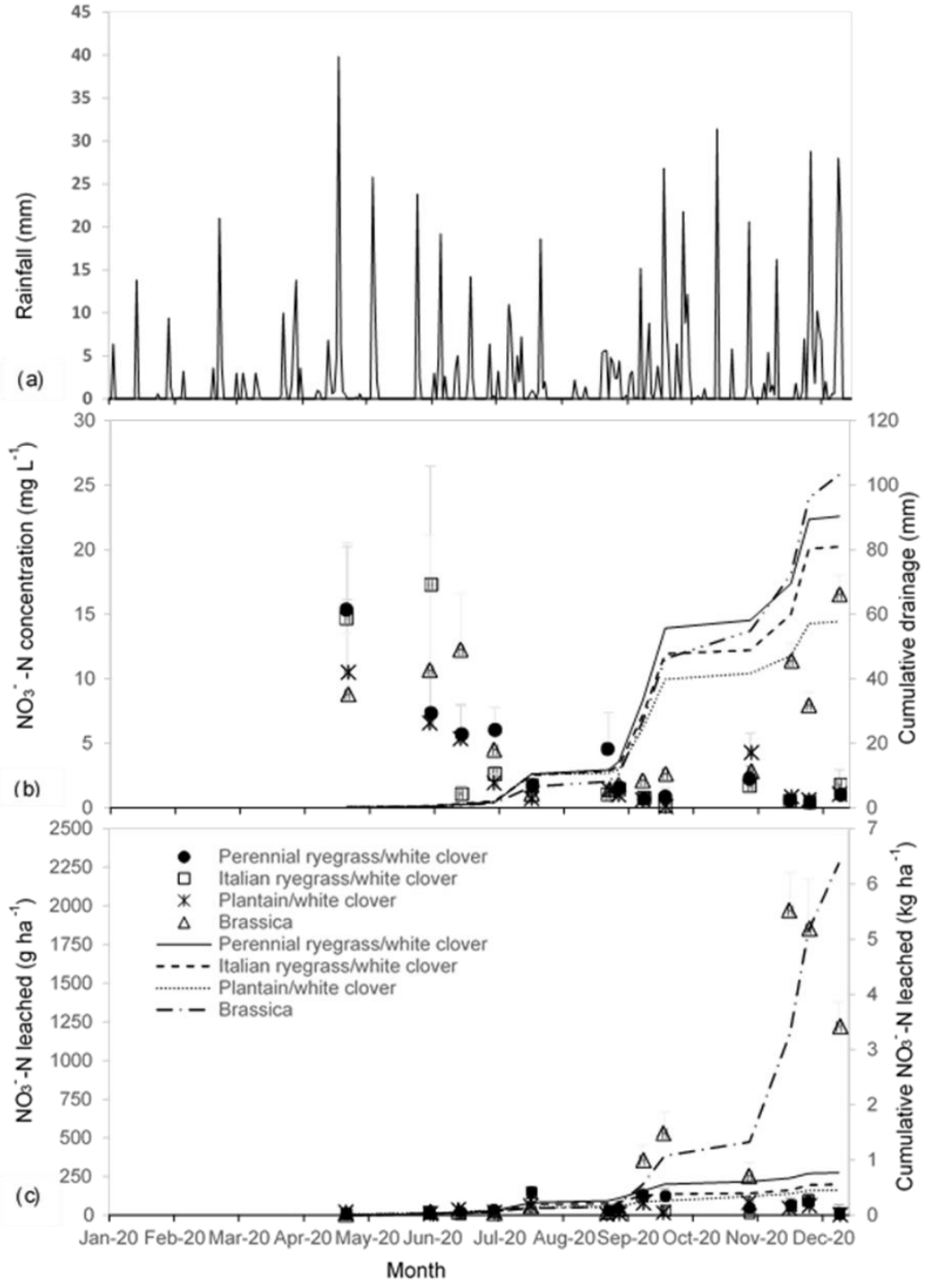

Figure 2 Rainfall (mm) (a) in 2020 (NIWA/Ag Research, Palmerston North meteorological station, 2 km from study site), mean NO_3^--N concentrations (mg L^{-1}) and cumulative drainage (mm) (b), and mean NO_3^--N losses (g ha^{-1}) and cumulative NO_3^--N losses (kg ha^{-1}) (c) from perennial ryegrass/white clover, Italian ryegrass/white clover, plantain/white clover and brassica in 2020.

Discussion

There was no effect of forage treatment on ewe or lamb LW. Therefore, the impacts of N leaching under different forage types can be directly compared without confounding effects of different animal performance. The lack of an effect of treatment on LW is perhaps unsurprising given that each forage treatment made up 24% of each farmlet system. The brassica treatment had no observed benefit to animal performance, although the crop was not utilised fully. The excess bulb could have been used to stock more sheep or to graze for a longer period, and potentially improve animal performance.

The winter of 2020 was relatively warm with less rainfall, compared with the last five years (NIWA 2021). The length of drainage season was longer than reported in other studies conducted near the current research site (Magesan et al. 1996; Hanly et al. 2017; Christensen et al. 2019). The drainage (mm) at the end of the season (October to December) was also higher than reported in the previous studies. The higher drainage (mm) measured for the brassica treatment plots was likely due to the lower evapotranspiration rates associated with bare soil during spring (Hanly et al. 2017).

The average NO_3^--N concentrations of drainage water collected from RGWC, IRWC and PWC treatments started high (above 5 mg L^{-1}) for early-drainage events and decreasing with successive drainage events over the winter months (Figure 2b), as observed by Heng et al. (1991) and Magesan et al. (1996). It is likely that the NO_3^--N that had accumulated in the soil under urine patches, prior to the commencement of drainage events in April and between the drainage events in April and June, contributed to early NO_3^--N leaching for the RGWC, IRWC and PWC treatments (Figure 2b). This demonstrates that grazing RGWC, IRWC and PWC treatments during late summer and autumn period is an important source of the NO_3^--N that accumulates in soil which, in turn, contributes to NO_3^--N leaching during the early stages of the drainage season. Due to the very low leaching losses observed in the present study, the N

accumulation under sheep grazing pastures appears to be much lower than the N accumulation under cattle grazing pastures (Rodriguez et al. 2020). The brassica treatment, however, had higher concentrations of NO_3^--N leached during the initial stage of the drainage season which may have been due to the mineralised N that accumulated as a result of renewing the forage (spraying herbicide and direct drilling) (Fraser et al. 2013).

Grazing of RGWC, IRWC and PWC treatments during spring or late winter did not increase the NO_3^--N concentrations (Figure 2b). Although drainage continued through into December, NO_3^--N concentrations remained <1.8 mg L^{-1}. This trend of drainage water NO_3^--N concentrations declining over the winter months has been previously reported in dairy cattle studies on the same soil type (Hanly et al. 2017; Christensen et al. 2019). Those studies suggested that lower NO_3^--N concentrations in spring and early summer were likely caused by a combination of factors, such as active pasture growth thereby increased NO_3^--N uptake and insufficient drainage volumes to move significant quantities of NO_3^--N into mole channels before the completion of the drainage season (Christensen et al. 2019).

The brassica treatment had greater NO_3^--N concentrations than other treatments at the end of the drainage season in December. The brassica plots were intensively grazed during winter after which the land was left bare for 17 weeks until re-sowing in late December. The lack of plant uptake likely resulted in higher amounts of NO_3^--N susceptible to N leaching under bare soil in subsequent drainage events. A dairy cow study has also shown that N leaching losses from forage-crop grazing during winter could be more than twice that under grazed RGWC (Monaghan et al. 2013). It is important to note that, in the current study, ewes began grazing the brassica plots in the latter part of the winter and had not completely eaten all the bulbs before set stocking for lambing. As a result, there is a risk of greater total NO_3^--N leaching if the ewes had been able to graze for a longer duration and harvest all the bulb material. It might

also be argued that the drainage season in 2020 was protracted, therefore, the losses from the brassica were potentially greater than might have occurred in a more typical year. A daily soil-water balance model analysed using 30 years of data, indicated that only three out of 30 years had greater quantities of drainage after mid-October (Hanly et al. 2017). In addition, resowing of the subsequent forage crop in 2020 was delayed until 27 December 2020 due to prolonged wet soil conditions. To determine the losses that occur when brassica plots are resown more typically in mid spring multiple years of research is required. The amounts of NO_3^--N leached per ha were much lower than those previously reported (8 to 50 kg N ha^{-1}) for sheep grazing RGWC on the same soil type (Heng et al. 1991; Magesan et al. 1996; White et al. 1998). Differences in the stocking rate and grazing time may explain the differences among the sheep studies. For example, White et al. (1998) stocked 40-50 sheep in an area of 0.125 ha for a week in May 1990 and 21 sheep for 5 days in July 1991 and reported losses of 35 and 43 kg N ha^{-1}, respectively, while in the current study, stocking rate was kept constant (46 sheep in an area of 0.4 ha), and the number of grazing days was adjusted according to the amount of forage available. Lower NO_3^--N loss measured in the present study compared to the previous studies could also be due to lower drainage (mm) recorded in 2020 than those reported in the previous studies. The values reported byHeng et al. (1991), Magesan et al. (1996) and White et al. (1998) appear to be very high (~40 kg N ha^{-1}) and more recent measures of leaching under dairy cows (Hanly et al. 2017) are more comparable to the present study (2 to 10 kg N ha^{-1}).

The N leaching values observed in the IRWC and PWC treatments were lower than that of RGWC treatment but did not differ significantly. Multiple years of studies with contrasting rainfall patterns are required to determine if differences might occur among forage treatments.

Conclusion

This study suggests N leaching under intensive sheep grazing of pasture-based forages (RGWC, IRWC, PWC) was very low on drained pallic soil. The grazing of a winter crop

(brassica) is likely to result in a marked increase in N leaching in years where resowing of subsequent forage is delayed until the end of spring. Further, none of the alternative forages improved animal performance above the levels achieved on RGWC pasture.

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References

Beef + Lamb New Zealand Economic Service 2021. On farm data & industry production Beef + Lamb New Zealand, New Zealand. Accessed: 8/3/2021. https://beeflambnz.com/data-tools/farm-classes

Blakemore L, Searle P, Daly B 1987. Methods for chemical analysis of soils. New Zealand Society of Soil Science, Lower Hutt, New Zealand. New Zealand soil Bureau scientific report: 80. 103 p.

Christensen CL, Hedley MJ, Hanly JA, Horne DJ 2019. Duration-controlled grazing of dairy cows. 2: Nitrogen losses in sub-surface drainage water and surface runoff. New Zealand Journal of Agricultural Research 62: 48-68.

Fraser P, Curtin D, Harrison-Kirk T, Meenken E, Beare M, Tabley F, Gillespie R, Francis G 2013. Winter nitrate leaching under different tillage and winter cover crop management practices. Soil Science Society of America Journal 77: 1391-1401.

Hanly JA, Hedley MJ, Horne DJ 2017. Effects of summer turnip forage cropping and pasture renewal on nitrogen and phosphorus losses in dairy farm drainage waters: A three-year field study. Agricultural Water Management 181: 10-17.

Haynes R, Williams P 1993. Nutrient cycling and soil fertility in the grazed pasture ecosystem. Advances in Agronomy 49: 119-199.

Heng L, White R, Bolan N, Scotter D 1991. Leaching losses of major nutrients from a mole-drained soil under pasture. New Zealand Journal of Agricultural Research 34: 325-334.

Hewitt A 1998. New Zealand Soil Classification. 2nd edition. Lincoln, New Zealand, Manaaki Whenua-Landcare Research New Zealand Ltd Press.

Hosomi M, Sudo R 1986. Simultaneous determination of total nitrogen and total phosphorus in freshwater samples using persulfate digestion. International Journal of Environmental Studies 27: 267-275.

Houlbrooke D, Horne D, Hedley M, Hanly J, Snow V 2004. A review of literature on the land treatment of farm-dairy effluent in New Zealand and its impact on water quality. New Zealand Journal of Agricultural Research 47: 499-511.

Humphrey M, Istok J, Lee J, Hevesi J, Flint A 1997. A new method for automated dynamic calibration of tipping-bucket rain gauges. Journal of Atmospheric and Oceanic Technology 14: 1513-1519.

Lucci G, Shepherd M, Carlson B, Lane M, Berry I 2013. Establishment of winter forage crops on pumice soils–experimental results and farmers' perspectives. Accurate and efficient use of nutrients on farms. Fertilizer and Lime Research Centre, Massey University, Palmerston North, New Zealand. Occasional Report, (26). 7 p.

Magesan G, White R, Scotter D 1996. Nitrate leaching from a drained, sheep-grazed pasture. I. Experimental results and environmental implications. Soil Research 34: 55-67.

Monaghan R, Smith L, De Klein C 2013. The effectiveness of the nitrification inhibitor dicyandiamide (DCD) in reducing nitrate leaching and nitrous oxide emissions from a grazed winter forage crop in southern New Zealand. Agriculture, Ecosystems & Environment. 175: 29-38.

Morris S, Kenyon P 2014. Intensive sheep and beef production from pasture - A New Zealand perspective of concerns, opportunities and challenges. Meat Science 98: 330-335.

Navarrete S, Kemp PD, Pain SJ, Back PJ, technology 2016. Bioactive compounds, aucubin and acteoside, in plantain (*Plantago lanceolata* L.) and their effect on in vitro rumen fermentation. Animal Feed Science and Technology 222: 158-167.

NIWA 2021. Annual Climate Summary 2020. NIWA (National Institute of Water and Atmospheric Research) National Climate Centre, New Zealand. Accessed: 8/4/2021. https://niwa.co.nz/climate/summaries/annual-climate-summary-2020

Rodriguez MJ, Kemp P, Navarrete S, Hanly J, Horne D, Bishop P 2020. NItrogen losses from plantain: What can we say?. Nutrient management in farmed landscapes. Fertilizer and Lime Research Centre, Massey University, Palmerston North, New Zealand. Occasional Report, (33). 8 p.

White R, Heng L, Magesan G 1998. Nitrate leaching from a drained, sheep-grazed pasture. II. Modelling nitrate leaching losses. Soil Research 36: 963-978.

Williams P, Haynes R 1990. Influence of improved pastures and grazing animals on nutrient cycling within New Zealand soils. New Zealand Journal of Ecology 14: 49-57.

Williams P, Haynes R 1994. Comparison of initial wetting pattern, nutrient concentrations in soil solution and the fate of 15 N-labelled urine in sheep and cattle urine patch areas of pasture soil. Plant and Soil 162: 49-59.

Woods R, Cameron K, Edwards G, Di H, Clough T 2016. Effects of forage type and gibberellic acid on nitrate leaching losses. Soil Use and Management 32: 565-572.

Appendix 2 Mean forage mass of treatments (approximately 2.9 ha) in non-drainage plot areas (each treatment included the following area: perennial ryegrass/white clover (RGWC): 2.91 ha of the area of RGWC; Italian ryegrass/white clover (IRWC): 2.49 ha of the area of RGWC plus 0.41 ha of the area of IRWC; plantain/white clover (PWC): 2.51 ha of the area of RGWC plus 0.41 ha of the area of PWC; brassica: 2.54 ha of the area RGWC plus 0.41 ha of the area of brassica) over Years 1 (July to December 2019), 2 (January to December 2020), and 3 (January to December 2021). Values are least-squares means.

																								SEM
															Year 1									
															29/07	13/08	27/08	10/09	24/09	8/10	22/10	5/11	19/11	4/12	17/12	
RGWC															3234	2695^{ab}	2307	2002	2035^{ab}	2060	2529^{a}	3062^{a}	3325^{a}	2639^{a}	2363	250
IRWC															3612	3396^{a}	2447	2010	2366^{a}	2332	3083^{a}	3084^{a}	2935^{ab}	2766^{a}	2426	250
PWC															2678	2164^{b}	1833	1427	1526^{b}	1571	1667^{b}	1782^{b}	2229^{bc}	2066^{ab}	2117	273
Brassica															3388	2399^{b}	2197	1749	1401^{b}	1536	1596^{b}	1718^{b}	1856^{c}	1671^{b}	1884	273
														Year 2												
	7/01	21/01	3/02	18/02	24/03	6/04	21/04	6/05	18/05	2/06	16/06	30/06	13/07	27/07	11/08	24/08	8/09	20/09	4/10	18/10	26/10	9/11	24/11	7/12	21/12	
RGWC	2551	2827	2453	2338	1892	1943	1712	1867	1929	1684	1488^{b}	1593	1744	1500	1443	1515	1395	1329	1422	1278	1550	2038	2510	2069	2481	250
IRWC	2396	2687	2328	2230	1579	1872	1751	1808	2124	1760	1732^{b}	1531	1908	1652	1417	1613	1441	1255	1262	1198	1424	1964	2277	2007	2161	250
PWC	2633	2811	2275	2233	1626	1760	1623	1623	1712	1684	1222^{b}	1281	1588	1487	1423	1415	1024	891	1111	935	1417	1824	1813	1801	1847	273
Brassica	2125	2111	1849	1770	1223	1259	1286	1464	1749	1446	2387^{a}	1053	1500	1241	1372	1818	1602	1194	1226	1164	1522	2023	2125	1961	2110	273
														Year 3												
	5/01	20/01	2/02	17/02	28/02	14/03	28/03	11/04	25/04	9/05	24/05	8/06	21/06	5/07	20/07	2/08	16/08	14/09	26/09	11/10	26/10	9/11	24/11			
RGWC	2719	2836	2636	2055	1885	1874	1998	1964	1930	2031	2161^{b}	2204	2098	1894^{ab}	2014^{ab}	1988	1709	1483	1584	1595	1669	1665	1715			250
IRWC	2425	2358	2608	1664	1699	1570	1605	1767	1750	1908	2328^{b}	2059	1933	1676^{b}	1692^{b}	1823	1572	1433	1466	1786	1610	1660	1738			250
PWC	2255	2663	2591	1850	1847	1605	1624	1711	1910	1848	2097^{b}	1912	1749	1451^{b}	1664^{b}	1678	1612	1168	1378	1573	1535	1592	1486			273
Brassica	2263	2342	2198	1618	1618	1640	1651	1820	2058	2123	3322^{a}	2328	2346	2585^{a}	2790^{a}	2459	2044	1773	1741	1781	1535	1497	1559			273

^{abc}subscripts with different letters indicate that means are significantly different between farmlets within the measuring date of a given year (P<0.05).

Appendix 3 Ash (g/kg DM), acid detergent fibre (ADF; g/kg DM), and organic matter digestibility (OMD; g/kg DM) contents of RGWC (perennial ryegrass/white clover), IRWC (Italian ryegrass/WC), PWC (plantain/WC), BL (brassica leaf) and BB (bulb or stem) measured in drainage plot areas (each treatment included the following areas: RGWC: 0.4 ha; IRWC: 0.4 ha; PWC: 0.4 ha; brassica: 0.4 ha) in winter and spring of Year 1 (July to December 2019) and summer, late-autumn, winter and spring of Years 2 (January to December 2020), and 3 (January to December 2021). Values are least-squares means.

		Year 1		Year 2				Year 3				SEM
		Winter	Spring	Summer	Late-Autumn	Winter	Spring	Summer	Late-autumn	Winter	Spring	
Ash	RGWC	96^{mn}	108^{efg}	78^{rs}	101^{ijklm}	103^{hijkl}	100^{jklm}	91^{o}	113^{cde}	108^{fgh}	92^{no}	1.9
	IRWC	92^{no}	108^{efg}	77^{s}	99^{klm}	105^{ghij}	101^{ijklm}	90^{o}	123^{b}	111^{def}	83^{pqr}	
	PWC	nm	114^{cde}	87^{op}	104^{ghijk}	nm	102^{ijklm}	99^{klm}	nm	106^{ghi}	89^{o}	
	Brassica											
	BL	130^{a}	-	-	-	117^{c}	-	-	93^{no}	99^{lm}	-	
	BB	87^{op}	-	-	-	46^{t}	-	-	79^{qrs}	83^{pq}	-	
ADF	RGWC	232^{ef}	233^{ef}	320^{b}	239^{ef}	173^{klm}	205^{g}	237^{ef}	175^{klm}	174^{klm}	194^{ghi}	5.1
	IRWC	264^{c}	207^{g}	348^{a}	167^{lm}	171^{lm}	201^{gh}	242^{def}	136^{pq}	146^{nop}	191^{hij}	
	PWC	nm	247^{de}	269^{c}	231^{f}	nm	178^{jkl}	180^{jkl}	nm	147^{nop}	186^{hijk}	
	Brassica											
	BL	162^{mn}	-	-	-	110^{r}	-	-	152^{no}	126^{q}	-	
	BB	177^{jklm}	-	-	-	108^{r}	-	-	151^{no}	140^{opq}	-	
OMD	RGWC	778^{f}	787^{ef}	611^{i}	746^{h}	836^{bc}	802^{e}	755^{gh}	828^{bc}	836^{bc}	830^{bc}	7.7
	IRWC	752^{h}	804^{de}	563^{j}	836^{bc}	839^{bc}	803^{e}	770^{fg}	840^{bc}	840^{bc}	840^{bc}	
	PWC		777^{fg}	738^{h}	770^{fg}	nm	803^{e}	826^{c}	nm	840^{bc}	833^{bc}	
	Brassica											
	BL	843^{bc}	-	-	-	876^{a}	-	-	850^{b}	840^{bc}	-	
	BB	828^{bcd}	-	-	-	884^{a}	-	-	835^{bc}	827^{c}	-	

[a-p]Superscripts with different letters indicate that means are significantly different among treatments within each parameter ($P<0.05$); nm: not measured as PWC were not grazed in winter of Years 1 and 2, and autumn of Year 3; -: no forage was present to sample as brassica was only present in winter of Years 1 and 2, and late-autumn and winter of Year 3.

Appendix 4 Effect of forage treatment (perennial ryegrass/white clover (RGWC): 100% of grazing area was RGWC; Italian ryegrass/white clover (IRWC): 24% of grazing area was IRWC and remaining 76% of area was RGWC; plantain/white clover (PWC): 24% of grazing area was PWC and remaining 76% of area was RGWC; brassica: 24% of grazing area was brassica and remaining 76% of area was RGWC) on ewe live weight and body condition score (BCS) at different event over three years (Year 1: July to December 2019; Year 2: January to December 2020; Year 3: January to December 2021). Values are least-squares means ± SEM.

	Live weight (kg)	BCS
Treatment		
RGWC	77.6±0.7	3.1±0.1
IRWC	77.2±0.8	3.2±0.1
PWC	77.8±0.7	3.3±0.1
Brassica	77.1±0.8	3.3±0.1
Year		
Year 1	76.6^{n}±0.5	3.4^{m}±0.07
Year 2	78.2^{m}±0.4	3.3^{m}±0.04
Year 3	77.5^{n}±0.4	2.9^{n}±0.04
Event		
Breeding	70.6^{z}±0.5	3.3^{x}±0.04
Docking	75.3^{y}±0.4	2.8^{z}±0.03
Set stock	88.3^{x}±0.4	3.7^{w}±0.03
Weaning	75.5^{y}±0.4	3.1^{y}±0.04

Within year, event and treatment, data within columns with different letters differ significantly ($P<0.05$).

Appendix 5 Effect of forage treatment (perennial ryegrass/white clover (RGWC): 100% of grazing area was RGWC; Italian ryegrass/white clover (IRWC): 24% of grazing area was IRWC and remaining 76% of area was RGWC; plantain/white clover (PWC): 24% of grazing area was PWC and remaining 76% of area was RGWC; brassica: 24% of grazing area was brassica and remaining 76% of area was RGWC) on lamb performance over three years (Year 1: July to December 2019; Year 2: January to December 2020; Year 3: January to December 2021). Values are least-squares means ± SEM.

	Lamb live weight (kg)	Lamb liveweight gain birth to weaning (g/day)
Treatment		
RGWC	15.6^{a}±0.7	237^{a}±3.6
IRWC	15.3^{b}±0.7	225^{b}±3.6
PWC	15.2^{ab}±0.7	226^{b}±3.5
Brassica	15.6^{a}±0.7	233^{a}±3.5
Year		
Year 1	15.8^{x}±0.7	249^{m}±3.6
Year 2	15.0^{z}±0.7	208^{o}±3.5
Year 3	15.4^{y}±0.7	234^{n}±3.3
Event		
Birth weight	5.1^{z}±0.7	
Docking weight	14.8^{y}±0.7	
Weaning weight	26.4^{x}±0.7	

Within year, event and treatment, data within columns with different letters differ significantly ($P<0.05$).

Appendix 6 Forage characteristics, chemical, mineral and botanical composition of perennial ryegrass/white clover (RGWC), Italian ryegrass/white clover (IRWC), plantain/white clover (PWC) and brassica in winter and spring of Year 1 (July to December 2019) and summer, late-autumn, winter and spring of Years 2 (January to December 2020), and 3 (January to December 2021). Values are least-squares means ± SEM.

	Forage treatment				Season				Year		
	RGWC	IRWC	PWC	Brassica	Summer	Late-Autumn	Winter	Spring	1	2	3
Forage characteristics											
Pre-grazing pasture mass (kg DM/ha)	3124^{b}±86	2913^{bc}±87	2826^{c}±104	9378^{a}±270	5388^{x}±142	4158^{w}±129	4464^{xy}±110	4232^{y}±105	5477^{m}±147	4832^{n}±99	3372^{o}±92
Apparent DMI (kg/ewe/d)	2.1±0.1	2.1±0.1	2.4±0.1	2.0±02	2.3±0.2	2.4±0.2	2.0±0.1	1.9±0.1	3.1^{m}±0.2	2.1^{n}±0.1	1.2^{o}±0.1
Apparent dietary N intake (g/d)	76.7^{a}±5.3	76.3^{a}±5.4	89.4^{a}±5.8	50.1^{b}±8.3	57.3^{x}±7.2	90.8^{w}±6.4	83.5^{w}±5.6	60.9^{x}±5.5	110.6^{m}±7.9	66.3^{n}±4.4	42.5^{o}±4.4
Chemical composition											
DM (%)	16.5^{a}±0.4	15.0^{b}±0.4	11.8^{c}±0.5	14.0^{b}±0.7	18.8^{w}±0.6	10.8^{z}±0.6	13.2^{y}±0.5	14.6^{x}±0.4	11.3^{o}±0.7	15.2^{n}±0.4	16.5^{m}±0.3
CP (g/kg DM)	227^{a}±7	227^{a}±7	233^{a}±8	71^{b}±11	125^{y}±9	221^{w}±8	236^{w}±7	176^{x}±7	192^{mn}±10	178^{n}±6	199^{m}±5
ME (MJ/kg)	10.9^{b}±0.1	11.0^{b}±0.1	10.9^{b}±0.1	11.5^{a}±0.1	9.9^{y}±0.1	11.2^{x}±0.1	11.8^{w}±0.1	11.4^{x}±0.1	10.4^{o}±0.1	11.0^{n}±0.1	11.9^{m}±0.1
SSS (g/kg DM)	107^{b}±6	111^{b}±6	109^{b}±7	333^{a}±11	151^{x}±8	145^{x}±8	184^{w}±7	180^{w}±6	154^{n}±10	153^{n}±5	188^{m}±5
SSS:CP	0.5^{b}±0.1	0.5^{b}±0.1	0.5^{b}±0.1	4.9^{a}±0.1	1.7^{w}±0.1	1.4^{x}±0.1	1.7^{w}±0.1	1.7^{w}±0.1	1.6±0.1	1.7±0.1	1.6±0.1
SSS:NDF	0.2^{b}	0.3^{b}	0.3^{b}	2.0±0.1	0.6^{y}	0.6^{xy}	0.8^{w}	0.7^{wx}	0.7±0.1	0.7	0.8
ADF (g/kg DM)	228^{a}±5	213^{b}±5	208^{b}±5	181^{c}±8	272^{w}±6	193^{x}±6	169^{y}±5	196^{x}±5	237^{m}±7	211^{n}±4	175o±4
NDF (g/kg DM)	453^{a}±9	417^{b}±9	374^{c}±11	239^{d}±15	448^{w}±12	357^{x}±12	316^{y}±10	363^{x}±10	406^{m}±15	381^{m}±8	326^{n}±8
OMD (g/kg DM)	771^{b}±7	781^{ab}±7	797^{a}±8	804^{a}±11	705^{y}±9	803^{x}±9	830^{w}±8	814^{wx}±7	767^{n}±11	774^{n}±6	824^{m}±6
Mineral composition											
Ca (mg/kg DM)	6399^{b}±401	5833^{b}±401	14819^{a}±429	15695^{a}±742	13087^{w}±652	10185^{x}±379	9299^{x}±529	10175^{x}±415	na	11783^{m}±495	9590^{n}±269
P (mg/kg)	4740^{a}±104	4698^{a}±104	4583^{a}±109	2596^{b}±193	3588^{x}±166	4756^{w}±99	4482^{w}±138	3791^{x}±108	na	4174±128	4134±69
Na (mg/kg)	3166^{b}±216	3541^{b}±216	7301^{a}±237	894^{c}±400	3660^{x}±360	4485^{w}±204	4062^{wx}±285	2695^{y}±223	na	4192^{m}±267	3260^{n}±147
Botanical composition											
Crop (% of total DM)	83.1^{a}±2.3	88.0^{a}±2.3	65.4^{b}±2.6	nm	66.1^{x}±2.9	84.7^{w}±3.1	85.1^{w}±3.2	79.5^{w}±2.0	85.5^{m}±4.1	73.4^{n}±1.9	77.7^{mn}±1.9
Dead (% of total DM)	11.3^{a}±1.2	11.7^{a}±1.2	5.6^{b}±1.3	nm	22.7^{w}±1.5	8.1^{x}±1.6	4.1^{xy}±1.6	3.3^{y}±1.1	10.5^{m}±2.1	14.9^{m}±1.0	3.2^{n}±1.0
Weed (% of total DM)	5.5^{b}±1.9	0.9^{b}±1.9	20.3^{a}±2.2	nm	7.4^{wx}±2.4	4.6^{y}±2.6	10.7^{wx}±2.6	12.9^{w}±1.7	5.4^{n}±3.5	7.1^{n}±1.6	14.3^{m}±1.6
White clover (% of total DM)	0.4^{b}±0.7	0.01^{b}±0.7	5.3^{a}±0.8	nm	2.6^{w}±0.9	1.4^{wx}±1.0	0.1^{x}±0.9	3.1^{w}±0.6	0.01^{n}±1.3	2.9^{m}±0.6	3.5^{m}±0.6

[a-z]Superscripts with different letters indicate that within each parameter, means are significantly different within forage treatment, season, and year ($P<0.05$); nm: brassica forages were not analysed for botanical composition; na: in first year mineral composition of the pastures were not measured.

Appendix 7 Urine, plasma and faecal nitrogen (N) compositions of breeding ewes grazing perennial ryegrass/white clover (RGWC), Italian ryegrass/white clover (IRWC), plantain/white clover (PWC) and brassica in winter and spring of Year 1 (July to December 2019) and summer, late-autumn, winter and spring of Years 2 (January to December 2020), and 3 (January to December 2021).Values are least-squares means ± SEM.

	Forage treatment				Season				Year		
	RGWC	IRWC	PWC	Brassica	Summer	Late-Autumn	Winter	Spring	1	2	3
Urine											
Urine N concentration* (g N/L)	4.5^{a}±0.3	3.3^{b}±0.3	1.9^{c}±0.4	1.8^{c}±0.5	5.0^{w}±0.4	1.6^{y}±0.4	1.9^{y}±0.3	3.0^{x}±0.3	2.1^{n}±0.4	3.1^{mn}±0.3	3.4^{m}±0.3
NH_3 (mmol/L)	4.6^{a}±0.4	2.4^{b}±0.4	0.7^{c}±0.5	2.4^{bc}±0.7	5.6^{w}±0.5	1.8^{xy}±0.5	0.7^{y}±0.4	2.0^{x}±0.4	1.7^{n}±0.5	3.3^{m}±0.4	2.6^{mn}±0.3
Urea N* (mmol/L)	119^{a}±1.1	87.0^{b}±1.1	58.5^{c}±1.1	27.1^{d}±1.2	107.0^{w}±1.1	49.4^{x}±1.1	52.4^{x}±1.1	59.4^{x}±1.1	51.8±1.1	70.1±1.1	71.0±1.1
Creatinine (mmol/L)	1.9^{a}±0.1	1.4^{ab}±0.1	0.8^{c}±0.2	1.3^{bc}±0.2	2.9^{w}±0.2	0.5^{y}±0.2	0.8^{y}±0.1	1.3^{x}±0.1	1.2±0.2	1.5±0.13	1.4±0.1
Creatinine excretion factor[1]	8.1±0.02	8.1±0.02	8.1±0.02	8.1±0.02	8.1±0.02	8.1±0.01	8.1±0.01	8.1±0.01	8.1±0.02	8.1±0.01	8.1±0.01
Estimated urine volume (L/ewe/d)	4.4^{b}±0.5	7.3^{a}±0.5	8.7^{a}±0.6	7.9^{a}±0.8	2.9^{z}±0.7	10.5^{w}±0.7	8.8^{x}±0.5	6.2^{y}±0.6	8.5^{m}±0.7	5.9^{n}±0.5	6.8^{n}±0.5
Urine N%	0.5^{a}±0.03	0.3^{b}±0.03	0.2^{c}±0.04	0.2^{c}±0.05	0.5^{w}±0.04	0.2^{y}±0.04	0.2^{y}±0.03	0.3^{x}±0.03	0.2^{n}±0.04	0.3^{mn}±0.03	0.3^{m}±0.03
Estimated urinary N excretion (g/ewe/d)[2]	14.7^{a}±0.8	15.3^{a}±0.8	13.9^{a}±0.9	3.84^{b}±1.3	5.9^{y}±1.0	16.2^{w}±1.0	14.4^{w}±0.8	11.2^{x}±0.8	11.0^{n}±1.0	9.4^{n}±0.7	15.3^{m}±0.7
Purine derivatives (PD)											
Uric acid (mmol/L)	0.8^{a}±0.1	0.7^{a}±0.1	0.4^{b}±0.1	0.6^{ab}±0.1	1.0^{w}±0.1	0.2^{y}±0.1	0.3^{y}±0.1	0.8^{x}±0.1	0.5^{n}±0.1	0.7^{m}±0.1	0.7^{m}±0.1
Allantoin (mmol/L)	5.5±3.0	3.6±3.0	3.0±2.8	3.8±2.3	7.9^{w}±1.5	1.4^{y}±1.5	2.4^{y}±1.4	4.2^{x}±1.4	3.0^{n}±1.5	4.6^{m}±1.4	4.4^{m}±1.4
Total PD (mmol/L)[3]	7.4^{a}±0.5	5.5^{b}±0.5	3.2^{c}±0.6	4.5±0.8	9.5^{w}±0.7	2.1^{y}±0.6	3.1^{y}±0.5	5.8^{x}±0.5	3.9^{n}±0.7	5.9^{m}±0.4	5.6^{m}±0.4
PD index[4]	104±31.3	110±28.3	113±24.1	93.6±16.6	72.5^{x}±14.0	114^{w}±13.7	112^{w}±13.5	122^{w}±13.5	100^{n}±13.8	95.7^{n}±13.0	120^{m}±13.5
Plasma											
Plasma urea N (mmol/L)	7.2^{a}±0.3	7.0^{a}±0.3	6.6^{a}±0.3	2.7^{b}±0.4	5.1^{x}±0.4	6.6^{w}±0.4	6.5^{w}±0.3	5.4^{x}±0.3	5.8^{mn}±0.4	5.4^{n}±0.2	6.5^{m}±0.2
Creatinine (μmol/L)	73.6^{a}±1.6	72.6^{a}±1.5	67.5^{b}±1.9	75.9^{a}±2.5	81.4^{w}±2.0	66.9^{x}±1.9	70.5^{x}±1.5	70.7^{x}±1.6	73.9±2.0	73.3±1.4	70.0±1.3
Uric acid* (μmol/L)	11.8±0.1	11.8±0.1	12.1±0.1	11.8±0.1	12.0±0.1	11.9±0.1	11.8±0.1	11.9±0.1	12.2^{m}±0.1	11.9^{n}±0.1	11.7^{o}±0.1
Estimated urinary N excretion (g/ewe/d)[5]	18.5^{a}±0.7	17.2^{ab}±0.7	16.3^{b}±0.8	6.6^{c}±1.1	11.8^{x}±1.0	16.4^{w}±0.9	17.1^{w}±0.7	13.4^{x}±0.8	13.8^{n}±1.0	13.7^{n}±0.6	16.5^{m}±0.6
Faecal N[1] %	3.1^{c}±0.05	3.3^{b}±0.05	3.6^{a}±0.06	2.4^{d}±0.09	2.4^{x}±0.06	3.2^{x}±0.06	3.5^{w}±0.05	3.3^{y}±0.06	na	3.1±0.04	3.1±0.04

[a-z]Superscripts with different letters indicate that within each parameter, means are significantly different within forage treatment, season, and year ($P<0.05$); na: faeces sample collected in first year was not analysed; * Back transformed urinary N and urea N concentrations (mean and SE); [1]Creatinine excretion factor (mg/kg LW) was calculated using Brody's equation: Mean daily creatinine excretion (mg) = (12.7 X LW $^{0.896}$); LW: live weight (Brody 1945; David et al. 2015); [3]Total PD = allantoin (mmol/L) + uric acid (mmol/L) + xanthine plus hypoxanthine (mmol/L) (Chen & Gomes 1992); [4]PD index $= \frac{\text{total PD (mmol/L)}}{\text{creatinine (mmol/L)}} \times \text{LW}^{0.75}$; Urinary N excretion (g/day) = creatinine excretion factor (mg/kg) × live weight (kg) × $\left(\frac{1}{\text{urinary creatinine (mg/kg)}}\right)$ × urine N (g/kg), based on David et al. (2015); [6]Urinary N excretion (g/day) = 1.2 X plasma urea concentration(g/L) × liveweight (kg), based on Kohn et al. (2005).

Appendix 8 Effect of perennial ryegrass/white clover, plantain and plantain clover mix on nitrogen (N) intake and its' excretion in urine and nitrate N losses in artificially drained, Tokomaru silt loam, conducted at the Massey University Dairy Farm No 4 in Palmerston North, New Zealand.

	RGWC	Plantain	Plantain clover-mix
Apparent dietary nitrogen (N) intake (g/cow/d)[1]	486.8	468.6	611.8
Urine N concentration (g N/L)[1]	4.3	3.3	3.6
Estimated urinary N excretion (g/cow/d)[1]	155.6	125.9	145.4
NO_3^--N loss (kg/ha/year)[2]	5.4	2.8	6.6

[1] 2017/2018 season data from Navarrete et al. (2022), [2]2017/2018 season data From Rodriguez et al. (2021).

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STATEMENT OF CONTRIBUTION
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We, the student and the student's main supervisor, certify that all co-authors have consented to their work being included in the thesis and they have accepted the student's contribution as indicated below in the Statement of Originality.

Student name:	Sarmini Maheswaran
Name and title of main supervisor:	Dr James Millner
In which chapter is the manuscript/published work?	Part of Chapter 2
What percentage of the manuscript/published work was contributed by the student?	90

Describe the contribution that the student has made to the manuscript/published work:

Sarmini has written and did the analysis for this publication work with the support from co-authors.

Please select one of the following three options:

- (●) The manuscript/published work is published or in press

  Please provide the full reference of the research output:
  Maheswaran S, Cranston LM, Millner JP, Horne DJ, Hanly JA, Kenyon PR, Kemp PD 2022. Effects of sheep grazing systems on water quality with a focus on nitrate leaching. Agriculture 12: 758. https://doi.org/10.3390/agriculture12060758.

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Student name:	Sarmini Maheswaran
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In which chapter is the manuscript/published work?	Chapter 4
What percentage of the manuscript/published work was contributed by the student?	90

Describe the contribution that the student has made to the manuscript/published work:

Sarmini undertook all of the field work, much of the the laboratory analysis including measurement of nitrate concentration in drainage water, statistical analysis of data and wrote the manuscript.

Please select one of the following three options:

- ○ The manuscript/published work is published or in press
  Please provide the full reference of the research output:
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Student name:	Sarmini Maheswaran
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In which chapter is the manuscript/published work?	Chapter 5
What percentage of the manuscript/published work was contributed by the student?	90

Describe the contribution that the student has made to the manuscript/published work:

Sarmini undertook all of the field work, much of the the laboratory analysis including measurement of nitrate concentration in drainage water, statistical analysis of data and wrote the manuscript.

Please select one of the following three options:

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STATEMENT OF CONTRIBUTION
DOCTORATE WITH PUBLICATIONS/MANUSCRIPTS

We, the student and the student's main supervisor, certify that all co-authors have consented to their work being included in the thesis and they have accepted the student's contribution as indicated below in the Statement of Originality.

Student name:	Sarmini Maheswaran
Name and title of main supervisor:	Dr James Millner

In which chapter is the manuscript/published work?	Chapter 6
What percentage of the manuscript/published work was contributed by the student?	90

Describe the contribution that the student has made to the manuscript/published work:

Sarmini undertook all of the field work, much of the the laboratory analysis including measurement of nitrate concentration in drainage water, statistical analysis of data and wrote the manuscript.

Please select one of the following three options:

- ○ **The manuscript/published work is published or in press**
  Please provide the full reference of the research output:
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Student name:	Sarmini Maheswaran
Name and title of main supervisor:	Dr James Millner
In which chapter is the manuscript/published work?	Appendix 1
What percentage of the manuscript/published work was contributed by the student?	80

Describe the contribution that the student has made to the manuscript/published work:
Maheswaran S, Cranston L, Millner J, Horne D, Hanly J, Kenyon P, Kemp P. 2021. Effects of alternative forages on nitrate leaching under intensive sheep grazing. New Zealand Journal of Animal Science and Production 81: 146-152.

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( ) **The manuscript is currently under review for publication**

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