# Interactions between pasture species and management and their implications for evaluating perennial ryegrass (*Lolium perenne* L.) cultivars in dairy systems

---

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

at
Lincoln University
by
Laura Ines Rossi Rodriguez

---

Lincoln University
2016

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

# Interactions between pasture species and management and their implications for evaluating perennial ryegrass (*Lolium perenne* L.) cultivars in dairy systems

by

Laura Ines Rossi Rodriguez

An index to rank perennial ryegrass cultivars based on their relative economic benefit to pasture-based dairy systems, has been developed in New Zealand (Chapman et al., 2016; DairyNZ) in recent years. Performance values in this system are calculated for the key trait of seasonal dry matter (DM) production, using data from cultivar evaluation trials conducted using perennial ryegrass monocultures and high nitrogen (N) fertiliser inputs. To determine if the index should account for genotype × management interactions, experiments with a common design were established in four regions of New Zealand in 2012. Results of the first two years of the Canterbury experiment are presented in this thesis. The experiment was conducted at the Lincoln University Research Dairy Farm, Lincoln, Canterbury, and used a split plot design with eight subplots randomly allocated within four main plots each replicated in five blocks. Main plots comprised all combinations of pasture sown with or without white clover receiving either low (100 kg N/ha) or high (325 kg N/ha) rates of nitrogen fertilizer annually, randomized within blocks. In the plus clover treatments, pastures were sown with a 50:50 mixture of two clover cultivars commonly used in dairy pastures. The eight perennial ryegrass cultivars were selected to provide contrasting phenotypes for two traits that may influence competition between grass and clover: morphology, and heading date. The morphological contrast was between high tiller density/fine leaf material (both diploids) and low tiller density/broad leaf material (both tetraploids). The heading date contrast was between mid-season and late-season heading date materials, all of them diploids. Each main plot was grazed by dairy cows following standard farm management practices. Total DM yield was estimated in each subplot before grazing by cutting, using a Haldrup forage harvester. Botanical and pasture nutritive value sampling was conducted pre-grazing in spring, summer and autumn each year. Ryegrass and clover population density were measured in autumn each year. Results of the first two years of the experiment show that seasonal and total annual yield of the High N treatments was greater than from the Low N treatments. With the exception of spring of the establishment year, seasonal and total annual yield of the plus clover treatments was greater than from the minus clover treatments. N and clover interactions were observed in summer of the first year, autumn of both years, and for the total annual yield from both years. In general, the Low N plus clover treatment yielded similarly to the High N treatments, and yielded significantly more DM than the Low N minus clover treatment. The effect of cultivar on DM yield was significant in spring and autumn in both years, in winter 2013, and in the annual total of the second year. The clover content of pastures was always greater in the Low N treatments compared with the High N treatments and was affected by the ryegrass cultivar during spring in both years and in the second summer. There were no significant interactions between N and cultivar for clover content during the two years of the experiment. The heading date contrast affected the white clover content of pastures during summer in both years and in autumn 2013, resulting in mid heading date cultivars having greater white clover content than late heading date cultivars. Despite the effects of cultivar and treatments on DM yield and clover content, no significant interactions were detected between clover inclusion/exclusion and perennial ryegrass cultivar, or between N level and perennial ryegrass cultivar on seasonal or annual total DM yield, with the exception of winter 2013. As a consequence no evidence of re-ranking emerged and therefore performance values in the DairyNZ Forage Value Index (DairyNZ) do not need adjustment to account for grass-clover interactions over time and their effects on total pasture DM yield.

The second experiment reported in this thesis was carried out with the objective of analysing how the perennial ryegrass and white clover characteristics affected their competitive ability, their proportion in the sward and the DM yield when grown in mixtures. The experiment was conducted at the Lincoln University Research Dairy Farm, Lincoln, Canterbury, and used a split plot design with four blocks. Main plots were two nitrogen levels (100 and 325 kg N/ha/year), randomised within blocks. Subplots were the pasture types (24), made up of a 4 × 4 factorial of 4 perennial ryegrass cultivars and 4 white clover cultivars (16 subplots) plus monocultures of each cultivar (8 subplots), randomised within main plots. Four perennial ryegrass cultivars were selected to create a range from fine to broader leaved materials and from open to denser cultivars. The four white clover cultivars were selected to create a range in leaf size, from small to large leaved. Total DM yield was estimated in each subplot by cutting, using a Haldrup forage harvester. Botanical composition was determined by dissecting a subsample collected from the harvested herbage at every harvest. Ryegrass and clover population density were measured four times during the experimental period (winter 2014 to autumn 2015). Photosynthetically active radiation (PAR ) intercepted by the canopy and canopy height were measured three times during the year. Total annual DM yield of the mixtures was greater in the High than in the Low N treatment. The inclusion of clover increased the total annual

DM yield under both N treatments, but the increment was greater under Low than under High N treatment. Only on one occasion was the white clover content of pasture affected by the interaction between perennial ryegrass and white clover cultivar, but not during the rest of the season. There were effects of perennial ryegrass and white clover cultivars on DM yield of the mixtures in some of the harvests during the year, but the total annual DM yield was similar for mixtures sown with different grass or different clover cultivars. With the exception of one occasion, no significant interactions were detected between perennial ryegrass cultivar and white clover cultivar on DM yield of the mixtures, meaning that the inclusion of different white clover phenotypes did not affect the DM yield of the mixture differently when associated to different perennial ryegrass phenotypes.

**Keywords:** Perennial ryegras, *Lolium perenne* L., white clover, *Trifolium repens* L., DairyNZ Forage Value Index, dairy, DM yield, N fertiliser, phenotype, heading date, tiller density, white clover growing points, botanical composition, quality, grazing, competition.

Dedication

To the memory of my beloved parents

# Acknowledgements

I would like to thank my two excellent supervisors, Dr. Grant R. Edwards and Dr. David F. Chapman for their guidance, support and encouragement during the course of my doctoral study. Their advice and contributions throughout the analysis and the writing up of this thesis  have been invaluable. I feel privileged to have had the opportunity to learn so much from them.

I would like to express my most sincere gratitude to the New Zealand dairy farmers and to DairyNZ for funding the project number RD 1414.

I wish to acknowledge Lincoln University; during my time at this institution I always found help and support from the members of the different teams.

I wish to extend my gratitude to the Lincoln University academics: Dr. Graham Barrell, Dr. Racheal Bryant, Dr. Jim Moir, Dr. Brendon Malcolm, Dr. Alistair Black, Dr. Farhad Dastgheib, Dr. Simon Hodge, Roy Edwards and Roger McLenaghen.

Many thanks to the members of the DairyNZ Academic Committee and to Jennie Burke. I would like to thank Dr. Julia Lee for her advice and help;  thanks to DairyNZ Newstead and DairyNZ Lincoln present and former technical team members: Dale Beker, Deanne Waugh, Hamish Hodgson, Anna Clement, Erin Garnett, Natalie McMillan, Angela Ravagnani, Cat O'Connor, Briar McGowan, Kirsty Martin for their assistance and support. Many thanks to DairyNZ Newstead and Lincoln offices staff; their help is greatly appreciated.

A special thank go to Barbara Dow, DairyNZ statistician, and Alison Lister, Learning Advisor in statistics at Lincoln University. Their teaching vocation, knowledge and dedication made statistics a more enjoyable subject. Their help with the analysis of the data is greatly appreciated.

Many thanks to the Lincoln University Research Dairy Farm (LURDF) staff: to the farm manager Jeff Curtis and to the present and former farm staff and technical team members: Jonathan Curtis, Helen Hague, Misato Iitaka, Sarah Taylor, Brian Maw, Bowen Evans, Adam Caldwell, Caleb Sixtus, Monique van Rossum, Diane Costello for their invaluable help.

Special thanks to the Field Service Centre, and Nursery present and former team members: Alan Marshall, David Jack, Daniel Dash, Shean Brown, Dr. Keith Pollock, Malcom Smith and Brent Richards, for their help and support. Thanks also to Neil Smith and Nigel Beale for their help.

Many thanks to the Library, Teaching and Learning and Information Technology Services departments. I would like to thank Sarah Tritt, Jan Thompson, Caitriona Cameron and Dean O'Connell for their invaluable help.

I wish to acknowledge the members of the Administration, Postgraduate and Enrolment teams: Robyn Wilson, Bernadette Mani, Anu Sharma, Alison Hind, Debbie Park and the late Jan Haldane. Their support and help was greatly appreciated.

Many thanks to the New Zealand Plant Breeding and Research Association (NZPBRA) members for their support.

I wish to acknowledge Aleisha Hansen and Chris Hardy (Plant Variety Rights Office of New Zealand) for their help.

I would like to acknowledge all the AgResearch scientists and technical team members with whom I had the opportunity to work during the time of my study: Dr. Gerald P. Cosgrove, Dr. Mark McNeill, Dr. Jim R. Crush, Dr. David E. Hume, Dr. David Stevens, Dr. Keith Widdup, Ben Harvey and Lily Ouyang.

I would like to acknowledge Dr. Michael O'Donovan, Dr. Mary McEvoy and PhD candidate Justin McDonagh, co-authors of a paper as well as to Dr. Michael Egan and PhD candidate Emma Louise Coffey, Teagasc Moorepark, for their support during my visit to the Republic of Ireland.

I wish to acknowledge Dr. Linda Lilburne and Trevor Webb from Landcare Research for their help.

Many thanks to Nigel Johnston for his work in one of the projects and for his advice.

I wish to acknowledge the former DairyNZ science interns: Charlotte Reed, Charlotte Robertson, Briar Robertson and Roshean Woods for their help.

Many thanks to the international students Caroline Varin, Marcel de Jong and Jan Sjoerd Mulder for their invaluable help.

Thanks to Lincoln University postgraduate students and students: Aimi Nabilah Hussein, Ao Chen, Krisada Boonnop, Lydia Farrell and Anita Ferris for their help.

Many thanks to all my DairyNZ Lincoln office workmates who always encouraged and supported me with their good wishes and kind words.

A special thank to John and Margaret Shearer and David and Sarah McLeod, dairy farmers, for their warm welcome when we arrived in New Zealand and for giving my family and I the opportunity to learn and experience the life and work on a dairy farm.

Thank you to my sister, brother in law and mother in law for their continuous support and to my extended family and friends in Uruguay and in New Zealand who always encouraged me to reach my goal.

To my husband Williams Leal and my children: Juan Manuel, Camila and Agustin Leal Rossi, all my love and gratitude. This thesis would not be possible without their help, patience and encouragement.

# Table of Contents

# List of Tables

# List of Figures

# Chapter 1
# Introduction

## 1.1 Background

The relevance of the dairy industry to the New Zealand economy can be clearly demonstrated by its contribution to the national Gross Domestic Product (GDP). In 2013 this contribution was NZD 5.035 billons, and represented 2.3 % of GDP (Statistics New Zealand). Typically, dairy earns over 40 % of New Zealand's primary industries' export value (Ministry for Primary Industries, 2015). In 2014-15, 21.3 billion litres of milk (containing 1.9 billion kilograms of milksolids) were processed by dairy companies. The number of herds in the same year was 11,970, with an average size of 419 cows; the total effective hectares of dairy land were 1.8 million, with an average farm size of 146 hectares. A record of 1,082 kg of milksolids per effective hectare was achieved in the 2014-15 season, with an average stocking rate of 2.87 cows/ha and a production per cow of 377 kg milksolids (Livestock Improvement Corporation Limited & DairyNZ Limited, 2015).

The strategies that have guided the investment and activities of this industry focus on actions to ensure dairy farming remains competitive and responsible. Competitive to continue being profitable over the long-term in a context of constrained dairy prices due to abundant milk supply and depressed short-term demand from the largest importers. Responsible through a good stewardship of resources and the aptitude to comply with environmental regulations (DairyNZ, Dairy Companies Association of New Zealand, & Federated Farmers, 2009; DairyNZ, Federated Farmers, Dairy Companies Association of New Zealand, & Dairy Women's Network, 2013; Ministry for Primary Industries, 2015).

To improve profitability at the farm level has been an objective of the industry strategies as well as to create and maintain industry information systems that serve the needs of all dairy farmers (DairyNZ et al., 2009; DairyNZ et al., 2013). On a per hectare basis farm profitability and pasture eaten are positively correlated (Clark, Caradus, Monaghan, Sharp, & Thorrold, 2007; Savage & Lewis, 2005; van Bysterveldt, 2005). Two of the avenues to increase pasture eaten are better management (grazing, fertiliser, etc.) and improved plant genetics. In this context is that the 2011 Forage Review group was established between DairyNZ and the New Zealand Plant Breeding and Research Association (NZPBRA) (DairyNZ & New Zealand Plant Breeding and Research Association, 2012). Some of the recommendations of the Forage Review were to align the dairy industry and plant improvement goals to achieve a sustainable, competitive and profitable dairy industry based on grazed forages, and to finalise and establish an independent forage value index.

In New Zealand, perennial ryegrass (*Lolium perenne* L.) and white clover (*Trifolium repens* L.) are the dominant species in the majority of sown pastures. Most of the herbage seed produced in the country is from grass, and perennial ryegrass is the largest component (Pyke, Rolston, & Woodfield, 2004), accounting for 51 % of the commercial grass seed sales (T. Chin, New Zealand Plant Breeding and Research Association, personal communication, 2016) and having the priority in the seed companies' research and development investment (DairyNZ & New Zealand Plant Breeding and Research Association, 2012). Therefore, this was the species selected to start working on a new forage evaluation system, and with which the impact of this new system would be more important. Meanwhile, white clover accounts for 62 % of the commercial legume seed sales.

Considering that dairy pasture renewal rates are estimated at about 5 % (DairyNZ & New Zealand Plant Breeding and Research Association, 2012) and for the season 2006 – 07 were estimated at 6.1 % of the total hectares in the dairy industry (K. Sanderson & Webster, 2009), or once every sixteen to twenty years, the decision about which cultivar to select becomes crucial. Although there is a long history of perennial ryegrass breeding in New Zealand, with evidence of genetic gain for annual yield of between 0.25 and 1.5 % per year (A. V. Stewart, 2006) and consistent gains of approximately 0.76 % per year after 1990 (Harmer, Stewart, & Woodfield, 2016), it is not clear what value this is delivering to farmers (DairyNZ & New Zealand Plant Breeding and Research Association, 2012). Therefore, in May 2012, the DairyNZ Forage Value Index (FVI) (DairyNZ) which ranks perennial ryegrass (and short-term ryegrass) cultivars based on their relative economic benefit to pasture-based dairy systems (Chapman, Bryant, McMillan, & Khaembah, 2012; Chapman et al., 2016; Chapman, Edwards, et al., 2015; Chapman et al., 2014), was launched. Initially it includes the key trait of seasonal dry matter (DM) yield for which performance values are calculated using data from cultivar evaluation trials conducted by the NZPBRA, the National Forage Variety Trial (NFVT) (New Zealand Plant Breeding and Research Association Inc., 2016). Cultivars are then ranked for their estimated profit index in the FVI. Traits such as nutritive value and persistence will be added in the future.

However, the NFVT trials are conducted using mostly perennial ryegrass monocultures and in general, high nitrogen (N) fertiliser inputs (3 % of mean dry matter harvested) (Easton et al., 1997; Easton et al., 2001), while the standard practice in New Zealand is sowing perennial ryegrass in a mixture with white clover. Although in mixed swards the white clover content is typically low, well below levels thought necessary for e.g. animal production benefits, these two species have the potential to influence each other when in association (Camlin, 1981), and their relationship and proportions in the sward are influenced by environmental and management factors (W. Harris, 1990; Schwinning & Parsons, 1996a, 1996b, 1996c). Therefore it was important to examine these issues and to determine if perennial ryegrass cultivars re-rank in terms of their comparative total DM yields when grown under different managements, and if the FVI system needed to take interactions with white clover into account. Moreover, greater efficiency of utilisation of metabolisable energy (ME) for growth and lower cost of ingestion when consuming clover than when consuming ryegrass (Nicol & Edwards, 2011), as well as a positive effect of the inclusion of clover on milk production (yield and solids) (Egan, Lynch, & Hennessy, 2015), explain the need to consider the consequences of different proportion of clover on animal nutrition and performance. These research questions are addressed in the Chapter 3 of this thesis.

Previous research have also shown that, in general, when perennial ryegrass and white clover cultivars with different phenotypes are grown in a mixture, the total annual yield of the pasture is similar due to substitution or compensatory effects between the two main components of the sward (Camlin, 1981; Connolly, 1968; Ledgard, Brier, & Upsdel, 1990; Reid, 1961; Widdup & Turner, 1983), but differences in botanical composition could emerge (Connolly, 1968; Rhodes & Harris, 1979; Widdup & Turner, 1983). Research has also emphasised in finding grass and clover plant characteristics that result in an improved white clover content in the sward (Collins & Rhodes, 1989; Elgersma, Nassiri, & Schlepers, 1998; Elgersma & Schlepers, 1997a; Frame & Boyd, 1986a; Gilliland, 1996) due to the multiple benefits that its inclusion brings to the production system. It has the ability to fix $N_2$, high nutritive and feeding value, and a seasonal growth complementary to grass growth that can result in an increased yield of the mixture compare to grass monoculture (W. Harris & Hoglund, 1977; Ledgard & Steele, 1992; Nicol & Edwards, 2011; Ulyatt, 1970; Walker, Orchiston, & Adams, 1954; Whitehead, 1970). This possible increased yield has positive implications when considering the relationship between pasture eaten and on-farm profitability. However, in New Zealand, the clover content on dairy pastures is relatively low (less than 20 % DM on an annual basis) (Caradus, Harris, & Johnson, 1996; Chapman, Parsons, & Schwinning, 1996; Ettema & Ledgard, 1992; Tozer et al., 2014), limiting the possibilities of exploiting the advantages of the grass/legume system (Chapman et al., 1996). The availability in the market of grass and clover cultivars with a range of phenotypes, plus the possibility of using irrigation on Canterbury farms, raises the question whether interactions between cultivars with different phenotypes could affect herbage yield and botanical composition and result on more productive mixtures of increased feeding value. This research question is addressed in the Chapter 4 of this thesis.

Together, answers from these research questions can also shed light on the value of white clover in mixtures, and how to increase white clover levels in grazed pastures in general.

## 1.2 Objectives

Therefore, the primary aim of the research described in this thesis was to analyse the interactions between pasture species and management and their implications for evaluating perennial ryegrass (*Lolium perenne* L.) cultivars in dairy systems; and to analyse how to increase total pasture eaten on New Zealand dairy farms through manipulation of grass-clover content.

The specific objectives of the research programme were to:

- compare the total DM yield (kg DM/ha) and ME density (MJ/kg DM) of swards based on different perennial ryegrass cultivars sown with and without white clover and receiving either low or high rates of N fertilizer application and to determine if they re-ranked in terms of their comparative total DM yields and ME densities when sown in mixed ryegrass/white clover swards compared to ryegrass monoculture swards.

- compare the total DM yield (kg DM/ha) of swards based on perennial ryegrass and white clover cultivars with different phenotypes grown in association and receiving either low or high rates of N fertiliser application.

- analyse the role of perennial ryegrass and white clover phenotypes in determining the botanical composition of the sward (white clover content expressed as % DM).

- determine which factors were affecting the competitive ability of the different perennial ryegrass and white clover phenotypes when grown in mixtures and receiving either low or high rates of N fertiliser application.

## 1.3 Thesis structure

This thesis comprises five chapters (Figure 1.1). Following this Introduction Chapter, a literature review is presented in Chapter 2. Chapter 3 reports the findings of the first two seasons of an experiment conducted in Canterbury, New Zealand, comparing the total DM yield (kg DM/ha) produced when perennial ryegrass cultivars were sown with and without white clover at low and high rates of N application under irrigation, and analysing if they re-ranked in terms of their comparative total DM yields. Chapter 4 presents the findings of one season of an experiment comparing the total DM yield (kg DM/ha) produced when perennial ryegrass and white clover cultivars with different phenotypes were grown in association at low and high rates of N application under irrigation, and the white clover content of the sward. In Chapter 5 overall conclusions of both experiments are presented as well as suggestions to improve white clover content of pastures through management and breeding objectives.

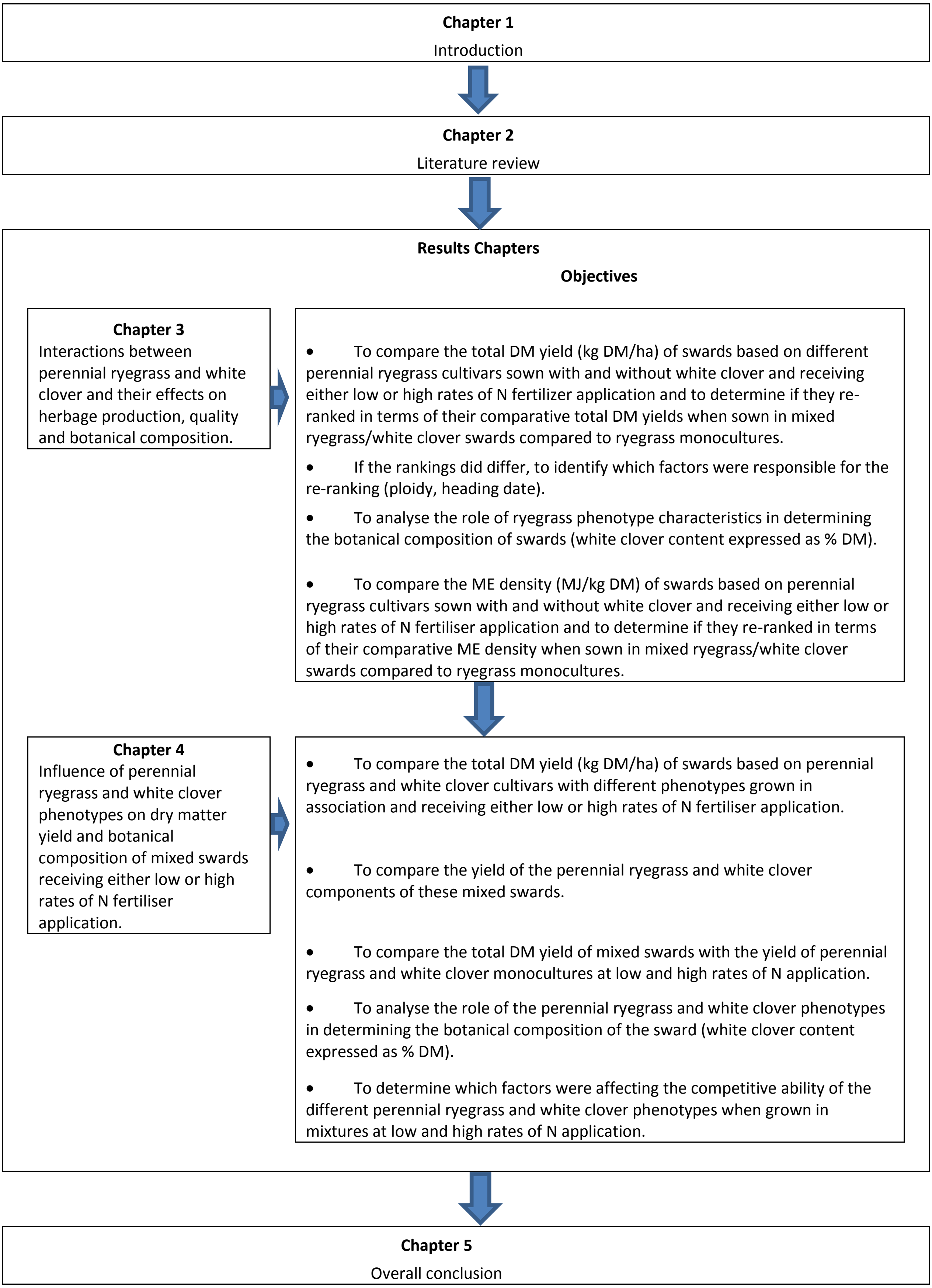


**Figure 1.1 Diagrammatic representation of the thesis structure.**

# Chapter 2

# Literature Review

## 2.1 Introduction

The dominant species in New Zealand dairy pastures are perennial ryegrass (*Lolium perenne* L.) and white clover (*Trifolium repens* L.). These species were introduced to the country in the 19$^{th}$ century, and both well-adapted to the New Zealand environment (Lee, Matthew, Thom, & Chapman, 2012; Mather, Melhuish, & Herlihy, 1996; A. V. Stewart, 2006). Breeding of improved cultivars started in the late 1920s and 1930s when the bases for the establishment of simple systems of perennial ryegrass and white clover dominant swards were developed (Hunt & Easton, 1989; Lee et al., 2012; Mather et al., 1996; A. V. Stewart, 2006).

The high yield and digestibility, easy establishment, persistence under different climatic and management conditions as well its tolerance to grazing has granted perennial ryegrass a dominant role in the pastoral farming in New Zealand (Hunt & Easton, 1989; Wilkins, 1991). However, the benefits of the grass legume association have been largely recognized and have secured white clover a secondary but not less important role in the mixture. It has: ability to fix $N_2$ via the symbiotic association with root nodule bacteria belonging to the genera *Rhizobium*, seasonal growth complementing the growth of grasses, high nutritive value and ability to improve animal feed intake and utilization rates, in addition to being tolerant of grazing (Caradus, Woodfield, & Stewart, 1996; S. L. Harris, Auldist, Clark, & Jansen, 1998; W. Harris & Hoglund, 1977; Ledgard & Steele, 1992; Martin, 1960; Walker et al., 1954; Whitehead, 1995). With all these merits, white clover became New Zealand's competitive advantage in the 1990s when it was the main source of N inputs and permitted the maintenance of a low-cost farming system (Caradus, 1990; Ledgard, Sprosen, Penno, & Rajendram, 2001).

But despite all the advantages that white clover brings to the mixture, studies in the 1970s and 1980s (O'Connor, 1982; O'Connor & Cumberland, 1973) showed that N availability was limiting pasture production and that there was response to N fertiliser application, especially in some areas of the country due to shorter growing season of the clover. These initial studies showed reduction in clover content due to N fertiliser use (O'Connor, 1982; O'Connor & Cumberland, 1973), effect that was also observed in later research when high or low levels of N fertiliser were applied (Caradus, Pinxterhuis, Hay, Lyons, & Hoglund, 1993; A. Davies & Evans, 1990; Egan et al., 2015; Enriquez-Hidalgo, Gilliland, & Hennessy, 2015; Frame & Boyd, 1986a, 1987b; Hennessy, Enriquez-Hidalgo, O'Donovan, & Gilliland, 2012; Ledgard, 2001; Ledgard, Sprosen, Steele, & West, 1995; Nassiri & Elgersma, 2002). Nevertheless, the need to provide additional feed in periods of shortage, the progressively earlier start of calving (Livestock Improvement Corporation Limited & DairyNZ Limited, 2015) in times of the year when low soil temperature may limit $N_2$ fixation (Hoglund, Crush, Brock, Ball, & Carran, 1979), and the increased intensification during the 1990s, justified the inclusion of N fertiliser in the production system (Ball & Field, 1985; Ledgard, Crush, & Penno, 1998; O'Connor, 1982).

Therefore, the current dairy production systems are based mostly on permanent pastures with a dominant perennial ryegrass component, a white clover component that usually does not exceed 20 percent of the sward (%DM on an annual basis) (Chapman et al., 1996; S. L. Harris, 1998; Tozer et al., 2014), and N fertiliser inputs, that in Canterbury, New Zealand, averaged 226 kg N/ha/year for the season 2014 – 15 (DairyBase® personal communication, January 2016).

On a per hectare basis farm profitability and pasture utilization are positively correlated (Clark et al., 2007; Savage & Lewis, 2005; van Bysterveldt, 2005), and pasture utilization is also positively affected by pasture grown and stocking rate (cows/ha) (Ramsbottom, Horan, Berry, & Roche, 2015). These findings have been supported by Dillon, Roche, Shalloo, and Horan (2005) work, showing that the cost of milk production/l decreases with an increase in grazed grass. Thus, to maximize both growth and utilization becomes crucial for the profitability of the farming business. The higher production limits for perennial ryegrass – white clover pastures in New Zealand have been indicated as about 15 t DM/ha per year or 20 t DM/ha per year when under irrigation (Clark et al., 2007; Clark, Matthew, & Crush, 2001). However, persistence of herbage yield is also an important productivity trait and has become an issue for farmers in many areas of New Zealand (Chapman, Muir, & Faville, 2015; Kerr, 2011); renewal of poor performing paddocks to increase production and profitability is one of the options available to farmers to overcome this limitation (Stevens & Knowles, 2011).

Considering that dairy pasture renewal rates are estimated at about 5 % (DairyNZ & New Zealand Plant Breeding and Research Association, 2012) and for the season 2006 – 07 were estimated at 6.1 % of the total hectares in the dairy industry (K. Sanderson & Webster, 2009), or once every sixteen to twenty years, the decision about which cultivar select becomes crucial. Therefore the importance of tools such as the DairyNZ Forage Value Index (FVI) (Chapman et al., 2016; DairyNZ) to support farmers to take these decisions.

DairyBase®: database information which purpose is to improve the financial understanding and performance of dairy farmers using a benchmarking approach. DairyBase® is owned and managed by DairyNZ on behalf of the dairy farmers of New Zealand.

## 2.2 Perennial ryegrass – the main component of New Zealand pastures

### 2.2.1 Plant development and sward characteristics

The tiller is the basic unit of growth of the perennial ryegrass plant. Its apical meristem is located below the soil surface, and consists of dividing cells that initiate new growth from which leaves develop in regular sequence on alternate sides of the apex. Each leaf attaches to the shoot apex at a point called the node, and the stem tissue which separates one node from the next is called the internode (Langer, 1973; Parsons & Chapman, 2000). In the vegetative state, internodes generally do not elongate so the true stem of the tiller is only a few millimetres in length. However, in the reproductive states, several younger internodes elongate rapidly to produce the flowering stem which supports the seedhead. The spikelets of the seedhead are formed by differentiation of bud primordia on the apex such that they are committed away from leaf production to reproductive development.

The leaf comprises two parts: the leaf blade, or lamina; and the sheath. The lamina is connected to the sheath, at its base. As each leaf grows inside the encircling sheaths of older leaves, a 'pseudo-stem' formed by the older sheaths develops, while the 'true stem' (apical meristem, nodes and internodes) remains located at the base (Langer, 1973; Parsons & Chapman, 2000). But the true stem also may branch forming 'tillers'. When the apical meristem produces a leaf, an axillary meristem develops on the opposite side of the internode, in the axil of the previous leaf. If this axillary bud becomes active, its apex produces leaves, and the replication of this process permits the increase in tiller numbers. In perennial ryegrass, the number of live leaves per tillers remains constant at approximately 3, because the rate of formation of leaves is similar to the rate of death (A. Davies, 1978). Once the tillering process starts, and the plant becomes larger, competition for resources (mainly light) within the plant or with adjacent plants takes place, and the pattern of tillering changes, resulting in a lower rate of production of tillers in relation to the rate of leaf appearance (site filling) (A. Davies & Thomas, 1983).

While the development of the aerial parts of the plant occurs, adventitious roots grow from nodes close to the soil surface, and with time, each tiller is able to produce its own network of roots (Langer, 1973).

In 1993 Chapman and Lemaire (1993, p. 96) stated:

> *Plant morphogenesis can be defined as the dynamics of generation and expansion of the plant form in space. It can be described in terms of the rate of appearance of new organs (organogenesis), their rate of expansion (growth), and their rate of senescence and decomposition.*

These authors mentioned that leaf appearance rate, leaf elongation rate and leaf life-span are the three main characteristics determining the morphogenesis of a vegetative grass sward, and indicated that although these characters are genetically determined, they could be modified by variation in factors such as temperature, N nutrition and water status amongst others (Chapman & Lemaire, 1993). The combination of the above mentioned three main characteristics determines the structural characteristics of the grass sward which are: leaf size, resulting from the leaf elongation rate and leaf appearance rate; tiller density related to leaf appearance rate by 'site filling'; and the number of living leaves per tiller, which depends on leaf-life span and leaf appearance rate. As these authors indicated, leaf size, tiller density and leaves per tiller determine the leaf area index (LAI; area of leaf in the canopy divided by the area of ground below) of the sward, which is the key determinant of light interception and regrowth dynamics (Chapman & Lemaire, 1993).

Previous research has linked tiller density and tiller size, through the self-thinning rule or size-density compensation response, named the -3/2 boundary rule, due to the negative slope of the line relating the logarithm of unit mass to the logarithm of population density (Sackville Hamilton, Matthew, & Lemaire, 1995). Size-density compensation occurs in response to modifications in the management of pastures and exemplifies the phenotypic plasticity of this species (Chapman & Lemaire, 1993).

Establishment and maintenance of tiller population is vital for pasture persistence (Edwards & Chapman, 2011). Despite tillers being formed continuously, spring is the time of the year when tiller appearance rate is high; however it is also a time of high tiller death rates. In New Zealand, peak tiller densities has been observed in late winter-early spring and increased tiller appearance rate has been reported before flowering in mid spring (Edwards & Chapman, 2011; Hunt & Field, 1979). Frequency, severity and timing of grazing are crucial factors in determining tiller population and consequently tiller size (Edwards & Chapman, 2011) but other environmental and endogenous factors also play important roles.

The impact of light intensity and temperature on the pattern of growth and quantity of tissue produced by plants was studied since the 1950s. Under controlled conditions Mitchell (1953a); (1953b) observed that when one or both of these factors increased, the number of days between the appearance of successive leaves decreased. The axillary buds in these new leaves could develop to visible tillers or remain dormant, depending on the quantity of light energy available; raising light intensity increased rate of tillering, but the same effect was obtained by lowering temperature, or applying these two conditions. However, Mitchell also highlighted that the effect of changes in light quantity, temperature or defoliation on bud development or inhibition is conditioned by the level of the other environmental factors and by genotype (Mitchell, 1953a, 1953b). Later work in the field by A. Davies and Thomas (1983) showed that the rate of leaf appearance increased linearly with mean soil temperature up to approximately 14°C, but the rate of production of tillers in relation to rate of leaf appearance (site filling) appeared to be independent of weather conditions (A. Davies & Thomas, 1983).

Other factors affecting tillering were added to the analysis later by other studies: water supply, mineral nutrition, photoperiod, endogenous factors such as genotype, flowering, growth regulators, and management factors such as cutting and grazing. However the common ground of the effect of light quality on site filling is present in many of these studies. For example in A. Davies and Thomas (1983) study, site filling was less complete in larger plants, indicating within-plant competition for light and the effect of shading at the base of the plant. Similar conclusions were reached by Deregibus, Sanchez, and Casal (1983): plants developed more tillers when they were illuminated by higher red/far-red ratios, without significantly modifying the photosynthetically active radiation, and concluded that branching of grasses was controlled by phytochrome activity (Deregibus et al., 1983). With increasing canopy growth, the capacity to produce new tillers and the light available per tiller decreased (Casal, Deregibus, & Sanchez, 1985). In later studies (using *Lolium multiflorum* Lam), Casal, Sanchez, and Deregibus (1987) found that adding low flux rates of red light at the base of the shoots increased tillering of plants that were exposed to low red/far-red ratios, irrespective of the ratios received by the rest of the plant. They suggest that these changes in the red/far-red ratio provide the signal that drives the plant response to competition for light (Casal et al., 1987).

Analysing the impact of grazing management on perennial ryegrass and white clover pastures, Korte, Watkin, and Harris (1984) also refer to the effect of shading at the base of the plant, when they explain greater tillering under the hard grazing treatment compared with lax grazing treatment. However, they also ascribe this higher tillering to greater assimilate availability, an argument that had been ruled out by A. Davies and Thomas (1983) as a reason for a cessation of tillering.

Simon and Lemaire (1987) studying the relationship between tillering of a vegetative grass stand and LAI, found that as soon as the LAI reached a value of 3, tillering rate slowed down, and then terminated rapidly at higher LAI. The increase in LAI and decrease of tillering is associated with an increase in the rate of leaf elongation, a phenomenon that can be interpreted as an adaptation to competition for light, where carbohydrate is preferentially allocated to elongation of leaves. However, despite genotypes with high leaf elongation rate and long laminae being associated with reduced site filling, this does not necessarily mean low tiller number per plant (Bahmani, 1999).

When analysing the effect of N, Simon and Lemaire (1987) found that this nutrient increased the number of tillers per plant at the beginning of the sward establishment, and as the LAI increased this effect disappeared. They concluded that in the absence of N deficiency the cessation of tillering was determined by the degree of self-shading of tiller buds (Simon & Lemaire, 1987). Langer (1963) however found that N affects the duration of tillering: plants inadequately supplied with N appear to stop producing new tillers at an early stage.

The need for a unifying theoretical synthesis of known effects of genetic and physiological factors and their interactions with the environment on control of tillering in grasses was recognized by Assuero and Tognetti (2010). Among the endogenous factors they cited biochemical changes, genetic control of tiller initiation and outgrowth, plant hormones (auxins, cytokinins, gibberellins), compounds such as strigolactone and ethylene, as well as assimilate availability. Among the environmental factors they cited light intensity and quality, photoperiod, temperature, water availability, and mineral nutrition. They also cited biotic factors such as mycorrhizae, endophyte and plant growth-promoting rhizobacteria as well as management factors such as grazing (Assuero & Tognetti, 2010).

### 2.2.2 Grazing and regrowth

Perennial ryegrass adaptation to grazing is facilitated by the position of its stem apex which lies close to the soil surface, and generally below grazing height, allowing the formation of leaves to continue after defoliation (Langer, 1973).

Management of grazing is a main determinant of herbage grown, and a balance between the amount of leaf area that remains in the sward after defoliation, regrowth to allow photosynthesis and the amount of leaf harvested to achieve a certain yield is needed to optimize pasture utilization (Parsons & Chapman, 2000). If the herbage is not harvested, leaves will die due to their rapid turnover. After grazing, rates of photosynthesis are reduced, respiration rate may exceed uptake of carbon, and the sward can lose mass, although new leaf tissue will be produced from reserves. This process may reduce allocation of assimilates to roots. The mobilization of reserves for regrowth after defoliation was analysed by Lee, Donaghy, Sathish, and Roche (2010) showing that stubble water-soluble carbohydrate content declined until the first new leaf had emerged, and replenishment took place during emergence of the second new leaf. These findings have been the basis of grazing recommendations for rotation lengths longer than the time to reach the 2-leaf stage of regrowth (Lee et al., 2010; Rawnsley et al., 2014). Once the canopy intercepts 95 – 100 % of the incident light, the 'optimum' leaf area index (LAI) is reached (Chapman & Lemaire, 1993; Donald, 1963) and 'gross 'photosynthesis reaches its maximum rate. After this, shade may create the conditions for a decline in gross photosynthesis. Respiration also increases during regrowth, accounting for about 25 % of gross photosynthesis (Parsons & Chapman, 2000); similarly the rate of senescence of leaf (per unit ground area) increases. Finally, the rate of senescence equals the rate of gross production (canopy gross photosynthesis minus respiration and root growth), and the rate of net accumulation of live tissue ($dW/dt$ – $W$ dry weight; $t$ time) decreases to zero.

### 2.2.3 Breeding objectives and evaluation methods

In 2011 A. Stewart and Hayes (2011, p. 32) stated:

> *The forage breeder's goal is to develop cultivars that will improve animal performance on farms.*

For this to happen, cultivars need to be productive, they have to be able to produce seed to be delivered to farmers and they also have to help minimise the impact of the production system on the environment (A. Stewart & Hayes, 2011). Traits such as total annual herbage yield, seasonal distribution of herbage yield, herbage quality, persistency as well as resistance to pests and diseases, and tolerance to freezing, drought and heat, are the main targets in perennial ryegrass breeding (Lee et al., 2012; A. Stewart & Hayes, 2011; Wilkins, 1991; Woodfield & Easton, 2004). A. V. Stewart

(2006), summarising previous findings, indicated that genetic gains for annual yield were estimated at between 0.25 and 1.5 % per year, but also pointed out that an increase in total mixture yield could be limited by the partial suppression of clover due to the increased yield of the ryegrass component.

To alter the seasonal distribution of yield, breeders have manipulated heading date of ryegrass (Lee et al., 2012; Wilkins, 1991). Earlier heading cultivars produce more feed in late winter while later cultivars provide higher quality herbage in late spring (Easton et al., 2002). Feed quality is an important factor in achieving improved animal performance.

High nutritive value means high DM digestibility and ME density, easy breakdown of forage into small particles by chewing, high non-structural carbohydrate content and high protein content (Lambert & Litherland, 2000; Wilkins, 1991). To improve herbage quality, breeders have also manipulated flowering behaviour, both timing of the main period in spring and the aftermath that occurs in summer. Retarding this process maintains quality of herbage longer during spring (Lee et al., 2012). Tetraploidy has also been used to improve quality (A. Stewart & Hayes, 2011). Doubling chromosome number by the use of colchicine (Morgan, 1976) has created cultivars with increased tiller, root and seed size, with larger cells, larger leaves, longer extended tiller height, and increased water soluble carbohydrate yield, but with lower tiller density and dry matter content (Lee et al., 2012; Neuteboom, Lantinga, & Wind, 1988; Wilkins, 1991). Breeding has also successfully increased the water soluble carbohydrate content of cultivars, creating 'high sugar grasses' that help to mitigate the effect of N on the environment and promote a more efficient use of N in the rumen (Edwards, Parsons, Rasmussen, & Bryant, 2007; Lee et al., 2012; A. Stewart & Hayes, 2011).

Meanwhile persistency of cultivars in pastures depends, amongst other factors, on the capacity to maintain a high tiller density, and the ability to tolerate various stresses (A. Stewart & Hayes, 2011). The use of new strains (AR1 and AR37) of the endophytic fungus *Epichloë festucae* var. *lolii* (formerly Neotyphodium lolii; Leuchtmann, Bacon, Schardl, White, & Tadych, 2014) has contributed to the delivery of plants able to persist better under stress conditions created by insects, and with reduced or no toxicity to grazing animals (Hume, Ryan, Cooper, & Popay, 2007; Thom, Popay, Hume, & Fletcher, 2013).

In New Zealand, pasture grass testing started in the late 1920's with the establishment of the Plant Research Station at Palmerston North (Hunt & Easton, 1989) and continued with the Department of Scientific and Industrial Research (DSIR) Grasslands Division (established in 1936). In the mid-1980s, the government reduced its participation in cultivar development, which was then taken up by private breeding companies (Hay & Lancashire, 1996). At the same time, and as a result of the same government policies (Lee et al., 2012), the compulsory cultivar testing scheme that had operated in New Zealand was abandoned, and in 1992, the Department of Scientific and Industrial Research (DSIR) was reconstituted into Crown Research institutes (Hay & Lancashire, 1996). Due to the need to deliver cultivars with proven benefits, a voluntary testing system called National Forage Variety Trial® (NFVT) was developed by the New Zealand Plant Breeding and Research Association Inc. (NZPBRA) and trials started in 1991 (New Zealand Plant Breeding and Research Association Inc.) in co-operation with AgResearch Grasslands. Perennial ryegrass evaluation trials run for three years and three months and cultivars, must have been through a minimum of three trials within region in order to be included in NFVT yield summaries approved by NZPBRA.

In 2011, an initiative between DairyNZ and the NZPBRA was established: the 2011 Forage Review group. One of the recommendations of this review, was to finalise the Forage Value Index (FVI) (DairyNZ) which ranks perennial ryegrass (and short-term ryegrass) cultivars based on their relative economic benefit to pasture-based dairy systems (Chapman et al., 2012; Chapman et al., 2016; Chapman, Edwards, et al., 2015; Chapman et al., 2014), and in 2012 the FVI was launched. This index includes the key trait of seasonal DM yield for which performance values are calculated using data from the NFVT trials. Cultivars are then ranked for their estimated profit index in the FVI. A similar index was developed in Ireland as well, including the traits: spring, midseason, and autumn grass DM yield, grass quality, first- and second-cut silage DM yield and sward persistency (McEvoy, O'Donovan, & Shalloo, 2011).

NFVT trials are usually conducted using perennial ryegrass monocultures and in general, high N fertiliser inputs (3 % of mean dry matter harvested, Easton et al., 1997; Easton et al., 2001), while the standard farm practice in New Zealand is to sow perennial ryegrass in a mixture with white clover. In 2001, Easton et al. (2001) reviewing the results of 17 trials established between 1991 and 1996 throughout New Zealand, found that although the relative mean yield of some cultivars varied across regions (Canterbury and the North Island), yields were mostly consistent and no evidence of interaction with management (pure grass or grass with clover) was detected. Nevertheless, management practices in New Zealand production systems have changed since the 1990s. An important increase in N fertiliser use during the decade 1991 – 2001 (MacLeod & Moller, 2006), the intensification of the dairy industry (Clark, 2011), the use of irrigation and the release of new perennial ryegrass and white clover cultivars have all created the conditions necessary for reconsidering the interactions between these two species in a mixed sward and the implications that these possible interactions may have on the relative ranking of perennial ryegrass cultivars based on their herbage DM yield.

## 2.3 White clover

### 2.3.1 Plant development

The initial *seedling* phase of development of the clover plant (Brock, Albrecht, Tilbrook, & Hay, 2000) ends when the embryonic shoot which grows with reserves stored in the cotyledons, unfolds the first simple leaf. Then a rosette of trifoliate leaves develops, photosynthesis increases and the seedling becomes independent of reserves. Branches (stolons) then develop in the axil of the crown leaves and grow horizontally. From the apical meristem (growing point) of each stolon a succession of leaves develop, formed by a trifoliate lamina subtended by a petiole attached to the originating stolon node. From these nodes adventitious roots may develop if the root primordia come into contact with soil moisture. Just below the leaflets is located the meristem that controls petiole extension and the final petiole length depends on the light environment within the sward (Langer, 1973; Parsons & Chapman, 2000).

Daughter stolons may develop from the single axillary bud in each node, increasing the population density. This second phase of development of the clover plant is the *taprooted phase* and lasts up to 2 years. With the death of the seminal taproot and primary stem axis starts the third stage of development, the *clonal growth phase*, when each clonal fragment depends on its own nodal root system. This is the typical growth unit of clover in permanent pastures (Brock et al., 2000; Brock & Hay, 2001). Another characteristic of this phase is the migration that results from the growth forward of the stolon and the death of the oldest portion, dispersing the clonal fragments through the sward (Parsons & Chapman, 2000).

### 2.3.2 Defoliation and regrowth

Defoliation affects both roots and aerial parts of the plants. Leaflets and part of the petiole are removed by cutting or grazing, while the terminal stolon growing points remain in general close to the ground (A. Davies, 1992). However, root function is also altered and root elongation stops; new leaves after defoliation are smaller initially, and their growth depends on carbohydrates and proteins translocated from other leaves and from reserves stored in stolons and roots. Carbohydrate levels in these organs fall after defoliation, and recover again once new leaves develop. Each set of new leaves will have longer petioles and larger laminae and the sward will continue growing to canopy closure (Frame & Newbould, 1986; Hart, 1987). During most of the year, the mean height of clover leaves is approximately 60 % of the neighbouring ryegrass plants (A. Davies, 1989), but not during winter, when clover leaves are positioned lower in the canopy (Woledge, Davidson, & Tewson, 1989).

The persistence of white clover in grazed pastures depends on stolon development and replacement (Caradus, Woodfield, et al., 1996). A high proportion of the stolon mass is buried during winter, by earthworm activity, treading and also by contraction of nodal roots (Cresswell et al., 1999). New stolons develop and establish during spring and summer, although initially the plants are smaller due to fragmentation, increasing in size towards summer reaching an equilibrium that lasts until winter (Caradus, Woodfield, et al., 1996). The establishment and longevity of new branches are increased by the presence of a root on the parental node (Pinxterhuis, 2000).

### 2.3.3 Cultivar characteristics

In New Zealand, the identification of white clover strains and ecotypes started in the 1920s and breeding efforts began in the 1930s (Caradus, Hay, & Woodfield, 1996; Woodfield & Caradus, 1994). Development and release of cultivars for different livestock classes (sheep and cattle) and management systems has occurred since the 1960s. According to the leaf size, cultivars are grouped into three main functional types: small, medium and large-leaved (Smetham, 1973). In general, small-leaved plants have prostrate growth habits, and higher stolon and growing point densities than larger leaved cultivars which have in general more erect habits and larger stolons. However, breeding has broken the traditional negative association between yield potential (linked to leaf size and upright habit) and persistence (linked to stolon growing point density) and developed larger leaved cultivars with increased stolon density (Caradus & Williams, 1989; Woodfield et al., 2003; Woodfield et al., 2001). While small leaved cultivars are more suitable for continuous sheep grazing, large leaved cultivars are more suitable for rotational cattle grazing (Caradus, Hay, et al., 1996; Woodfield & Caradus, 1994). Improvements in performance of clover due to breeding have been estimated at between 6 and 14.9 % per decade (Woodfield, 1999; Woodfield & Caradus, 1994).

### 2.3.4 Contribution to herbage DM yield, nutritive value and $N_2$ fixation

Legumes have the ability to fix $N_2$ via the symbiotic association with root nodule bacteria belonging to the genera *Rhizobium* (Whitehead, 1995). Soil N status, legume persistence and production, and competition with the associated grass are the main factors indicated by Ledgard and Steele (1992) as influencing $N_2$ fixation. In general, the amount of $N_2$ fixed follows clover yield (Caradus, 1990), and it is inhibited by increasing levels of inorganic N in the soil (Ledgard & Steele, 1992). Hoglund et al. (1979) reviewing grazing trials conducted in New Zealand, found that clover N fixation efficiency (ratio of measured N fixation to measured clover DM, kg N/t DM) varied among sites and within sites between seasons and years, and was positively correlated to soil C/N ratio but only weakly related to soil mineral N availability. Average total $N_2$ fixation in grazed grass- clover pastures in temperate regions of the world has been reported as approximately 80 – 100 kg N/ha/year (range 10 – 270 kg N/ha/year) by Ledgard (2001). Annual $N_2$ fixation in New Zealand has been indicated to be around 184 kg N/ha (ranging from 107 to 392 kg N/ha/year) by Hoglund et al. (1979), or between 82 and 291 kg N/ha/year by Ledgard et al. (1990). Other studies conducted in the country and summarized by Ledgard and Steele (1992, p. 139, Table 1) show a greater variability in the level of nitrogen fixation. Therefore the contribution of clover to the increase in the N available for plant growth has been well documented.

Yield gains due to the inclusion of clover in the sward have been reported in the literature. Ledgard et al. (1990) studying the effect of clover cultivar on herbage production and N fixation under dairy cow grazing without N fertiliser application, found that grass only plots yielded 11 and 20 % less than all mixture treatments in the first and second year of their experiment, respectively. Reid (1983), investigating the effect of different rates of N fertiliser (from 0 to 750 kg N/ha/year) on monocultures of S.23 perennial ryegrass and Blanca white clover and on the mixture of both cultivars, found that in the first year of the experiment, the mixture yielded more than the ryegrass monoculture at all N rates, and up to 500 and 250 kg N/ha in the second and third year respectively. Enriquez-Hidalgo, Gilliland, and Hennessy (2016) in a three years study involving swards of perennial ryegrass and perennial ryegrass with white clover receiving up to 240 kg N/ha/year under grazing, found that the inclusion of clover increased herbage yield by 12 – 44 %. However, no effect of the inclusion of clover on herbage production has also been reported. Egan et al. (2015) found no difference in the total herbage production of perennial ryegrass swards receiving 250 kg N/ha/year and perennial ryegrass – white clover swards receiving 150 or 250 kg N/ha/year.

Meanwhile, the improved herbage nutritive value due to the inclusion of clover in the sward has also been described in previous research. Higher N content in clover than in perennial ryegrass plants was recorded by Davidson and Robson (1986) working with simulated swards of grass and clover monoculture and mixtures, grown under low or high N levels. Although they did not find evidence of transfer of fixed N from the clover to the grass, the N percentage in grass was higher in mixture than in monoculture, indicating greater N availability for grass growth in mixed swards. As a result an increase in the herbage crude protein level is expected with an increased proportion of clover in the sward.

Reviewing the results of field experiments in New Zealand where pasture quality had been assessed as sheep liveweight gain, and expressed relative to perennial ryegrass (considered as 100), Ulyatt (1970) indicated that white clover relative liveweight gain was 186, but mentioned that this value could be higher in other areas and seasons. Ulyatt (1970) stated that pasture quality is a function of intake and nutritive value and concluded that white clover, and lucerne, were of higher quality than the grasses included in the studies. Similarly, S. L. Harris et al. (1998) in experiments to investigate the effect of diets based on perennial ryegrass and different percentages of clover (20, 50 or 80 %DM) on milk production of cows housed indoors, observed that higher clover content increased the nutritive value of the diet, and resulted in increased protein and energy intakes, and milk yields. In the first experiment, they observed that crude protein increased from 116 to 200 kg/kg DM, neutral detergent fibre decreased from 543 to 417 g/kg DM, and ME increased from 107 to 115 MJ/kg DM with an increase in clover content from 20 to 80 % DM. Diets containing white clover as 50 % DM had intermediate protein, fibre and energy contents, while pure clover diets had the highest protein, metabolisable energy and lowest neutral detergent fibre, and pure ryegrass had the opposite. Egan (2015) and Egan et al. (2015) recorded greater milk solids production from cows grazing perennial ryegrass and white clover mixtures receiving 150 or 250 kg N/ha/year compared to perennial ryegrass monoculture receiving 250 kg N/ha/year, and the largest difference occurred during the second half of the grazing season, when the clover content was at its highest and led to greater DM intake. In a grazing experiment, S. L. Harris, Clark, Auldist, Waugh, and Laboyrie (1997) also found increased milk yield from cows grazing mixtures of C4 grasses with higher clover proportion and attributed this increase to greater intakes and higher nutritive value of the clover.

Moreover, greater efficiency of utilisation of ME for growth and lower cost of ingestion when consuming clover than when consuming ryegrass have been stated as important reasons for the improved animal performance on clover compared to ryegrass in a review of previous research conducted by Nicol and Edwards (2011). They also established that the digestibility and ME content of both species is similar at young vegetative stage (Nicol & Edwards, 2011). However, maturation in clover has less detrimental effects in plant composition than in grass (Waghorn & Clark, 2004), and as a result, mixed pastures maintain higher quality than grass monoculture if the grazing is delayed during spring and summer.

## 2.4 Environmental factors affecting growth

Grass and clover respond similarly to external growth factors (Parsons & Chapman, 2000). Leaves of both species appear at similar rate in the range of 10 to 25°C under similar management, but when the sward is taller grass leaves appeared more slowly, while clover leaf appearance rate is unaffected by grazing intensity. However it takes longer for clover leaves to complete their expansion in the taller swards (Parsons, Harvey, & Woledge, 1991).

Optimum temperatures for ryegrass and white clover growth were investigated by Mitchell in the 1950s (Mitchell, 1956b) under controlled environment conditions. For ryegrass, the optimum temperature is between 18 and 21°C, while for white clover it is 24°C. These dissimilar temperatures explain the different seasonal patterns of growth which for ryegrass is higher in spring while for clover is higher in summer (Brougham, 1959; W. Harris & Hoglund, 1977). They also explain the decline observed in the growth of the legume during winter in New Zealand conditions (Mitchell, 1956b).

Increasing temperature in the range of 5 – 25°C increases the rate of leaf appearance and extension in grass, and although temperature has less effect on site filling, the number of tillers producing leaves is greater (Parsons & Chapman, 2000). In clover, the rate of leaf appearance also increases with increasing temperature, and although site filling decreases with temperatures above 10°C, the net effect is that branching increases. Soil temperature at 10 cm depth was the main climatic variable examined by Pinxterhuis (2000) that was associated with clover growth, and the linear phase of the growth curve was in the range of 7 to 21°C.

Water stress affects both grass and clover. Leaf appearance rate and tiller production are reduced in grass and the rate of leaf expansion is slowed by water stress in both species. Furthermore, white clover does not control water loss efficiently (Hart, 1987), thus under water stress conditions white clover loses fresh weight, followed by a decrease in other functions of the plant (Hart, 1987).

Solar radiation plays a fundamental role in determining herbage yield and sward composition, through photosynthesis and photomorphogenic responses of the sward components (Ballare & Casal, 2000). Although competition for light accentuates when the canopy begins to close, the red to far-red ratio (R:FR) of light is modified by the canopy before shading becomes significant, causing changes in plant morphology by altering the distribution of photoassimilates (Ballare & Casal, 2000; H. Smith, 2000). Increased axis elongation and reduced branching are some of the plant responses to reduced R:FR ratio. Acceleration of senescence of older leaves by shading is also another consequence of changes in light quality with canopy closure (Ballare & Casal, 2000; Mitchell & Calder, 1958). In clover, the increase in shading and reduction in the R:FR ratio increases petiole length and specific leaf area, decreases the proportion of nodes that produce a branch stolon, but has little effect on the photosynthetic capacity of successive leaves (Caradus & Chapman, 1991; Dennis & Woledge, 1983; Solangaarachchi & Harper, 1987; Thompson & Harper, 1988). Increased petiole length allows the young clover leaves to reach canopy areas with favourable light environment (Boller & Nosberger, 1985).

## 2.5 Nitrogen: effect on perennial ryegrass and white clover plants.

The role of N in plant development and growth is fundamental; it is a component of proteins, enzymes, nucleic acids and chlorophyll.

*Effect of N fertiliser supply on perennial ryegrass plants*

Tiller production, leaf area and root growth are affected by N supply (Whitehead, 1970). Rate of leaf extension is increased by N and as a result of larger leaves, increased area for photosynthesis is available (Hollington & Wilman, 1985; Parsons & Chapman, 2000; Pearse & Wilman, 1984; Wilman & Wright, 1983b). Pearse and Wilman (1984) observed that, in the early stages after N application in summer, net gain in green laminae length and weight per tiller doubled or trebled with the application of 22 kg N/ha or 66 kg N respectively, compared with nil N application. Previous research has shown that the number of tillers per plant increases as a result of an increment in N supply at low LAI (leaf area index) or on single plants. However, under dense sward conditions or longer interval between harvests , some of the extra tillers formed could be short-lived; a decline in relative tillering rate occurs as R:FR ratio underneath the canopy decreases and site filling falls (Hollington & Wilman, 1985; Parsons & Chapman, 2000; van Loo, Schapendonk, & Devos, 1992; Whitehead, 1970; Wilman, Koocheki, Lwoga, Droushiotis, & Shim, 1976; Wilman & Wright, 1983b). Therefore, the effect of N on tiller number of denser swards is less than on single plants (Whitehead, 1970). S. L. Harris, Thom, and Clark (1996) found increased tiller density and perennial ryegrass plant density in ryegrass/white clover swards when more N was applied, in Hamilton, New Zealand. Significant increases due to N in leaf number/plant, leaf dry weight/plant and dry weight/leaf was also found in their study. Increased tiller number due to N application was also found in New Zealand by Bahmani, Thom, Matthew, Hooper, and Lemaire (2003). However, the rate of leaf appearance appears to be little affected by N supply (S. L. Harris, Thom, et al., 1996; Robson & Deacon, 1978; Whitehead, 1970; Wilman & Wright, 1983b). Under conditions of N deficiency, root growth increases with N supply, but above a moderate level of N root weight decreases. As a consequence, in general shoot/root ratio increases with increments in N, because increments in root growth are less than increments in shoot growth (Whitehead, 1970). Root elongation and root number decrease when more N is available, but root diameter increases (Whitehead, 1970). As a consequence of greater photosynthesis per unit leaf area after defoliation and increased leaf area, the growth of N-fertilised swards is greater than that of unfertilised swards (Woledge & Pearse, 1985).

*Effect of N supply on white clover plants*

Dinitrogen fixation decreases when N fertiliser is applied to the legume, and this is associated with a reduction in the number and size of root nodules, and the partial substitution of fixed $N_2$ by mineral N uptake from the soil, which has a lower metabolic cost (Cowling, 1961; Crush, Cosgrove, & Brougham, 1982; Enriquez-Hidalgo et al., 2016; Ryle, Powell, & Gordon, 1979; Whitehead, 1995). This reduction in $N_2$ fixation however, was not reflected in a decrease of white clover monoculture yield in a study conducted by Cowling (1961). In mixed swards, increased petiole length, inhibition of branching, a reduction in the number of rooted nodes and the diameter and dry weight of stolons, and an increase in mortality of growing points occur after N fertiliser application due to competition with grass and the alteration of the light environment (Dennis & Woledge, 1987; S. L. Harris, Clark, Waugh, & Clarkson, 1996; Laidlaw & Withers, 1998; Pinxterhuis, 2000; Whitehead, 1995). However, clover leaflet size was not increased by N application in the range of 0 to 600 kg N/ha/year in Hollington and Wilman (1985) study. Similarly, no increase in clover leaf size was recorded by S. L. Harris and Clark (1996) when applying N fertiliser in the range of 0 to 200 kg N/ha/year to mixed swards. However, a certain tolerance to applied N was reported by Wilman and Asiegbu (1982b), when they observed that medium large-leaved varieties increased petiole length more than small and medium-small leaved cultivars when 224 kg N/ha were applied to a mixed sward, compared with nil N application. Moreover, the larger negative effect of applied N on stolon length in the smaller leaved varieties in their study, suggest that medium large-leaved varieties appeared more tolerant to applied N than smaller varieties (Wilman & Asiegbu, 1982b).

*Use of N fertiliser*

In the 1960's fertiliser use in New Zealand was mostly restricted to non-nitrogenous fertilisers, and the supply of this nutrient for grass growth in mixed pastures, was secured by $N_2$ fixation (Ball, 1969). However, studies in the 1970s and 1980s (O'Connor, 1982; O'Connor & Cumberland, 1973) showed that N availability was limiting pasture production and that there was response to N fertiliser application, especially in some areas of the country due to shorter growing season of the clover.

These initial studies showed a reduction in clover content due to N fertiliser use (O'Connor, 1982; O'Connor & Cumberland, 1973), an effect that has been observed in many subsequent studies when high or low levels of N fertiliser were applied (Caradus et al., 1993; A. Davies & Evans, 1990; Egan et al., 2015; Enriquez-Hidalgo et al., 2015; Frame & Boyd, 1986a, 1987a; Hennessy et al., 2012; Ledgard, 2001; Ledgard et al., 1995; Nassiri & Elgersma, 2002). However, the need to provide additional feed in periods of shortage and the increased intensification of farming systems during the 1990s, justified the inclusion of N fertiliser in the production system (Ledgard et al., 1998; O'Connor, 1982).

Variation in response to N has been reported in the literature, and is related to differences in factors such as soil temperature, N supply by the soil, season, pasture composition and N application rate. In the late 1970s Ball, Molloy, and Ross (1978) reported response efficiencies for the year of 8 – 10 kg DM/kg N, after application of 112 or 448 kg N/ha to a ryegrass – white clover pasture. A similar average response of 10 kg DM/kg N was reported by Ledgard et al. (2001) for the five years of an experiment when two rates of N fertiliser were applied (200 and 400 kg N/ha/year). Meanwhile, Ball and Field (1982) studying the effect of pasture characteristics, season and grazing management on the responses to N in New Zealand, indicated that season and weather affect growth rates and subsequently the potential demand for N by the pasture, but also the rate of supply of N from all sources. They reported efficiencies of 5 kg DM/kg N after the application of 45 kg N/ha in May or 2.8 kg DM/kg N after the application of 180 kg N/ha in the same month, and efficiencies of the same magnitude after similar N applications in June, increasing to a maximum of 32.9 kg DM/kg N after application of 45 kg N/ha in August or 19.1 kg DM/kg N after the application of 180 kg N/ha in the same month. The efficiencies reported in Ball and Field (1982) study decreased after the application of N in September. Clark and Harris (1996), also in New Zealand, showed responses to N fertiliser of 21 and 13 kg DM/kg N for 200 and 400 kg N/ha/year respectively, averaged over two years. Meanwhile, Glassey, Roach, Lee, and Clark (2013) reported an apparent N response of 16 kg DM/kg N applied to mixed ryegrass-white clover pasture in New Zealand.

## 2.6 Interactions between perennial ryegrass and white clover in a mixed sward

The use of white clover as a sustainable source of N in New Zealand dairy production has given the system a competitive advantage. Although intensification of production has added N fertiliser into the farm management practices, it is unlikely that the use of white clover will be abandoned.

The coexistence of both species in the sward is a consequence of their different responses to N (Schwinning & Parsons, 1996a, 1996b), amongst other factors. In conditions of low N availability, white clover is able to maintain high photosynthesis capacity and meristematic function (Parsons &

Chapman, 2000) while ryegrass may be at a disadvantage due to N deficiency. In contrast, under high N availability, although both species are able to increase N uptake, the grass is able to translate this extra N into morphological changes that favour competition for light and negatively affect the adjacent clover plants (Black, Laidlaw, Moot, & O'Kiely, 2009; Collins, Fothergill, Macduff, & Puzio, 2003; Laidlaw & Withers, 1998).

Increased N supply from the soil as a result of $N_2$ fixation creates the conditions for the development of an 'exploitation' interaction (W. Harris, 1990; Schwinning & Parsons, 1996a, 1996b). This type of interaction is characterised by the occurrence of cycles in which the increased N will favour grass dominance since it benefits more per unit increase in mineral N than the legume does (Schwinning & Parsons, 1996c; Thornley, Bergelson, & Parsons, 1995). The increase in grass growth eventually depletes the pool of this nutrient in the soil, promoting the development of another cycle of legume dominance. This type of interaction allows the coexistence and self-regulation of both species in the community (Schwinning & Parsons, 1996a, 1996b) reaching a dynamic equilibrium at an average proportion in the sward of approximately 70 % grass and 30 % clover in the absence of N fertiliser use (W. Harris, 1990; W. Harris & Thomas, 1973).

Most of the N fixed by the legume is translocated into the clover plant, and returns to the soil in animal excreta (mainly urine, but some in dung) or is made available to grasses by underground transfer via senescence of plant roots and litter and subsequent mineralisation (Ball, 1969; Ledgard, 1991, 2001; Ledgard & Steele, 1992; Walker et al., 1954). As a result of grazing, uneven and patchy distribution of N occurs at the field scale (Schwinning & Parsons, 1996a, 1996b). This uneven distribution plays an important role in the stability of the clover component of the pasture, because it creates different areas in the pasture that are 'out of phase' respect to grass or legume dominance (Chapman et al., 1996; Schwinning & Parsons, 1996a, 1996c).

Coexistence of both species is also facilitated by their different seasonal growth rates due to their respective optimum temperatures for growth (Brougham, 1959; W. Harris, 1990; W. Harris & Hoglund, 1977; W. Harris & Thomas, 1973; Mitchell, 1956b; Turkington & Harper, 1979a, 1979b). Moreover, the occurrence of the phenomenon described as mid-summer yield depression of ryegrass (Anslow, 1965; W. Harris, 1990), reduces competition from the grass at this time and favours clover growth. This, together with the ability of white clover to fix atmospheric $N_2$ (Ledgard, 1991) facilitates the development of systems in which both species compete for 'different space' according to the de Wit (1960) definition. Under these conditions the mixture can theoretically provide more herbage than the average of the two monocultures (W. Harris, 2001; Sackville Hamilton, 2001).

### 2.6.1 From coexistence to competition

The above mentioned coexistence of ryegrass and clover in the mixed sward, although involving competition for some resources, could be threatened by management factors that favour one of the species more than the other. Such is the case of the application of N fertiliser which increases the competitive advantage of ryegrass over clover, shifting the relationship between the two species from one of coexistence, towards more aggressive competition for light.

Therefore it is pertinent to consider what 'competition' means in this context.

Competition has been the subject of much previous research and is defined in several ways. For Grime (1974, p. 27):

> *Competition may be defined as the attempt by neighbouring plants to utilise the same units of light, water, mineral nutrients or space.*

Grime (1974) also adds *stress* and *disturbance* as the other determinants of the species composition of plant communities. Stress inhibits the development of a large standing crop by restricting primary production (usually imposed by the physical environment), while disturbance acts by damage to the vegetation (derived from the activities of grazing animals, pathogens or from human activities) (Grime, 1974). According to Grime (1973) *'competitive'* species share four features: tall stature, a growth form that allows extensive and intensive exploitation of the environment above and below ground, a high maximum relative growth rate, and a tendency to deposit a dense layer of litter on the ground surface (Grime, 1973).

For Begon, Harper, and Townsend (1986, p. 214):

> *competition is an interaction between individuals, brought about by a shared requirement for a resource in limited supply, and leading to a reduction in the survivorship, growth and/or reproduction of at least some of the competing individuals concerned.*

Tilman (1990) stated that there are two major mechanisms of plant competition, one of them is resource competition and this competition can be subdivided into competition for soil resources and competition for light. The second type of competition is interference involving allelopathic mechanisms. His theory also predicts that the species with the lowest minimum resource requirement will be the superior competitor and has been discussed in opposition to Grime's (1974) theory which predicts that the species with the greatest capacity for resource capture will be the superior competitor (Grace, 1990). Another alternative view of competition was added by Tow and Lazenby (2001) proposing that a plant will be competitively superior if it has the capacity to capture resources faster than others. A review of how the complex competitive interactions involved in the grass-legume ecosystem have influenced the results from competition experiments has been conducted by Sackville Hamilton (2001).

The already mentioned competition for light is an example of the complex dynamics occurring in mixed swards. Rhodes and Stern (1978) stated that relative abilities of grass and clover to compete for light vary with management factors such as fertiliser, harvesting or grazing treatments, and conclude that light cannot be considered as a factor in isolation.

According to Haynes (1980), height is probably the most important characteristic of plants that determines their competitive ability for light. Therefore, in competing for this resource, plants use their ability to reach the top of the pasture canopy resulting in longer leaves in grasses and leaves with longer petioles in clover (Section 2.5). As shown by Hill and Michaelsonyeates (1987) canopy height in ryegrass and clover are positively correlated, indicating an active response between the two species to intercept more light. The more-erect plagiophile leaves of the grasses suit the situation of direct sunlight above light saturation at the top of the canopy, allowing light to reach lower levels of the sward (Haynes, 1980). Meanwhile the planophile horizontal orientation of the leaflets of clover makes this species more prone to shading (W. Harris, 2001) and this is aggravated by the fact that grasses tend to be taller than clover. As a result, some authors have suggested that clover is a poorer competitor for light than ryegrass (e. g. Haynes, 1980). Adding to the competitive ability of plants for light is the area of the laminae and the angles of the laminae relative to the horizontal (Haynes, 1980). Under controlled environment conditions, Faurie, Soussana, and Sinoquet (1996) found that swards of perennial ryegrass and white clover under high levels of N supply increased their height from 30 to approximately 40 cm and that grass leaf area density ($m^2/m^3$, determined by the stratified clipping technique) was greatest in the top centimetres of the canopy, while under low levels of N supply clover had the greatest leaf area density in the upper layers. The curvature of the grass leaves, measured by the mean leaf blade angle relative to the horizontal, showed that the upper layers of tall canopies under the higher N supply were partly formed by horizontal grass leaves (Faurie et al., 1996). Through simulation using field data and results from experiments conducted under controlled environment conditions Faurie et al. (1996) found that in a mixed sward, clover captured relatively more photosynthetically active radiation (PAR) per unit area than grass under the low N supply, but not at higher N supply. In the controlled environment experiment they found that the radiation use efficiency (RUE) of clover was less than that of ryegrass, probably due to the larger amount of PAR captured. Nevertheless, a compensation between efficiency and proportion of PAR capture occurs. In mixtures under high N, although the advantage of clover in capturing PAR decreased, its RUE increased.

Studying the effect of different levels of N on Wimmera ryegrass (*Lolium rigidum* Gaud.) - subterranean clover (*Trifolium subterraneum* L.) seedling swards, Stern and Donald (1962) concluded that increased grass yield and leaf area due to greater N availability reduced the light intensity reaching the clover leaf canopy resulting in reduced growth of clover. Later work by Dennis and Woledge (1982) found that white clover leaves from plants artificially protected from shading in a mixed perennial ryegrass – white clover sward did not have significantly different photosynthetic capacities from leaves in the undisturbed sward, and successive clover leaves were longer and received full light close to the upper layers of the canopy. Davidson, Robson, and Dennis (1982) measured the photosynthetic potential of leaves grown in mixed swards with or without N application and found that when no N was applied, the upper layers of the sward were dominated by clover leaves which had high photosynthetic potential. However, after the first N application in spring, the clover component of the LAI decreased and the leaves had lower photosynthetic potential than leaves in the zero N treatment, an effect that could be also due to the lower temperatures in spring, not optimum for clover growth. Nevertheless, later leaves reached higher in the canopy, achieving high photosynthetic potential, despite receiving more N applications during summer, and therefore the authors conclude that the effect of N in decreasing clover content could not be explained by increasing shading by grass when LAI increased. Moreover, there was no significant difference in the individual lamina area and petiole length between N treatments and each stolon had a similar number of leaves. Thus, swards in the with N treatment must have contained fewer stolon growing points (Davidson et al., 1982; Dennis & Woledge, 1985). Results of a study by A. Davies and Evans (1990) were consistent with this assumption. Later work by Woledge (1988) on irrigated ryegrass – white clover swards growing with or without N fertiliser application in spring, showed that clover leaves were not overtopped by grass leaves, and that the relative growth rate of clover in the with N swards was as great as that of grass and greater than grass in the without N swards. Clover had a higher mean leaf photosynthesis rate per unit leaf area than grass, but a smaller ratio of leaf area to total above-ground dry weight than grass. Moreover, in swards receiving N fertiliser, the cost of producing longer petioles to reach the top of the taller canopy might impose a restriction on the production of lamina area or stolons. Woledge (1988) therefore concluded that clover is not a weaker competitor for light than grass, and suggested that other factors such as defoliation might be playing a role in the decrease in clover content in the long term due to N fertiliser application. The greater proportion of leaf area in the upper layers of the canopy compared to ryegrass means that when the sward is defoliated, the clover may lose a larger proportion of its leaf area than grass.

As a result of these factors, the clover content of mixed swards in most cases declines when N fertiliser is applied. Frame and Boyd (1986a) observed a reduction in mean white clover DM production over three years from 4.48 t DM/ha without the use of N fertiliser to 2.82 t DM/ha with the application of 150 kg N/ha/year. A. Davies and Evans (1990) also found higher white clover percentage in the herbage of unfertilised plots than in N fertilised plots. Similarly, in Caradus et al. (1993) study, the application of 225 kg N/ha/year to perennial ryegrass – white clover mixed swards depressed clover yield by 38 % and clover proportion in the sward by 45 %. Meanwhile, a reduction in clover content (% DM) from 43 to 12 % by the application of 150 kg DM/ha/year was reported by Nassiri and Elgersma (2002).

The timing and frequency of defoliation can play important roles in the outcome of this competition by allowing more or less light to reach lower levels of the canopy or by restricting the ability of taller plants to shade more prostrate species (W. Harris, 1990; Haynes, 1980). Therefore the more prostrate and stoloniferous species or cultivars will benefit from more frequent and intensive grazing, while species or cultivars with a more erect habit will benefit from less frequent and less intensive grazing (Haynes, 1980). In an experiment conducted to examine the effects of high N fertiliser application rates (0, 200 and 400 kg N/ha/year) and increased ryegrass production on clover growth, persistence, morphology and N fixation activity, S. L. Harris and Clark (1996) found that at a low stocking rate (3.2 cows/ha), clover content declined from 16.8 % under 0 N to 10.6 % when 200 kg N/ha/year was applied, and to 2.2 % when 400 kg N/ha/year was applied. However, at a higher stocking rate (4.5 cows/ha) the clover content was 14.9 % in the 200 kg N/ha/year treatment, close to the 15.4 % in the 0 N treatment, but it was 6.8 % in the 400 kg N/ha/year. These results indicated that N fertiliser had a smaller effect on clover content when pasture utilisation was improved, especially in spring (S. L. Harris & Clark, 1996)

Competition for other resources such as nutrients and water also takes place in the sward. Generally, grasses have longer, thinner and more finely branched roots than clover, as well as longer and more frequent root hairs. This could give the grass a competitive advantage over the clover in water and nutrients uptake (P. S. Evans, 1977; W. Harris, 1990).

## 2.7 Interactions between perennial ryegrass and white clover cultivars and effects on DM yield and white clover content

Studies attempting to improve herbage production and sward clover content by combining different perennial ryegrass and white clover cultivars have been conducted previously. These studies also sought evidence for how the association in mixed swards could affect, from a cultivar evaluation perspective, the ranking of cultivars derived from monoculture swards.

In the 1960's results of a study by Cowling and Lockyer (1965) using seven species or varieties of grass and a mixture of three of them sown in pure grass swards and receiving four N fertiliser application rates, or in association with white clover, showed that annual yield of the eight grass-clover mixtures did not differ significantly and that the grass and clover component of the mixture were inversely related. Another important conclusion of this work was that the yield of grasses when sown in mixture followed a similar order to their yield in monoculture. Similarly, Williams, Abberton, Evans, Thornley, and Rhodes (2000) observed that grass yields of different species and varieties showed similar ranking when grown in mixture with white clover and in monoculture.

Then, Connolly (1968) assessed the DM and crude protein production of six white clover varieties each sown with three perennial ryegrass varieties. No significant interaction was found between grass and clover variety. Although some of the clover cultivars grew better than others, this was not reflected in an increased yield of the mixture because in general the swards with more vigorous clover had less grass than the swards with the poorer varieties. There were differences in the ryegrass seasonal production; however, there was no difference in the total annual yields of mixtures with different varieties. The earlier New Zealand ryegrass variety had a higher clover content than S.23 and Glasnevin, but this difference was in general too small to be significant (Connolly, 1968).

Chestnutt and Lowe (1970) reviewing the results from earlier research (expressed as relative amounts of clover in association with different ryegrass cultivars) indicated that there were no marked differences between ryegrass cultivars in their compatibility with white clover.

The interest for including the ecological combining ability of grasses and legumes in the selection process was highlighted by W. Harris (1977). The results of the experiment assessing seventy different swards including grasses and legumes in mixture and monoculture under two N levels, did not reveal a situation where a grass maintained high yield and high legume content. However, the author stated that over a longer period, the beneficial combining ability may be achieved by differences in seasonality of production (W. Harris, 1977)

Meanwhile, Rhodes and Harris (1979) comparing herbage production of mixed swards of ryegrass and white clover varieties of contrasting morphology and monocultures, found that sward composition could differ as a result of the use of different clover varieties, and that defoliation management could modify the influence of variety on composition. Their results also showed that breeding for increase stature may have changed the harvest index, at the expense of stolon material; this may have improved competitive ability during establishment, but may have led to a subsequent decline in competitive ability (Rhodes & Harris, 1979; Rhodes & Mee, 1978).

A later study by Camlin (1981) assessing the competitive relationships between three white clover cultivars with different leaf size (from small to medium-large) and ten perennial ryegrass cultivars (from early to late season ryegrass) receiving N fertiliser (200 – 240 kg N/ha/year) revealed that the medium-large leaved cultivar was more aggressive towards grass, produced a greater contribution to total herbage yield and depressed the yield of some of the companion grass cultivars. However, due to substitution effects between clover and grass components, the differences in the total herbage yield were reduced during the second and third year of the study. The results also showed that the compatibility of the ryegrass cultivars with clover was inversely related to persistence. The author concluded that (Camlin, 1981, p. 169):

> *The interactions revealed in the experiment showed that both ryegrass and clover cultivars have the potential to influence each other when in association although, with minor exceptions, total annual yields were similar for all grass and clover mixtures at the moderately high level of N applied.*

Another important conclusion of this work was that total herbage yield tended to reflect the yield of the grass component, while the clover played a secondary role.

However, not all studies have revealed yield substitution; Elgersma and Schlepers (1997b) found that mixtures including the large-leaved variety Alice had a significantly higher total herbage yield and the highest clover yield in a study where two varieties of perennial ryegrass with contrasting growth habits were sown in mixtures with three white clover varieties differing in leaf size under cutting and without N fertiliser application.

Moreover, Williams, Abberton, Thornley, and Rhodes (2001) found differences in perennial ryegrass, white clover and total yield of the mixture when different clover cultivars, all of small leaf size were grown in mixed swards. They also observed that the relationship between grass and clover yield varied between a cutting and grazing management regime. A negative correlation was observed under cutting, providing evidence of competitive effects, but this correlation was not observed under grazing.

Other previous studies have focussed on the performance of white clover when grown in monoculture versus grown in mixture. Widdup and Turner (1983) assessing herbage accumulation and botanical composition of four morphologically-contrasting white clover cultivars (from small to large leaved) sown in monoculture or in association with perennial ryegrass (Grassland Ruanui or Nui) under grazing, found that the small leaved clover yielded the least in monoculture and mixture while the large leaved cultivar yielded the most, probably due to the different harvest index of the clover cultivars. Interestingly, when comparing clover yield in monoculture and mixtures, the reduction in clover yield due to the association with grass was greater for the small-leaved than for the large-leaved cultivar. Nui ryegrass was a stronger competitor than Grassland Ruanui, as demonstrated by the lower yields of all clover types when in association with the former. This fact could be the consequence of its more erect growth habit, resulting in a more open pasture where clover was more exposed to selective grazing. In general, the lowest clover yield was associated with the largest grass yield and vice-versa; as a consequence of this compensatory effect, the mixtures produced similar total herbage (Widdup & Turner, 1983).

Results from a study conducted by Ledgard et al. (1990) assessing herbage yield of swards sown in mixtures of Ellett ryegrass and five white clovers (four cultivars and the resident white clover), or in clover monoculture, showed that total annual pasture production was similar for all grass-clover swards, although some clover cultivars grew stronger in certain seasons, contributing to a greater total yield production during that season.

Many previous studies suggest that the yield of mixed swards follows the same ranking order of the yield of the dominant grass component. In studies by D. A. Davies, Fothergill, and Morgan (1993), although no re-ranking occurred (based on annual total herbage yield) when three ryegrass cultivars were sown as grass-only swards and grass/clover swards and managed under grazing, the results showed that larger differences between cultivars occurred in mixtures than in grass-only swards, due to differences in the compatibility between grass cultivars and white clover. Therefore the authors remark the need to assess varieties for this attribute under a realistic grazing management (D. A. Davies et al., 1993). In these three years studies, the grass-only plots received 200 kg N/ha per year, while the mixed sward plots received 75 kg N/ha/year only during the first year. Under these conditions of lower N supply, the clover had the opportunity to make a great contribution to the sward, although the herbage production of the mixed sward (average of the three years) was only 65% of that of grass only swards.

### 2.7.1 Reasons behind an improved combining ability

The influence of coexistence of the components of a grass-clover mixture and its implications for herbage yield and clover content was studied by D. R. Evans, Hill, Williams, and Rhodes (1985). After these studies, Collins and Rhodes (1989) conducted an experiment to examine the nature and agronomic significance of variation in compatibility in perennial ryegrass-white clover mixtures, to define selection criteria for breeding programmes. The results showed substantial differences in clover yields in different mixtures and changes in the yield ranking of clover according to the companion grass. The authors suggested that variation in spatial arrangement of plant parts (spatial compatibility), and in seasonal growth patterns were behind the differences in grass-clover compatibility. In Collins and Rhodes (1989) study, mixtures with an early flowering ryegrass often had greatest clover yield, a result attributed to a decline in the competitive ability of the grasses at the start of flowering as reported by Rhodes (1970) which gave the clover a competitive advantage early in the growing season (Collins & Rhodes, 1989). Meanwhile, the effect of grass morphology, one of the determinants of spatial compatibility, had been reported by Rhodes and Ngah (1983) indicating that erect grasses allow better clover growth than prostrate or lax leaved grasses. This argument however, contradicts the explanation offered by Widdup and Turner (1983) for their results with the cultivars Grassland Ruanui and Nui under grazing conditions.

With the objective of examining the effect of ryegrass cultivar morphology (ploidy and heading date) and seed rate on herbage production of grass-clover mixtures with and without N application, Frame and Boyd (1986a) compared two intermediate-heading cultivars (one diploid and one tetraploid) and two late cultivars (one diploid and one tetraploid) sown with white clover. The authors concluded that (Frame & Boyd, 1986a, p. 359):

> *Modern highly-productive perennial ryegrass varieties do not differ substantially in compatibility with white clover but tetraploids permit better clover performance than diploids.*

A later study by D. A. Davies and Fothergill (1990) examining the contribution of a small-leaved white clover cultivar when sown in association with three contrasting perennial ryegrass cultivars (one very early-flowering diploid, one late-flowering tetraploid and one late-flowering diploid), showed large differences in the growth and persistence of white clover, which yielded least when grown with the late-flowering diploid.

The influence of different grass morphology on clover survival under grazing was assessed by Gilliland (1996) in a study using four white clover varieties differing in leaf size grown in binary mixtures with 33 perennial ryegrass varieties of differing maturity, ploidy, yield potential and morphological characteristics and receiving N fertiliser. They concluded that (Gilliland, 1996, p. 65):

> *Tetraploid varieties were significantly more compatible with white clover than diploids, with the early and intermediate tetraploids being the least aggressive towards clover.*

They also added:

> *Assessment of grass variety production and morphological characteristics revealed that sward density was the overriding factor determining grass/clover compatibility. Further examination by principal component analysis revealed that among the grass varieties, a growth pattern of higher spring and lower summer yield potential was an additional factor contributing to high clover compatibility in this study.*

However, the role of tiller density and ploidy in determining sward clover content is not clear. Elgersma and Schlepers (1997a) observed slightly more clover in a mixed sward including an erect diploid than in swards including a tetraploid ryegrass, although both of these swards contained greater clover content than in mixtures with a prostrate diploid ryegrass. Therefore the authors suggest that factors other than ryegrass tiller density affect clover content in mixed swards.

Assessing the performance of one hundred and fifty eight cultivars and breeding lines of white clover in mixed swards, to determine which leaf and stolon characteristics were the best predictors of clover content in mixed swards under rotational grazing, Caradus and Mackay (1991) found that leaf number and leaf size rather than stolon growing point density were the best predictors of proportion of clover in the sward.

Root morphology has also been considered in the analysis of competition (Collins, Fothergill, MacDuff, & Rhodes, 1997; Collins et al., 2003; Collins, Fothergill, & Rhodes, 1996); Collins et al. (1997) found that in a mixed sward under low N condition the root length distribution differed amongst ryegrass cultivars, resulting in grass roots placed more in direct contact (and competition) with clover roots for some cultivars, more than others.

# Chapter 3

# Interactions between perennial ryegrass and white clover and their effects on herbage production, quality and botanical composition

*Some results from the first year of this experiment were published in the Proceedings of the 5th. Australasian Dairy Science Symposium, 19-21 November 2014, Hamilton, New Zealand 259-262.*

## 3.1 Introduction

Perennial ryegrass (*Lolium perenne* L.) and white clover (*Trifolium repens* L.) are the basis of New Zealand dairy production systems. The benefits of this association have been largely recognized (S. L. Harris et al., 1997). However, management and environmental factors as well as intrinsic characteristics of the relationship between these two species have limited the contribution of white clover, which content rarely exceeds 20 % of the sward (DM, on an annual basis) (Chapman et al., 1996; S. L. Harris, 1998; Tozer et al., 2014). Therefore, the role of perennial ryegrass as the dominant component of the pasture is crucial to the sustainability and profitability of the system.

In New Zealand, most of the herbage seed produced is from grass, and perennial ryegrass is the largest component (Pyke et al., 2004), being the species that has the priority in the seed companies' research and development investment (DairyNZ & New Zealand Plant Breeding and Research Association, 2012). Improvements in production traits such as yield, quality and persistence (A. Stewart & Hayes, 2011) have been some of the objectives of the breeding companies; but they have also been a priority for the dairy industry, which could benefit from these improvements. Therefore, in 2011, an initiative between DairyNZ and the New Zealand Plant Breeding and Research Association (NZPBRA) was established: the 2011 Forage Review group. Amongst the recommendations of this review (DairyNZ & New Zealand Plant Breeding and Research Association, 2012), was to finalise the Forage Value Index (FVI; DairyNZ) which ranks perennial ryegrass (and short-term ryegrass) cultivars based on their relative economic benefit to pasture-based dairy systems (Chapman et al., 2012; Chapman et al., 2016; Chapman, Edwards, et al., 2015; Chapman et al., 2014), and in 2012 the FVI was launched. This index includes the key trait of seasonal DM yield for which performance values are calculated using data from cultivar evaluation trials conducted by the NZPBRA, the National Forage Variety Trial (NFVT). Cultivars are then ranked for their estimated profit index in the FVI.

However, the NFVT trials are conducted using perennial ryegrass monocultures and in general, high N fertiliser inputs (3 % of mean DM harvested, Easton et al., 1997; Easton et al., 2001), while the standard practice in New Zealand is sowing perennial ryegrass in a mixture with white clover. Knowing that these two species have the potential to influence each other when in association (Camlin, 1981), and that their relationship and proportions in the sward are influenced by environmental and management factors (W. Harris, 1990; Schwinning & Parsons, 1996a, 1996b), it was important to examine this issue and to determine if the FVI system needed to take interactions with white clover into account. Further, it was relevant to determine how other inputs, such as N fertilizer, affect the interaction. Therefore, experiments with a common design were established in four regions of New Zealand (Species Interaction trials) in 2012. Results of the first two years of the Canterbury experiment are presented in this thesis.

Based on previous research (Camlin, 1981), the working hypothesis was that the relative ranking of the perennial ryegrass cultivars in terms of their comparative total DM yields would not change when sown with white clover under the high and low N fertiliser application rates used in this experiment.

## 3.2 Objectives

The objectives of this study were:

- To compare the total dry DM yield (kg DM/ha) of swards based on different perennial ryegrass cultivars sown with and without white clover and receiving either low or high rates of N fertilizer application and to determine if they re-ranked in terms of their comparative total DM yields when sown in mixed ryegrass/white clover swards compared to ryegrass monocultures.

- If the rankings did differ, to identify which factors were responsible for the re-ranking (ploidy, heading date).

- To analyse the role of ryegrass phenotype characteristics in determining the botanical composition of swards (white clover content expressed as % DM).

- To compare the ME density (MJ/kg DM) of swards based on perennial ryegrass cultivars sown with and without white clover and receiving either low or high rates of N fertiliser application and to determine if they re-ranked in terms of their comparative ME density when sown in mixed ryegrass/white clover swards compared to ryegrass monocultures.

## 3.3 Materials and Methods

### 3.3.1 Site description

The experiment was conducted from the 27th March 2012 (treatment establishment) to the 31st May 2014 (cessation of measurements) at the Lincoln University Research Dairy Farm (LURDF), Lincoln, Canterbury, New Zealand (latitude 43°38’10.26”S; longitude 172°27’42.91”E; altitude 12 m a.s.l.).

The soils at the site are Wakanui silt loam and Wakanui silt loam on sandy loam. They are mottled immature pallic soils according to the New Zealand soil classification (Hewitt, 2010) and Aquic

Haplustept fine silty, mixed, mesic soils according to USDA classification (Soil survey staff, 1998); both soil types are imperfectly drained. Wakanui silt loam was the predominant soil in two of the five replicates of the experiment, and is described as having low water logging vulnerability, medium bypass flow and low N leaching vulnerability (Landcare Research, 2015). Wakanui silt loam on sandy loam was the predominant soil on the other three replicates and is described as having medium water logging vulnerability, high bypass flow and medium N leaching vulnerability (Landcare Research, 2015).

The area used for the experiment (1.15 ha total) had been part of an organic cropping farm until autumn 2011 when it was sown in a perennial ryegrass and white clover mixture that remained for one year.

### 3.3.2 Meteorological conditions

Historical data from the Broadfield meteorological station located 1 km north of the site show a mean annual rainfall of 599 mm and a mean air temperature of 11.7°C for the period 1981 to 2010 (National Institute of Water and Atmospheric Research, 2015). Total rainfall for the experimental period was 72 and 284 mm higher than the historical mean for 2012 – 13 and 2013 – 14 respectively with the extra rain falling mainly during winter and autumn (Figure 3.1 and Table A.1 in Appendix A). Mean temperature for both seasons was 0.2 and 0.5 higher than the historical mean for the respective years.

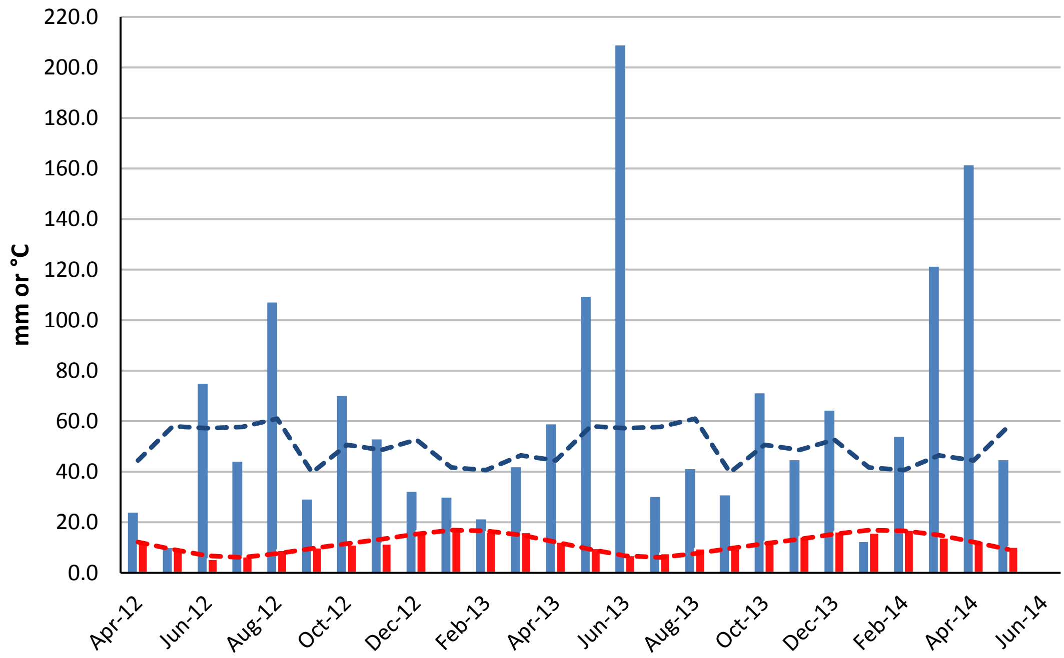


**Figure 3.1 Monthly total rainfall (mm) and mean air temperature (°C) during the seasons 2012 – 13 and 2013 – 14 and historical data (1981 to 2010). Monthly total rainfall (blue bar), mean air temperature (red bar), mean monthly rainfall historical data (dashed blue line), mean monthly temperature historical data (dashed red line).**

Total Penman potential evapo-transpiration (mm) (National Institute of Water and Atmospheric Research, 2015) during spring and summer exceeded total rainfall and irrigation, creating an accumulated soil water deficit of 274 mm and 218 mm between September and February in 2012 – 13 and 2013 – 14 respectively (Figure 3.2).

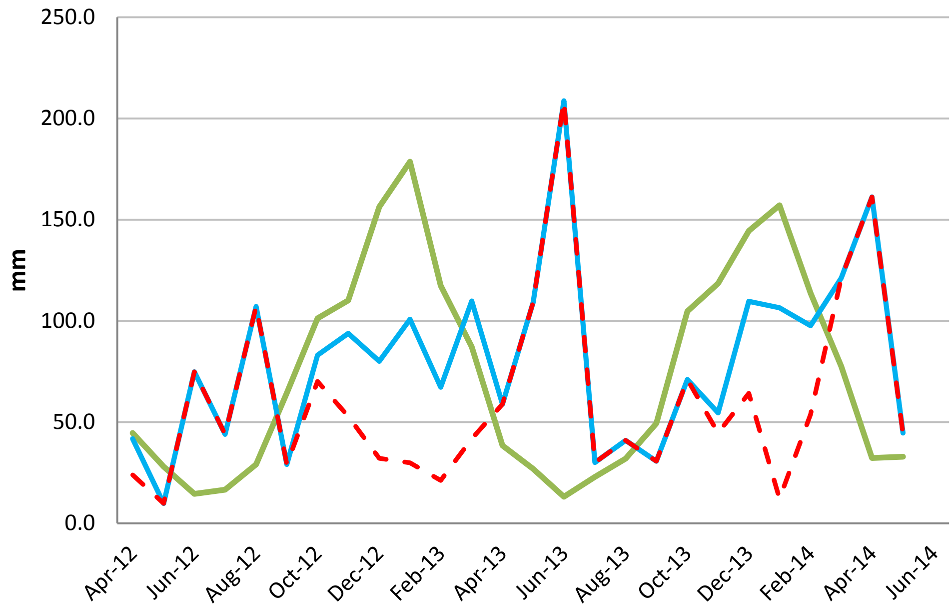


**Figure 3.2 Total rainfall, rainfall plus irrigation and total Penman potential evapo-transpiration during the period April 2012 – May 2014 (mm). Total rainfall (dashed red line), Total rainfall + irrigation (solid blue line) and Total Penman potential evapo-transpiration (solid green line).**

### 3.3.3 Trial design and treatments

The experiment used a split plot design with eight subplots randomly allocated within four main plots each replicated in five blocks. Main plots (518 $m^2$) comprised all combinations of pastures sown with ("plus") or without ("minus") white clover receiving either "low" or "high" rates of N fertiliser, randomised within blocks. Subplots (65 $m^2$) comprised eight perennial ryegrass cultivars. Each subplot was 18 m long by 3.6 m wide. Each main plot was 18 m long by 28.8 m wide and was fenced to allow control of the frequency and intensity of grazing by dairy cows (Figure A.1 in the Appendix A shows the layout of the experiment).

***Main plots***

The rates of N fertiliser applied annually were either low (100 kg N/ha) or high (325 kg N/ha). The high N level is above the average of the N applied in the Canterbury region during the farming season 2011 – 12 (229 kg N/ha/year, DairyBase® personal communication, January 2016) while the low N is below this average, and low enough to create a large difference between N treatments. In the plus clover treatments, pastures were sown with a 50:50 mixture of Kopu II and Tribute, large and medium-large leaved clover cultivars respectively, commonly used in dairy pastures, while the minus clover treatment was sown as a grass monoculture.

***Subplots***

The eight perennial ryegrass cultivars (Table 3.1) were selected to provide contrasting phenotypes for two traits that may influence competition between grass and clover: morphology, and heading date. The morphological contrast was between high tiller density/fine leaf material (‘dense’, cultivars Prospect AR37 and Abermagic AR1, both diploids) and low tiller density/broad leaf material (‘open’, cultivars Base AR37 and Bealey NEA2/6, both tetraploids). The heading date contrast was between mid-season (cultivars Commando AR37 and Kamo AR37) and late-season (cultivars One50 AR37 and Alto AR37) heading date materials, all of them diploids. (Note, there were two cultivars per contrast level).

**Table 3.1 Phenotypic contrasts, and details of the perennial ryegrass cultivars' characteristics**

| Phenotypic contrast | Cultivar | Endophyte[1] | Ploidy | Heading date | Tiller habit (Dense or open) | Leaf habit (width x length) |
|---|---|---|---|---|---|---|
| Dense/fine | Abermagic AR1 | AR1 | Diploid | Late (+19) | Dense | Narrow x Short |
| Dense/fine | Prospect AR37 | AR37 | Diploid | Late (+12) | Dense | Medium to wide x Medium to long |
| Open/broad | Base AR37 | AR37 | Tetraploid | Very late (+22) | Open | Medium to wide x Medium |
| Open/broad | Bealey NEA2/6 | NEA2/6 | Tetraploid | Very late (+25) | Open | Medium x Medium to long |
| Mid | Commando AR37 | AR37 | Diploid | Mid (+1) | Dense | Medium to broad x Medium to long |
| Mid | Kamo AR37 | AR37 | Diploid | Mid (0) | Dense | Medium x Medium |
| Late | Alto AR37 | AR37 | Diploid | Late (+14) | Dense | Medium x Medium |
| Late | One50 AR37 | AR37 | Diploid | Late (+20) | Dense | Medium to broad x Medium to long |

Note to Table: Heading date - time when 50 % of plants have emerged seedhead in a typical year and it is defined relative to cultivar Nui (heading at date zero, 22 October each year). Maturity groups used for classification (after Lee et al., 2012) were: mid-season maturing (day 0 to +6), late-season maturing (day +7 to +21), very late-season maturing (day +22 to +25). Information about ploidy, leaf width and length is based on the Objective Description of Variety or from the trials in which the cultivar has been used as comparator (Kamo) (Plant Variety Rights Office of New Zealand, personal communication, July 2013, April 2015). Heading dates in this Table are based on commercial information (PGG Wrightson Seeds, 2015).

[1] *Epichloë festucae* var. *lolii* ; formerly *Neotyphodium lolii* (Leuchtmann et al., 2014).

### 3.3.4 Site preparation and baseline measurements

On 24th February 2012 soil preparation for establishment of the trial started with the spraying of Roundup® 360 (360 g/litre glyphosate) at 3 litres/ha using the penetrant Accelerate™ (polyether modified polysiloxane 75 %) at 100 ml/100 litres of water. The paddock was then ploughed (ploughshare) on 6th March, power harrowed and rolled on 13th March, dutch harrowed and rolled on 14th March and heavy rolled on 26th March, one day before sowing of the trial.

Soil nutrient status was assessed pre and post-cultivation (5th and 16th March 2012 respectively). Forty soil cores (2.5 cm diameter to 7.5 cm depth) were collected from each replicate along a diagonal. Samples were bulked, then dried at 25°C for five days prior to analysis. Before cultivation soil nutrient concentrations were within or above the range to sustain near maximum pasture production (Roberts & Morton, 2009) or surpassing the critical level to achieve pasture concentrations to ensure animal health (Edmeades & O'Connor, 2003; Edmeades & Perrott, 2004) (Table 3.2). However, after cultivation, the levels of phosphorous (P) and potassium (K) dropped below the biological optimum. Soil organic matter content (Organic carbon × 1.724) was assessed six months after the sowing of the trial using the same sampling regime described above. The mean organic matter content was 3.9 %.

**Table 3.2 Mean soil pH and nutrient status prior to (5th March 2012) and after cultivation (16th March 2012) except for soil organic matter which was sampled on 2nd October 2012.**

| | Soil properties | | Target soil |
|---|---|---|---|
| | Pre-cultivation | Post cultivation | test |
| pH [1] | 6.1 | 5.8 | 5.8 – 6 [5] |
| Ca - Calcium MAF QT [1] | 10.2 | 11.0 | > 1.5 [6] |
| P - Olsen Phosphate µg/mL [2] | 24.8 | 13.3 | 20 - 30 [5] |
| K - Potassium MAF QT [1] | 7.3 | 3.9 | 5 – 8 [5] |
| $S(SO_4)$ - Sulphate Sulphur ppm [3] | 20.0 | 21.3 | 10 – 12 [5] |
| Mg - Magnesium MAF QT [1] | 13.7 | 14.7 | 8 – 10 [5] |
| Na - Sodium MAF QT [1] | 8.7 | 7.9 | > 5 [7] |
| Organic matter (%) [4] | – | 3.9 | – |

[1] (Blakemore, Searle, & Daly, 1987; Cornforth, 1980) [2] (Ammerman, 2003; Cornforth, 1980) [3] (Watkinson & Perrott, 1990) [4] (Rayment & Lyons, 2011) [5] (Roberts & Morton, 2009) [6] (Edmeades & Perrott, 2004); [7](Edmeades & O'Connor, 2003).

The number of germinable buried seeds was estimated from soil samples collected on 16th March following the same procedure used for soil fertility sampling, and using the same soil corer. Approximately 2.2 kg of soil was collected from each replicate. The soil was thoroughly mixed, then spread out in the glasshouse and watered at regular intervals to stimulate seed germination. Seedlings were counted and identified by species on two occasions: 17th April and 4th May. On average a total of 50 seeds germinated in each sample (2547 seeds/m$^2$); 44.8 % (1141 seeds/m$^2$) of these were from weed grasses (mostly *Poa trivialis* L. and *Poa annua* L.), 38.8 % (988 seeds/m$^2$) were broadleaf weeds (mostly *Capsella bursa-pastoris* L. and *Stellaria media* L.) and 16.4 % (418 seeds/m$^2$) were legumes (mostly *Trifolium repens* L.). No ryegrass seeds germinated during the time of the test.

### 3.3.5 Pasture establishment

Perennial ryegrass cultivars were sown on 27$^{th}$ March using a cone seeder, at a row spacing of 15 cm and depth of approximately 1 cm. White clover was sown by hand (onto the soil surface) on 28th March, and the entire area was rolled by a Cambridge roller on 30$^{th}$ March.

All perennial ryegrass seed was treated with Poncho®, systemic insecticide that protects seedlings from Argentine stem weevil, black beetle and grass grub larvae for up to six weeks post-emergence. The white clover seed was coated with Superstrike®, coating containing Rhizobia, molybdenum, lime, a nematicide and Poncho®.

Sowing rates for perennial ryegrass were equivalent to 20 kg/ha for the diploid cultivars and 28 kg/ha for the tetraploids due to their greater seed weight, giving an average of 940 viable seeds/m$^2$. White clover sown was a 50:50 mix of the cultivars Tribute (medium-large leaved) and Kopu II (large leaved), at a total rate equivalent to 4 kg/ha of bare seed (2 kg of each cultivar).

The purity, germination and endophyte status of the seed lines used, as assessed by grow-out tests, are shown in Table 3.3.

**Table 3.3 Seed analyses**

| Cultivar | Purity (%) | Germination (%) | Endophyte infection frequency (%) |
|---|---|---|---|
| Kamo AR37 | 99.7 | 95 | 84 |
| Commando AR37 | 99.9 | 98 | 81 |
| Prospect AR37 | 99.6 | 95 | 84 |
| One50 AR37 | 98.3 | 85 | 90 |
| Base AR37 | 99.8 | 93 | 93 |
| Bealey NEA2/6 | 99.6 | 91 | 86 |
| Abermagic AR1 | 99.9 | 94 | 90 |
| Alto AR37 | 99.5 | 96 | 71 |
| | | | |
| Kopu II | 99.9 | 99 | Not applicable |
| Tribute | 99.9 | 97 | Not applicable |

Considering the thousand-seed weight of the different species (approximately 2 g for the diploid cultivars, 3 g for the tetraploid cultivars and 0.6 g for the uncoated white clover), the sowing rates and the germination % (Table 3.3), the total number of viable seeds sown/m$^2$ was between 840 (Prospect AR37) and 980 (Commando AR37) for perennial ryegrass, and 650 for the mixture of the white clovers. All seed lines established successfully, however a snow fall event on 6$^{th}$ June 2012 followed by freezing temperatures caused frost-heave damage to the surface-sown white clover. Therefore, the same white clove mixture was re-sown by hand at a rate equivalent to 6 kg/ha of bare seed on 7th September to compensate for plant losses and ensure a strong white clover presence in the resultant pastures.

Endure® (50 gr/kg metaldehyde) slug bait was applied (5.5 kg/ha) on the 4th April to prevent slug damage to the plants. On the 6th april the paddock was irrigated with 18 mm of water using a lateral irrigator to assist seedling establishment.

### 3.3.6 Grazing and management

#### Grazing

Each main plot was grazed by dairy cows following standard farm management practices when the herbage mass was between 2500 and 3300 kg DM/ha (between the 2 and 3 leaf stage of regrowth from spring to autumn, although occasionally grazing occurred before the 2 leaf stage to avoid canopy closure). The target post-grazing residual was 4 – 5 cm sward height (approximately 1500 – 1750 kg DM/ha). The number of cows required to graze each main plot was calculated based on the available herbage between the pre-grazing mass and the target residual, and the expected animal intake. During 2012 – 13, nine grazing events occurred in all treatments between the end of August 2012 and the end of May 2013. During 2013 – 14 ten grazing events occurred in the high N treatments (with and without white clover), and nine in the low N plus white clover treatment, between August 2013 and May 2014. The low N minus clover treatment was grazed eight times between September 2013 and May 2014. Typically each main plot was grazed by 10 - 11 cows, during half day (12 – hour grazing system).

#### Mowing

The high N treatments were mown once during spring 2013 (26 November) and the low N treatments once during summer 2013 – 14 (6 December the low N plus clover treatments and 13 December the low N minus clover treatments), to reduce heterogeneity in the swards resulting from atypical (compared to commercial scale grazing systems) patterns of dung and urine return and consequent rejection of spoiled areas by grazing cows. No mowing was implemented during the first year of the experiment.

#### Herbicide application

In September 2012 Preside™ herbicide (active ingredient 800 g/kg flumetsulam) was applied to remove broadleaf weed seedlings. The dose used was 50 g/ha Preside™ plus Uptake™ (582 g/L paraffinic oil and 240 g/L alkoxylated alcohol non-ionic surfactants) spraying oil applied at 1 L/ha, in 230 L/ha of water. In March 2013, the minus clover treatments were sprayed with Banvel® 200 (active constituent 200 g/L dicamba) to remove clover; the dose used was 2 L/ha. At the same time, the plus clover treatments were sprayed with Preside™ at 61.7 g/ha plus Uptake™ spraying oil applied at 1 L/ha, in 230 L/ha of water, to achieve similar broadleaf weed control. The same herbicide treatments applied in March were applied in December 2013 again.

### Nitrogen fertiliser

All N fertiliser was applied as urea (46% N). In the low N treatments, 25 kg N/ha was applied in September, December, March and May during both years. In the high N treatments during the first year (2012 – 13), N was applied at a rate of 32.5 kg N/ha after each of the 9 grazing events and again at the end of May, while during the second year (2013 – 14) N was applied after each of the 10 grazing events at the same rate. Fertiliser was applied to each individual subplot using a hand-held broadcast spreader.

### Maintenance fertilizer

Rates of maintenance fertilizer applied were determined annually from soil fertility test results. Based on the results of the sampling conducted post-cultivation in March 2012 (Table 3.2), 10 % Potash Super (0-8.1-5 + 18 Ca + 9.9 S) was applied to the entire area in June 2012 at a rate of 400 kg/ha. Following a soil test conducted in October 2012, the same amount of 10 % Potash Super was applied again in December 2012. Based on the results of soil sampling conducted during August 2013, Sulphur Super 15 (0-8.6-0 + 19.2 Ca + 14.8 S) was applied at a rate of 1 t/ha in spring 2013. In March 2014, 50 % Potash Super (0-4.5-25 + 10 Ca + 5.5 S) was applied at a rate of 500 kg/ha to the entire area. Due to lower K levels in one replicate, these plots also received Potassium chloride (0-0-50) at a rate of 500 kg/ha also in March 2014.

### Irrigation

The experiment was irrigated according to the schedule organized for the farm by the LURDF management team. During the first year when a lateral move irrigator was used, 287 mm of water were applied in the period October 2012 to March 2013. During the second year the farm was irrigated with a centre pivot irrigator and 194 mm of water were applied in the period November 2013 to February 2014 (Table A.1 in Appendix A).

## 3.3.7 Measurements

### a) Herbage mass and DM yield: direct cutting

Total herbage mass was estimated in each subplot before every grazing (except for the first grazing in August 2012) by cutting a 9 $m^2$ strip (6 m long × 1.5 m width) to 5.5 cm above ground level, using a Haldrup forage harvester (Haldrup F-55, Denmark) (Woodward, Waugh, Roach, Fynn, & Phillips, 2013). To avoid harvesting the same strip area in consecutive grazings, the position of this strip was rotated within each subplot. The fresh weight of the cut herbage was recorded and a subsample was collected to determine DM content (DM %). This subsample was weighed, then oven-dried for not less than 72 hours at 60 – 65°C, and weighed after drying. DM yield (kg DM/ha) was calculated from the fresh weight of the harvested herbage and the DM %. Data for individual harvests were allocated to seasons as follows: winter (June to August), spring (September to November), summer (December to February), and autumn (March to May).

### b) Herbage mass and DM yield: rising plate meter (RPM)

Herbage mass was estimated using a rising plate meter (Jenquip, Feilding, New Zealand) before and after every grazing (L'Huillier & Thomson, 1988; Litherland et al., 2008). The procedure involved walking in a "W" pattern across each subplot taking 40 readings to estimate pasture height (measured in units of 0.5 cm of compressed pasture height) one day before and after each grazing. The general calibration (Equation 1), was used to provide an estimate of herbage mass. When mowing was needed during the season 2013 – 14, plating post- grazing was conducted after the mowing was completed.

**Equation 1**

Herbage mass (kg DM/ha) = RPM units × 140 + 500

### c) Botanical composition and pasture nutritive value

Botanical and pasture nutritive value (NV) sampling was conducted pre-grazing in spring (November 2012, October 2013) summer (January 2013, January 2014) and autumn (April/May 2013, April/May 2014) each year. The dried material from the subsample that was used for DM % determination in the herbage cutting method (3.3.7 a) was ground through a 1 mm sieve (ZM200 rotor mill, Retsch GmbH, Hann, Germany), then analysed for organic matter digestibility (OMD), crude protein (CP), lipid, ash, lignin, acid detergent fibre (ADF), neutral detergent fibre (NDF), soluble sugars & starch (SSS) and metabolisable energy (ME) content, using near infrared spectroscopy (NIRS) (Corson, Waghorn, Ulyatt, & Lee, 1999). An additional subsample taken from the harvested material (3.3.7 a) was used for botanical composition determination. This subsample, of approximately 15 g was dissected into: live perennial ryegrass, live white clover, live other species and dead material of all species. Fresh material of each fraction was oven dried for not less than 72 hours at 60 – 65°C before weighing, to determine the percentage contribution of each component to the total DM of the sample.

### d) Perennial ryegrass and white clover population density

The perennial ryegrass and white clover population density was measured in autumn each year (May 2013 and May 2014). For this purpose a 5 cm × 20 cm (100 $cm^2$) frame was randomly positioned at five locations in each subplot and the number of perennial ryegrass tillers (Lee et al., 2016) and white clover growing points within each frame was counted. Care was taken to avoid locating frames in areas where drill rows overlapped or in areas affected by urine burn or dung, and in areas within 3 m of the ends of each subplot.

## e) Endophyte infection frequency

Endophyte frequency in perennial ryegrass populations was assessed using samples collected in the field in autumn each year. Sampling was restricted to three blocks and only to the Low N plus white clover treatment, since it is unlikely that the main treatments would affect endophyte status, and this treatment combination represented typical farm management better than the other three treatment combinations. Fifty vegetative ryegrass tillers were randomly selected in each subplot (taking care that all tillers come from different plants), and removed at or slightly below the soil surface with some roots attached. Tillers were placed on ice, and later stored in a refrigerator until ready to blot. In the lab, all dead material, decaying outer leaves and any soil particles were removed, and a scalpel was used to cut the tiller approximately 2 mm above the base, close to the growing point where the endophyte hyphae concentration is greatest. Sap from each tiller was absorbed on blotting paper in a pre-determined grid pattern before being analysed for endophyte presence using the method described by W. R. Simpson, Schmid, Singh, Faville, and Johnson (2012). The percentage of tillers infected with endophyte was calculated for each subplot.

## f) Reproductive development

During October, November and December 2013, plant development stage was assessed for all ryegrass cultivars, in three blocks of the High and Low N minus clover treatments. Sampling dates were 15$^{th}$ October (22 days after grazing for the High N treatment and 33 days after grazing for the Low N treatment) and 5$^{th}$ November (13 days after grazing for the High N treatment and 19 days after grazing for the Low N treatment) for both N treatments, 9$^{th}$ December for the Low N treatment (31 days after grazing) and 17$^{th}$ December for the High N treatment (24 days after grazing). The procedure involved collecting 300 – 400 tillers by cutting to ground level, and scoring plant development stage on a subsample of 30 randomly selected tillers according to the Moore et al. (1991) indices for the elongation – stem elongation (E0 to E4) and reproductive – floral development (R0 to R4) stages. Mean Stage Count (MSC) was calculated based on the formula presented by Moore et al. (1991) adjusted as per Equation 2 to simplify the comparison between cultivars (Wims, Lee, Rossi, & Chapman, 2014a). The maximum possible value for the adjusted MSC is 200.

**Equation 2**

$$\text{Adjusted MSC} = (\text{MSC}-2) \times 100$$

Tillers in each development stage were counted, bulked, oven dried and weighed to calculate their contribution to the total tiller sample, as a % of total tiller number and % of the total tiller dry weight.

## g) Light interception, canopy height, botanical composition and DM production during regrowth

In the second year of the trial, during one regrowth period in spring and in summer, photosynthetically active radiation (PAR, 400-700 nm) above and below canopy was measured for 3 of the perennial ryegrass cultivars (Bealey NEA2/6, Kamo AR37 and Prospect AR37), to calculate the proportion of light intercepted by the canopy. At two randomly selected positions inside the area that had been cut with the Haldrup harvester in the previous grazing, light measurements were conducted using a 0.8 m long AccuPAR ceptometer model LP-80 (Decagon Devices, Inc.). Measurements were conducted between 10:00 and 14:30 (daylight saving), under clear sky; if the conditions changed to cloudy during the measurements, readings were stopped and resumed once the sky was clear again. In each position, PAR above (average of three readings) and under the canopy (from each side of the harvested area which was 1.5 m wide) was measured. The percentage of PAR intercepted by the pasture, PAR interceptance (Russell, Jarvis, & Monteith, 1989) in each position was calculated by difference using the measurements taken above and below the canopy. The mean for the two measurement areas was calculated to represent the light intercepted by the canopy of the subplot. After light measurements were completed, perennial ryegrass and white clover height was measured in the same two positions, using an automated sward stick (Jenquip, Feilding, New Zealand), similar to the method described by Bluett and Macdonald (2002). The procedure involved measuring the height of the undisturbed perennial ryegrass leaves at ten randomly selected points inside the 1.5 m strip where light measurements were conducted, and then the same procedure was applied for white clover. Mean for the heights recorded in the two positions was calculated for each subplot. Finally, hand shears were used to cut all herbage in the 1.5 m long strip to ground level in the same two areas where light and canopy height measurements were conducted. All harvested herbage was dissected into live perennial ryegrass, live white clover and other material (including dead matter of all species). These samples were oven dried for not less than 48 hours at 60°C to calculate kg DM/ha and botanical composition (% DM). The mean for the two measurement areas was calculated to represent the subplot.

Sampling dates in spring were: for the High N plus or minus clover treatments 20$^{th}$ November 2013 (27 days after grazing), for the Low N plus clover treatment 29$^{th}$ November (30 days after grazing) and for the Low N minus clover treatment 7$^{th}$ December (29 days after grazing). In summer, and following the same order, sampling dates were: 4$^{th}$ February 2014 (19 days after grazing), 3$^{rd}$ February (23 days after grazing) and 8$^{th}$ February (21 days after grazing).

### h) Leaf regrowth stage

During the second year of the experiment, leaf regrowth stage was assessed on 10 randomly selected tillers per subplot in one block before every grazing using the method of Donaghy (1998). Since there is no evidence of important differences between cultivars or N levels (in the range used in this trial) in leaf appearance rate (A. Davies, 1971, 1978; Luxmoore & Millington, 1971; van Loo et al., 1992), sampling was confined to one block to track seasonal trends and help inform grazing management decisions.

### 3.3.8 Data analysis

#### Total adjusted DM yield

The total DM yield harvested was adjusted to account for the differences in the post-grazing residual left by the cows among the different cultivars (section 3.4.3 of this Chapter). For this purpose, the difference between the residual post – harvest (1750 kg DM/ha in the first three harvests and 1900 kg DM/ha in the following harvests), and the post-grazing residual from the previous grazing in each subplot, was added or subtracted to the DM harvested, depending if the subplot had been grazed lower or higher than the cutting height. In this way, most of this adjusted DM yield is the actual harvested herbage, but it also includes a small fraction that considers the preference showed by the cows for some of the cultivars, which, if not considered, could bias the results. In the text total DM yield and total adjusted DM yield are used as synonyms.

#### Chesson-Manly index

The Chesson-Manly (CM) Index (Chesson, 1983; Smit, Tamminga, & Elgersma, 2006; Solomon, Macoon, Lang, Vann, & Ward, 2014) which relates consumption to forage availability as a measure of relative preference, was calculated using the DM estimated with the rising plate meter pre and post-grazing and based on the formula developed by Manly, Miller, and Cook (1972) and Chesson (1983), and used by Smit et al. (2006) and Solomon et al. (2014) (Equation 3).

**Equation 3**

$$\alpha_i = \frac{\ln\left[1 - (\text{consumed}_i / \text{available}_i)\right]}{\sum_{j=1}^{m} \ln\left[1 - (\text{consumed}_j / \text{available}_j)\right]}, \quad i = 1, \ldots\ldots, m$$

In this formula, consumed $_i$ is the amount of consumed herbage of cultivar *i* and available *i* is the available herbage of the same cultivar at the beginning of the grazing event; *m* is the number of cultivars available to choose (8 in this experiment) and the denominator term is the sum of all numerator terms.

#### Farming seasons

The first year of the experiment comprised the farming season 2012 – 2013, starting on 1$^{st}$ June 2012 and ending 31$^{st}$ May 2013. The second year comprised the farming season 2013 – 2014, starting on 1$^{st}$ June 2013 and ending 31$^{st}$ May 2014.

#### Statistical analysis

Analysis of variance was performed on all data using GenStat 17 (VSN International, 2014) with cultivar, nitrogen and clover treatments and their interactions as fixed effects, and block, main plot within block and subplot as random effects. Least significant differences (LSD) at the 5% level were used to declare differences among means. Contrasts among the cultivars were included in the analysis of variance using the COMPARISON function in GenStat with a matrix of 6 contrasts of the cultivars (Dense versus Open, Prospect AR37 versus Abermagic AR1, Base AR37 versus Bealey NEA2, Mid versus Late, Commando AR37 versus Kamo AR37, One50 AR37 versus Alto AR37); the aim of this analysis was to gain an insight into possible interactions with treatment.

Botanical composition data were analysed before and after angular transformation. Visual assessment of residual plots was conducted; when a transformation was necessary *P* values and letters (to indicate significant differences) presented in the Tables are from the analysis of transformed data. Percentages and SED from the analysis of untransformed data are included for ease of interpretation.

Tiller and white clover population density data were analysed before and after square root transformation. Visual assessment of residual plots was conducted; when a transformation was necessary *P* values and letters (to indicate significant differences) presented in the Tables are from the analysis of transformed data. Means and SED from the analysis of untransformed data are included for ease of interpretation.

Regression analyses were conducted between tiller density and seasonal autumn yield within N × Clover treatment, for each year using GenStat 17 (VSN International, 2014). Regression analyses were also conducted between seasonal yield for each cultivar in the minus clover treatments and seasonal white clover percentage (and white clover yield) in pastures sown with the same ryegrass cultivar in the plus clover treatments, analysed within N treatment, using GenStat 17 (VSN International, 2014). The white clover yield was calculated based on the seasonal yield of the mixture and the white clover percentage in the sampling conducted during the same season. Additionally, regression analyses were conducted between tiller density in perennial ryegrass monocultures and white clover percentage (and white clover yield) in mixtures, and between tiller density in mixtures and white clover percentage (and white clover yield) in mixtures, within N treatment, in autumn each year using GenStat 17 (VSN International, 2014). Regression analyses were also conducted between tiller density and white clover growing point density either combined or pooled within treatment, and between white clover growing point density and white clover percentage (and white clover yield) within N treatment, in autumn each year using GenStat 17 (VSN International, 2014). Moreover, regression analyses were conducted between white clover percentage in each season and seasonal white clover yield within N treatment using GenStat 17 (VSN International, 2014).

Analysis of variance of the adjusted Mean Stage Count (MSC) was conducted before and after square root transformation using GenStat 17 (VSN International, 2014). Visual assessment of residual plots was conducted; untransformed means and SED are presented in the Tables for ease of interpretation. However, *P* values and letters are from the analysis of transformed data. The contribution of reproductive tillers to the total tiller sample expressed as % of the total tiller number and % of the total sample dry weight and their angular transformations were analysed. Visual assessment of residual plots was conducted; untransformed means and SED are presented in the Tables but *P* values and letters are from the analysis of transformed data.

Repeated measures analyses were conducted on the adjusted DM yield, white clover percentage, angular transformation of the white clover percentage, tiller density and Chesson-Manly index, using the AREPMEASURES procedure in GenStat 17 (VSN International, 2014). Since there were significant interactions between treatments and season, results of the analysis of variance for individual seasons are presented. Repeated measures analysis was also conducted on the endophyte infection frequency data using the same procedure, showing no interaction between year and Cultivar.

For the post-grazing mass (kg DM/ha), repeated measurements through time were analysed using spline models within the linear mixed model framework as described by Verbyla, Cullis, Kenward, and Welham (1999). Treatment, cultivar, treatment by cultivar interaction, the linear trend of time and the interaction of treatment and cultivar with the linear trend of time were included in the model as fixed effects; block, main plot within block, subplot, linear trend of time within subplot, spline, the interaction of subplot with spline and the interaction of treatment and cultivar with spline were included as random effects. Residual maximum likelihood (REML) in GenStat 16.2 (VSN International, 2013) was used to fit these models. This method of analysis essentially fits straight lines to the data initially (the linear trends) and estimates the differences in the slopes of these lines for the treatments and cultivars. Curvature in addition to the linear trend is then included in the model (the spline terms) and treatment and cultivar differences in curvature are determined. These are represented by the interactions of the spline term with treatment and cultivar. The fitted curves are hence determined by combining the linear trend and the curvature in addition to this for each treatment cultivar combination.

## 3.4 Results

### 3.4.1 Total DM yield: cultivar level

The effects of treatments, and their interactions, on seasonal and annual total adjusted DM yield (kg DM/ha) are presented in Table 3.4.

There was no evidence of significant interactions between Cultivar, N treatment and Clover treatment on seasonal or annual total adjusted DM yield. Therefore, only main effects and first-order interactions are presented in subsequent sub-sections.

**Table 3.4 Seasonal and annual total adjusted DM yield (kg DM/ha) from pastures sown with or without clover, and receiving high (325 kg N/ha) or low (100 kg N/ha) N fertiliser annually.**

| | | Spring 2012 | | Summer 2012 - 2013 | | Autumn 2013 | | Total season 2012 - 2013 | | Winter 2013 | | Spring 2013 | | Summer 2013 - 2014 | | Autumn 2014 | | Total season 2013 -2014 | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Nitrogen treatment | High | 4695 | | 4105 | | 2885 | | 11685 | | 1950 | | 4220 | | 3740 | | 2970 | | 12880 | |
| | Low | 3505 | | 3325 | | 2275 | | 9105 | | 1095 | | 3200 | | 3035 | | 2535 | | 9865 | |
| Clover treatment | + clover | 4110 | | 4380 | | 2970 | | 11460 | | 1680 | | 3935 | | 4035 | | 3125 | | 12780 | |
| | – clover | 4090 | | 3050 | | 2185 | | 9325 | | 1360 | | 3485 | | 2740 | | 2375 | | 9965 | |
| SED | | 247.0 | | 153.5 | | 82.1 | | 382.6 | | 53.2 | | 91.0 | | 263.1 | | 102.3 | | 364.2 | |
| Perennial ryegrass cultivar | One50 AR37 | 4000 | bcd | 3825 | | 2690 | ab | 10515 | | 1815 | a | 3855 | a | 3645 | | 3055 | a | 12375 | a |
| | Prospect AR37 | 4180 | abc | 3855 | | 2810 | a | 10845 | | 1835 | a | 3820 | a | 3515 | | 2910 | ab | 12075 | ab |
| | Bealey NEA2 | 3870 | cd | 4035 | | 2770 | a | 10675 | | 1680 | ab | 3575 | ab | 3575 | | 2860 | ab | 11685 | abc |
| | Alto AR37 | 4180 | abc | 3510 | | 2560 | abc | 10250 | | 1545 | b | 3570 | ab | 3445 | | 2725 | bcd | 11290 | bcd |
| | Base AR37 | 3690 | d | 3640 | | 2600 | abc | 9930 | | 1600 | b | 3440 | b | 3285 | | 2790 | bc | 11120 | cd |
| | Abermagic AR1 | 4170 | abc | 3660 | | 2360 | c | 10185 | | 1120 | d | 3770 | a | 3485 | | 2580 | de | 10955 | cd |
| | Kamo AR37 | 4390 | a | 3440 | | 2505 | bc | 10335 | | 1300 | c | 3860 | a | 3110 | | 2645 | cde | 10915 | cd |
| | Commando AR37 | 4310 | ab | 3755 | | 2340 | c | 10410 | | 1280 | cd | 3800 | a | 3030 | | 2455 | e | 10565 | d |
| SED | | 197.8 | | 200.3 | | 134.5 | | 369.4 | | 83.7 | | 146.5 | | 224.1 | | 104.2 | | 400.3 | |
| N x clover treatment | High N + clover | 4600 | | 4360 | a | 3035 | a | 12000 | a | 2065 | | 4370 | | 4245 | | 3195 | a | 13875 | a |
| | High N – clover | 4790 | | 3850 | b | 2730 | b | 11370 | a | 1830 | | 4070 | | 3240 | | 2750 | b | 11890 | b |
| | Low N + clover | 3615 | | 4400 | a | 2905 | ab | 10920 | a | 1300 | | 3500 | | 3830 | | 3060 | ab | 11685 | b |
| | Low N – clover | 3390 | | 2250 | c | 1645 | c | 7285 | b | 890 | | 2905 | | 2235 | | 2005 | c | 8040 | c |
| SED | | 349.3 | | 217.1 | | 116.1 | | 541.1 | | 75.3 | | 128.6 | | 372.0 | | 144.7 | | 515.0 | |
| *P* value | N effect | < 0.001. | | < 0.001. | | < 0.001. | | < 0.001. | | < 0.001. | | < 0.001. | | < 0.05 | | < 0.01 | | < 0.001. | |
| | Clover effect | 0.941 | | < 0.001. | | < 0.001. | | < 0.001. | | < 0.001. | | < 0.001. | | < 0.001. | | < 0.001. | | < 0.001. | |
| | Cultivar effect | < 0.05 | | 0.079 | | < 0.01 | | 0.301 | | < 0.001. | | < 0.05 | | 0.067 | | < 0.001. | | < 0.001. | |
| | N x clover interaction | 0.420 | | < 0.001. | | < 0.001. | | < 0.01 | | 0.127 | | 0.136 | | 0.285 | | < 0.05 | | < 0.05 | |
| | N x cultivar interaction | 0.870 | | 0.062 | | 0.181 | | 0.386 | | < 0.05 | | 0.726 | | 0.582 | | 0.739 | | 0.852 | |
| | Clover x cultivar interaction | 0.794 | | 0.665 | | 0.903 | | 0.731 | | < 0.05 | | 0.342 | | 0.846 | | 0.696 | | 0.682 | |

SED = standard error of the difference between means. Different letters within a column indicate statistical differences.

## Main effect of N

During both years, seasonal and total annual yield of the High N treatments was greater than from the Low N treatments in all cases (Table 3.4). High N treatments yielded 28 % and 31 % more on average than the Low N treatments in the first and second year respectively. In 2012 – 13, most of this increase (76 %) occurred during spring and summer. In 2013 – 14, there was also an increase in the DM yield during winter (not included in measurements in 2012 – 13), while the contribution of autumn to the total increment for the season was only 15 %.

## Main effect of clover

During the spring of the establishment year (2012) there was no effect of clover on the adjusted DM yield. Thereafter, seasonal and total annual yield of the plus clover treatments was consistently greater than from the minus clover treatments (Table 3.4). Mean yield of the plus clover treatments was 23 % and 28 % greater than the minus clover treatments in the first and second year respectively. Most of this increase occurred in summer and autumn, which together accounted for 99 % and 72 % of the total increase during the first and second year respectively.

## N × clover interactions

N and clover interactions were observed in summer of the first year, autumn of both years, and for the total annual yield from both years. In general, the Low N plus clover treatment yielded similarly to the High N treatments, but yielded significantly more DM than the Low N minus clover treatment (Table 3.4).

N x clover interactions were driven by differences between clover treatments in the apparent efficiency of the total pasture growth response to the additional 225 kg N/ha per year applied in the High N treatments compared to the Low N treatments. The apparent N response efficiencies are shown in Table 3.5 for situations where significant N x clover interactions occurred.

**Table 3.5 Apparent response to N expressed as kg DM/kg of additional N applied in the High N treatment compared with Low N treatment plus or minus clover for seasons where significant N x clover interactions occurred.**

| Apparent response to N (kg DM/kg of additional N) | Summer | Autumn | Annual |
|---|---|---|---|
| High N - clover versus Low N - clover season 2012 - 13 | 20.2 | 13.2 | 18.8 |
| High N + clover versus Low N + clover season 2012 - 13 | -0.5 | 1.6 | 5.0 |
| High N - clover versus Low N - clover season 2013 - 14 | --- | 10.2 | 14.2 |
| High N + clover versus Low N + clover season 2013 - 14 | --- | 1.9 | 6.9 |

Minus clover treatments always had greater N response efficiency than plus clover treatments, which were relatively unresponsive to N in summer and autumn, and in total annual yield. However the plus clover treatments did response to extra N in spring (average of both years 15.6 kg DM/kg N, versus 21.8 in minus clover treatments).

### Main effect of cultivar

Cultivar differences in adjusted DM yield were significant in spring and autumn in both years, and in winter 2013 (Table 3.4). There was a trend toward significance in summer in both years. Cultivar did not affect total annual yield in the first year, but there was a significant effect in the second year.

Prospect AR37, Bealey NEA2 and One50 AR37 were generally the highest yielding cultivars while Kamo AR37, Abermagic AR1 and Commando AR37 were generally among the lowest yielding cultivars from autumn 2013 onwards (Table 3.4).

The range between the highest and lowest yielding cultivar for annual total adjusted DM yield was 0.9 t DM/ha and 1.8 t DM/ha for the first and second years respectively.

### Interactions between cultivar and clover, and cultivar and N

No significant interactions were detected between clover inclusion/exclusion and perennial ryegrass cultivar, or between N level and perennial ryegrass cultivar on seasonal or annual total DM yield, with the exception of winter 2013 (Table 3.4).

In winter 2013, although the yield of all cultivars increased in the presence of clover, this increase was only significant for One50 AR37, Prospect AR37, Base AR37 and Kamo AR37 (Figure 3.3, SED between treatments – 122.8 kg DM/ha).

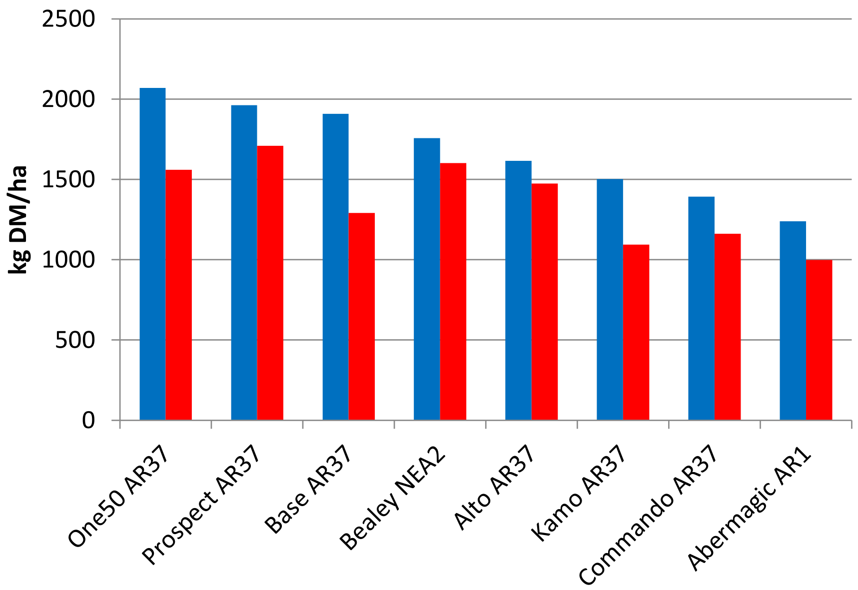


**Figure 3.3 Cultivar x clover treatment interaction in winter 2013: Adjusted DM yield (kg DM/ha) from pastures of the plus or minus clover treatments (mean of High and Low N treatments). Plus (blue bar) or minus (red bar) clover treatments. SED between clover treatments – 122.8 kg DM/ha.**

Also in winter 2013, a scaling interaction between cultivar and N treatment was evident such that the range between the highest yielding cultivar (One50 AR37; 2366 kg DM/ha) and the lowest yielding cultivar (Abermagic AR1; 1477 kg DM/ha) was greater in the High N treatment than in the Low N treatment (Prospect AR37; 1389 kg DM/ha versus Abermagic AR1 – 760 kg DM/ha the lowest cultivar) (Figure 3.4). Although the yield of all cultivars increased significantly under High N treatment compared to Low N treatment (SED between N treatments – 122.8 kg DM/ha), Kamo AR37, Commando AR37 and Abermagic AR1 responded less strongly, and were the lowest yielding cultivars at both N levels (SED within treatment – 118.4 kg DM/ha).

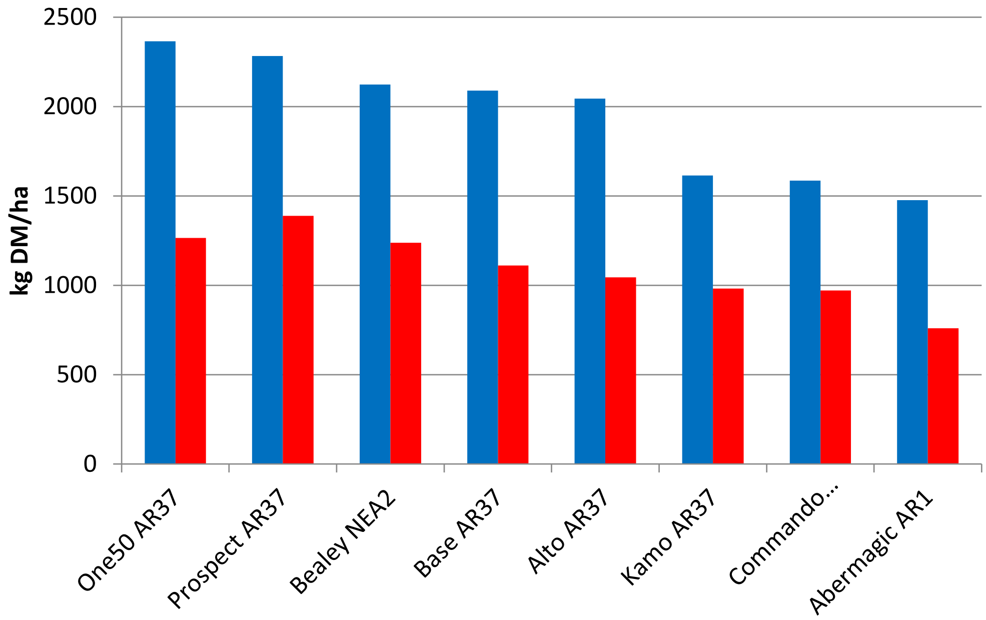


**Figure 3.4 Cultivar x N treatment interaction in winter 2013: Adjusted DM yield (kg DM/ha) from pastures receiving High or Low rates of N fertiliser annually (mean of plus and minus clover treatments). High (blue bar) or Low (red bar) rates of N fertiliser. SED within N treatments – 118.4 kg DM/ha.**

### 3.4.2 Total DM yield: phenotypic contrast level

Seasonal and annual total adjusted DM yields (kg DM/ha) for the phenotypic contrasts are presented in Table 3.6.

**Table 3.6 Seasonal and annual total adjusted DM yield (kg DM/ha) from pastures sown with perennial ryegrass cultivars with contrasting morphology and heading date.**

| Perennial ryegrass contrasts | | Spring 2012 | Summer 2012 - 2013 | Autumn 2013 | Total season 2012 - 2013 | Winter 2013 | Spring 2013 | Summer 2013 - 2014 | Autumn 2014 | Total season 2013 -2014 |
|---|---|---|---|---|---|---|---|---|---|---|
| Morphology | Dense | 4175 | 3760 | 2585 | 10515 | 1480 | 3795 | 3500 | 2745 | 11515 |
| | Open | 3780 | 3840 | 2685 | 10305 | 1640 | 3505 | 3430 | 2825 | 11405 |
| Heading date | Mid | 4350 | 3600 | 2420 | 10370 | 1290 | 3830 | 3070 | 2550 | 10740 |
| | Late | 4090 | 3670 | 2625 | 10385 | 1680 | 3715 | 3545 | 2890 | 11830 |
| SED | | 139.9 | 141.6 | 95.1 | 261.2 | 59.2 | 103.6 | 158.5 | 73.7 | 283.1 |
| *P* value | Morphology | < 0.01 | 0.572 | 0.285 | 0.417 | < 0.01 | < 0.01 | 0.663 | 0.278 | 0.693 |
| | Heading date | 0.063 | 0.620 | < 0.05 | 0.966 | < 0.001. | 0.266 | < 0.01 | < 0.001. | < 0.001. |
| | Morphology x N | 0.639 | < 0.01 | 0.806 | 0.202 | 0.286 | 0.313 | 0.462 | 0.508 | 0.465 |
| | Morphology x Clover | 0.502 | 0.223 | 0.675 | 0.241 | 0.237 | 0.732 | 0.702 | 0.863 | 0.770 |
| | Heading date x N | 0.111 | 0.444 | 0.102 | 0.876 | < 0.001. | 0.880 | 0.678 | 0.873 | 0.592 |
| | Heading date x Clover | 0.363 | 0.500 | 0.347 | 0.825 | 0.959 | 0.669 | 0.760 | 0.884 | 0.780 |

SED = standard error of the difference between means.

### Morphological contrast

Mean DM yield of cultivars representing the dense phenotype (Prospect AR37 and Abermagic AR1, both diploids) was greater than that of cultivars representing the open phenotype (Bealey NEA2 and Base AR37, both tetraploids) during spring in both years while the reverse was observed in winter 2013 (Table 3.6).

However, significant differences in yield of the two cultivars used to represent the dense phenotype were detected in winter 2013, autumn in both years and in the total annual yield for the season 2013 – 14. In all cases, Prospect AR37 yielded more than Abermagic AR1, while the yield of the two open cultivars was intermediate between them (Table 3.4). Moreover; significant differences in yield of the two open cultivars were observed in summer of the first year ($P = 0.050$) and in the total annual yield for 2012 – 13 ($P = 0.047$), when Bealey NEA2 yielded more than Base AR37. As a result of these differences (particularly within the dense phenotype) the morphological contrast lacks the internal consistency required to draw robust conclusions.

### Heading date contrast

In the first year of the experiment, the mean DM yield of cultivars representing the mid and late heading dates was similar, except in autumn 2013, when late cultivars (Alto AR37 and One50 AR37, both diploids), yielded more than mid heading date cultivars (Commando AR37 and Kamo AR37, both diploids). The main effect of heading date strengthened over time, with late cultivars yielding more than mid cultivars during the second year (except in spring 2013) (Table 3.6).

Although some significant differences in the DM yield of the two cultivars representing the late heading date contrast were observed (in winter 2013, autumn 2014 and total annual 2013 – 14), both cultivars yielded more than the two mid heading date cultivars during those seasons. Consequently, the performance of cultivars within the heading date contrast was internally consistent, allowing more confidence to be placed in the conclusions drawn from this comparison.

### Interactions between perennial ryegrass contrasts and treatments

Only two significant interactions between phenotype contrasts and treatments were observed.

In summer 2012 – 13 the interaction was between plant morphology and N treatment ($P < 0.001$, Table 3.6). Under High N, mean yield from both plant morphology contrasts was similar, but under Low N, mean yield from cultivars with an open tillering habit and broad leaves was greater than from cultivars with a dense tillering habit and fine leaves (Table 3.7).

**Table 3.7 Interaction between plant morphology and N treatment during summer 2012 – 13.**

| Morphology | High N | | Low N | |
|---|---|---|---|---|
| Dense | 4405 | a | 3111 | b |
| Open | 4108 | a | 3569 | a |
| SED within N treatment | | 200.3 | | |
| SED between N treatment | | 216.5 | | |

Different letters within a column indicate statistical differences.

This interaction was accompanied by a trend towards an interaction ($P$ = 0.053) between N and the two cultivars representing the dense morphology; Prospect AR37 yielded significantly more than Abermagic AR1 under High N, but they yielded similarly under Low N. This interaction was also present during autumn 2013 ($P$ = 0.017), summer 2013-14 ($P$ = 0.038) and was close to significance in the annual total for 2012 – 13 ($P$ = 0.054).

During winter 2013, the interaction was between heading date phenotypes and N treatment ($P$ < 0.001, Table 3.6). Under both N treatments, late heading phenotypes yielded more than mid heading phenotypes, but the increment in yield when more N was available was greater for the late phenotypes (1051 kg DM/ha) than for the mid heading phenotypes (623 kg DM/ha, Table 3.8).

**Table 3.8 Interactions between heading date and N treatment during winter 2013.**

| | High N | | Low N | |
|---|---|---|---|---|
| Mid | 1600 | b | 977 | b |
| Late | 2206 | a | 1155 | a |
| SED within N treatment | | 83.7 | | |
| SED between N treatment | | 86.8 | | |

Different letters within a column indicate statistical differences.

### 3.4.3 Post-grazing herbage mass

The effect of cultivar on post-grazing herbage mass was significant throughout the experiment ($P$ < 0.001). There were also significant interactions between treatment and time ($P$ <0.001), and cultivar and time ($P$ <0.001), both linear trend and spline i.e. curvature, but no significant three way interaction between treatment, cultivar and time. This means that it is valid to present the cultivar by time interaction as an average over treatments (Figure 3.5). The treatment by time interactions will not be considered in the analysis of results, since the main treatments plots were not always grazed at the same time or under the same conditions and as a consequence it could be influenced by management decisions.

A seasonal pattern in post-grazing mass was observed: it was low in early spring, increased through late spring-summer and declined over autumn. In general, the post grazing mass achieved was higher than the target (1500 - 1750 kg DM/ha), especially in summer. Bealey NEA2 , Base AR37 (both tetraploids) and Abermagic AR1 were the cultivars grazed lowest; after the first spring of the experiment Kamo AR37 tended to be grazed lower as well. During the second year of the experiment, cultivar differences in post-grazing mass became smaller and almost disappeared at the end of the study.

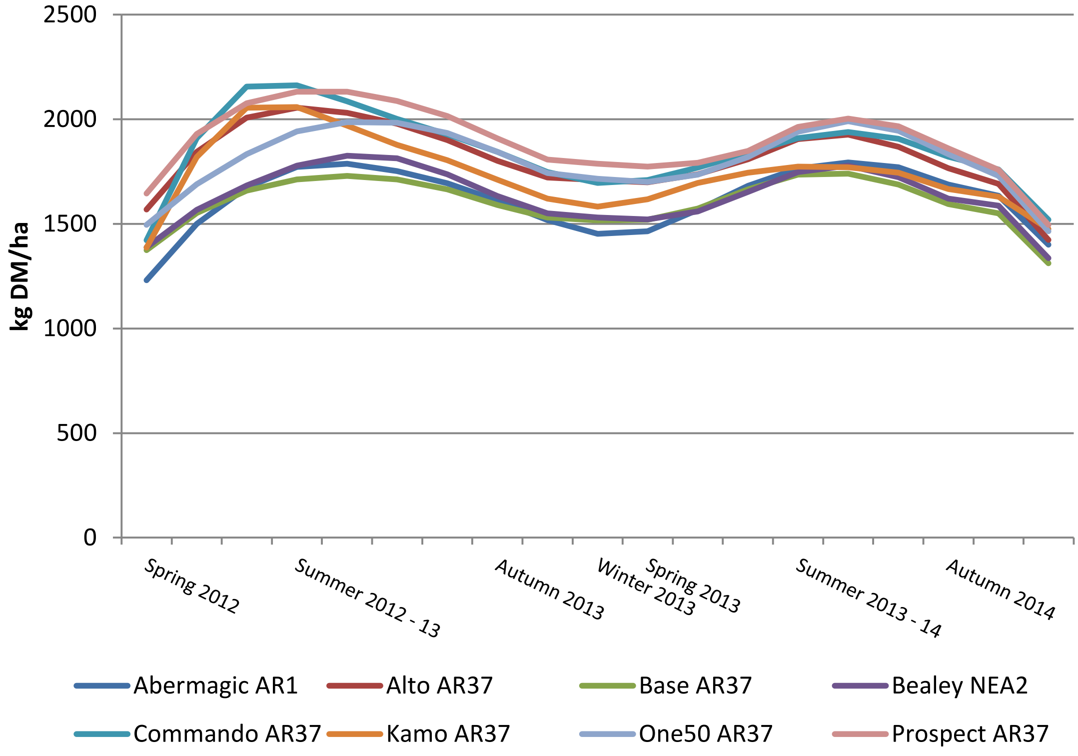


**Figure 3.5 Post-grazing herbage mass (kg DM/ha) – Fitted curves (average for all treatments).**

The Chesson-Manly index (expressed as %) for the year 2012 – 13 and 2013 – 14 showed a significant cultivar effect in 12 of 18 grazings (grazing 13 in which the plating post-grazing was conducted after mowing is not included in the analysis) (Tables 3.9 and 3.10). A higher index indicates a higher apparent grazing preference for that cultivar. Although the results were not consistent during both years, the two tetraploid cultivars tended to be in the most preferred group.

**Table 3.9 Chesson-Manly index (expressed as %) - year 2012 - 13**

| | | Grazing number | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 1 | | 2 | 3 | | 4 | | 5 | | 6 | 7 | | 8 | | 9 | | |
| Perennial ryegrass cultivar | Abermagic AR1 | 11.3 | c | 11.7 | 13.1 | ab | 13.3 | ab | 12.9 | ab | 12.9 | 14.6 | a | 13.2 | ab | 11.8 | c | |
| | Alto AR37 | 13.5 | ab | 12.8 | 12.3 | bcd | 12.2 | b | 11.7 | cd | 11.7 | 11.7 | de | 12.3 | bc | 12.5 | abc | |
| | Base AR37 | 13.7 | ab | 12.8 | 12.4 | bcd | 13.6 | ab | 13.6 | a | 13.4 | 13.8 | abc | 13.3 | ab | 12.3 | bc | |
| | Bealey NEA2 | 13.5 | ab | 11.8 | 13.5 | a | 13.8 | a | 13.5 | a | 13.4 | 13.9 | ab | 13.6 | a | 12.8 | ab | |
| | Commando AR37 | 10.2 | c | 13.4 | 12.9 | abc | 10.7 | c | 12.1 | bc | 11.6 | 11.0 | de | 11.7 | c | 11.7 | c | |
| | Kamo AR37 | 10.1 | c | 13.5 | 11.8 | d | 10.6 | c | 12.7 | abc | 12.8 | 12.1 | cd | 12.6 | abc | 12.7 | abc | |
| | One50 AR37 | 13.1 | b | 10.9 | 12.0 | d | 14.1 | a | 12.5 | abc | 12.7 | 12.6 | bcd | 11.7 | c | 12.8 | ab | |
| | Prospect AR37 | 14.5 | a | 13.0 | 12.1 | cd | 11.8 | c | 11.0 | d | 11.5 | 10.3 | e | 11.7 | c | 13.4 | a | |
| SED | | 0.64 | | 0.91 | 0.43 | | 0.73 | | 0.58 | | 0.84 | 0.89 | | 0.55 | | 0.50 | | |
| *P* value | | <.001 | | 0.057 | <.001 | | <.001 | | <.001 | | 0.099 | <.001 | | <.001 | | < 0.05 | | |

SED = standard error of the difference between means. Different letters within a column indicate statistical differences.

**Table 3.10 Chesson-Manly index (expressed as %) - year 2013 – 14**

| | | Grazing number | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 10 | | 11 | | 12 | | 13 | 14 | | 15 | 16 | | 17 | 18 | 19 |
| Perennial ryegrass cultivar | Abermagic AR1 | 10.0 | e | 12.5 | bc | 13.4 | ab | — | 13.4 | a | 13.0 | 12.6 | abc | 12.8 | 12.2 | 13.4 |
| | Alto AR37 | 12.6 | b | 12.3 | bcd | 11.7 | cd | — | 11.9 | b | 12.2 | 12.4 | bc | 12.3 | 12.6 | 12.5 |
| | Base AR37 | 13.6 | a | 12.2 | bcd | 12.9 | ab | — | 12.8 | ab | 13.1 | 13.8 | a | 12.5 | 12.6 | 11.5 |
| | Bealey NEA2 | 14.1 | a | 11.6 | d | 12.6 | bc | — | 13.8 | a | 12.4 | 12.9 | ab | 13.6 | 12.7 | 13.5 |
| | Commando AR37 | 11.7 | c | 12.9 | b | 13.1 | ab | — | 11.7 | b | 12.3 | 12.9 | ab | 12.2 | 12.4 | 12.2 |
| | Kamo AR37 | 10.8 | d | 13.8 | a | 13.8 | a | — | 11.7 | b | 12.9 | 12.1 | bc | 12.7 | 13.0 | 11.8 |
| | One50 AR37 | 13.4 | ab | 12.0 | cd | 11.4 | d | — | 12.8 | ab | 12.4 | 11.6 | c | 11.8 | 12.2 | 13.5 |
| | Prospect AR37 | 13.8 | a | 12.6 | bc | 11.2 | d | — | 12.0 | b | 11.8 | 11.9 | bc | 12.1 | 12.2 | 11.6 |
| SED | | 0.41 | | 0.36 | | 0.52 | | | 0.64 | | 0.69 | 0.66 | | 0.58 | 0.40 | 0.94 |
| *P* value | | <.001 | | <.001 | | <.001 | | — | < 0.01 | | 0.506 | < 0.05 | | 0.088 | 0.471 | 0.130 |

SED = standard error of the difference between means. Different letters within a column indicate statistical differences.

### 3.4.4 Botanical composition: cultivar level

Large differences in the white clover and perennial ryegrass content of pastures were evident throughout the study. These differences were driven by the main treatment combinations (High N plus clover, High N minus clover, Low N plus clover, Low N minus clover), and reinforced by management (spraying out clover in the minus clover pastures) (Tables A.3 and A.4 in Appendix A). Mean white clover content in the minus clover treatment was less than 5 % of total DM on an annual basis, and on a seasonal basis it was generally less than 2 % (with the exception of summer 2012 – 13 when clover reached 5.6 % DM averaged across no clover treatments).

#### Main effect of clover

The inclusion of clover decreased significantly the perennial ryegrass content of pastures throughout the two years ($P$ <0.001 at every season with the exception of spring 2012 in which $P$ = 0.002, Tables A.3 and A.4 in Appendix A). Mean ryegrass content in the minus clover treatment was 87.5 % while in the clover treatment it was 75.8 % of total DM (average of the two years).

The effect of clover treatment on clover content was highly significant at every season ($P$ < 0.001). Mean clover content in the minus clover treatment was 1.2 % and in the plus clover treatments it was 16.6 % total DM (average of the two years).

The content of other species was also affected by the presence or absence of clover. With the exception of spring 2013 in which both clover treatments had similar content (3.2 % in the plus clover treatment and 2.5% of total DM in the minus clover treatment, $P$ = 0.760), during the rest of the two years the presence of clover decreased significantly the content of other species (2.4 % in the plus clover treatment and 4.8 % of total DM in the minus clover treatment, average of the two years, Tables A.3 and A.4 in Appendix A).

Meanwhile the presence or absence of clover affected the content of dead matter only during summer in both years, but not in other seasons. Mean dead content in the plus clover treatment was 5.5 % and in the minus clover treatment it was 9.0 % of total DM in summer 2012 – 2013 ($P$ = 0.002). In the second summer the dead content in the plus clover treatment was 3.9 % and in the minus clover treatment it was 6.6 % of total DM ($P$ <0.001). Average dead content for the two years was 5.1 % in the plus clover treatment and 6.5 % total DM in the minus clover treatment. The differences observed between plus and minus clover treatments in percentage of dead matter during summer could be an indication of a better grazing efficiency in the grass/clover mixture than in grass monoculture.

Analysis of major trends that could affect botanical composition of the mixtures and the relationships between ryegrass and clover is confined to the plus clover treatments only, due to the botanical composition in the minus clover treatment being controlled by treatment combinations.

***Plus clover treatments***

In the plus clover treatments only, a general seasonal trend was observed (Tables 3.11 and 3.12); the white clover percentage was higher in summer, and this was associated with a lower perennial ryegrass percentage.

### Main effect of N

The clover content of pastures was always greater in the Low N treatments compared with the High N treatments, while the reverse generally applied for the perennial ryegrass content. N treatment also affected the content of other species and dead matter in some seasons but these were always minor components of the pasture (≤ 8.5% of total DM).

### Main effect of cultivar

The white clover content of pastures varied with perennial ryegrass cultivar during spring in both years and in summer 2013 - 14. During spring 2012, pastures based on Bealey NEA2, Base AR37 and Abermagic AR1 contained more white clover than pastures sown with Alto AR37, Commando AR37 and Prospect AR37 (Table 3.11). In spring 2013, pastures based on Bealey NEA2 contained more white clover than pastures based on One50 AR37 and Prospect AR37 (Table 3.12). The range between the highest and lowest white clover content was close to 9 % in both springs. During the first spring, the three cultivars with the highest white clover percentage (Bealey NEA2, Abermagic AR1 and Base AR37), also had the lowest percentage of dead matter. However, Abermagic AR1 and Bealey NEA2 were also the cultivars with the highest percentage of other species. Meanwhile, during summer 2013 – 14, the three cultivars with higher white clover percentage were Kamo AR37, Commando AR37 and Bealey NEA2, and the cultivar with lowest clover content was Prospect AR37. The range between the highest and lowest white clover content was close to 15 % in both summers.

### Interactions between N and cultivar

There were no significant interactions between N and cultivar for clover content during the two years of the experiment. However, there was a trend towards a N × cultivar interaction (*P* value = 0.091) during summer 2012 – 13, when clover content was greater in the Low N pastures compared with the High N pastures for all the cultivars, with the exception of Abermagic AR1, where clover content did not differ between N treatments.  Abermagic AR1 had the highest clover content under the High N treatment (22 %), but the lowest content under the Low N treatment (38 %).

**Table 3.11 Botanical composition (% of DM) of pastures (plus clover treatments only) during 2012 - 13.**

| | | Spring 2012 | | | | Summer 2012 - 13 | | | | Autumn 2013 | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | PRG% | WC% | Other% | Dead% | PRG% | WC% | Other% | Dead% | PRG% | WC% | Other% | Dead% |
| Nitrogen treatment | High | 81.6 | 5.4 | 6.9 | 6.1 | 77.8 | 14.6 | 2.3 | 5.3 | 87.2 | 5.2 | 1.8 | 5.8 |
| | Low | 77.4 | 12.3 | 4.5 | 5.8 | 42.9 | 50.5 | 0.9 | 5.7 | 68.5 | 23.0 | 0.1 | 8.4 |
| SED | | 1.6 | 2.2 | 1.0 | 0.9 | 5.7 | 6.8 | 0.7 | 0.9 | 4.1 | 2.5 | 0.6 | 1.0 |
| Perennial ryegrass cultivar | Abermagic AR1 | 72.2 | 11.9 a | 12.0 a | 3.9 d | 61.7 | 30.2 | 2.5 | 5.5 | 77.7 | 16.9 | 1.3 | 4.0 c |
| | Alto AR37 | 81.8 | 4.7 b | 5.3 bc | 8.2 ab | 64.3 | 29.5 | 0.7 | 5.6 | 82.6 | 9.8 | 0.9 | 6.6 abc |
| | Base AR37 | 80.8 | 11.5 a | 4.3 bc | 3.5 d | 65.4 | 30.1 | 0.5 | 3.9 | 79.8 | 13.1 | 0.9 | 6.1 bc |
| | Bealey NEA2 | 74.9 | 13.5 a | 7.4 ab | 4.2 cd | 59.5 | 34.8 | 1.5 | 4.2 | 79.1 | 14.9 | 0.9 | 5.1 c |
| | Commando AR37 | 85.2 | 5.7 b | 3.2 bc | 5.9 bcd | 55.7 | 35.5 | 1.4 | 7.4 | 75.9 | 16.3 | 1.2 | 6.7 bc |
| | Kamo AR37 | 81.8 | 8.6 ab | 2.4 c | 7.2 abc | 51.5 | 42.2 | 1.4 | 4.9 | 71.8 | 18.1 | 0.7 | 9.5 ab |
| | One50 AR37 | 78.9 | 8.6 ab | 7.4 abc | 5.1 bcd | 59.5 | 30.8 | 3.2 | 6.5 | 78.4 | 11.9 | 0.9 | 8.7 ab |
| | Prospect AR37 | 80.1 | 6.2 b | 3.7 bc | 9.9 a | 65.4 | 27.3 | 1.4 | 5.9 | 77.8 | 11.8 | 0.6 | 9.8 a |
| SED | | 4.0 | 2.9 | 2.9 | 1.5 | 5.2 | 4.8 | 1.2 | 1.2 | 4.1 | 3.5 | 0.5 | 1.7 |
| *P* value | N effect | 0.051 | < 0.05 | < 0.05 | 0.909 | < 0.01 | < 0.01 | 0.064 | 0.636 | < 0.05 | < 0.01 | < 0.05 | < 0.05 |
| | Cultivar effect | 0.068 | < 0.05 | < 0.05 | < 0.01 | 0.114 | 0.084 | 0.338 | 0.092 | 0.267 | 0.084 | 0.422 | < 0.01 |
| | N x cultivar interaction | 0.842 | 0.496 | 0.927 | 0.168 | 0.230 | 0.091 | 0.925 | 0.129 | 0.620 | 0.834 | 0.850 | 0.175 |

SED = standard error of the difference between means. Different letters within a column indicate statistical differences. In this table % and SED are from the analysis without angular transformation, but *P* value and letters are from the analysis with angular transformation.

**Table 3.12 Botanical composition (% of DM) of pastures (plus clover treatments only) during 2013 - 14.**

| | | Spring 2013 | | | | Summer 2013 - 14 | | | | Autumn 2014 | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | PRG% | WC% | Other% | Dead% | PRG% | WC% | Other% | Dead% | PRG% | WC% | Other% | Dead% |
| Nitrogen treatment | High | 87.8 | 5.7 | 3.7 | 2.8 | 79.7 | 11.7 | 2.9 | 5.7 | 86.1 | 6.0 | 0.8 | 7.2 |
| | Low | 83.5 | 11.0 | 2.6 | 2.8 | 58.8 | 36.4 | 2.6 | 2.2 | 78.6 | 17.6 | 0.3 | 3.5 |
| SED | | 2.0 | 2.0 | 0.9 | 0.5 | 2.3 | 1.8 | 0.6 | 0.8 | 0.8 | 1.2 | 0.2 | 0.9 |
| Perennial ryegrass cultivar | Abermagic AR1 | 85.7 b | 8.6 ab | 3.9 | 1.8 c | 66.6 c | 24.3 abcd | 4.8 | 4.3 | 84.9 abc | 10.9 | 0.5 | 3.8 bcd |
| | Alto AR37 | 85.4 bc | 8.4 ab | 2.9 | 3.2 ab | 70.4 abc | 25.7 abc | 1.2 | 2.7 | 80.1 c | 13.9 | 0.6 | 5.4 abc |
| | Base AR37 | 84.9 bc | 7.9 ab | 4.4 | 2.8 abc | 75.1 ab | 18.7 cd | 2.3 | 3.9 | 86.2 a | 9.9 | 0.4 | 3.5 cd |
| | Bealey NEA2 | 80.4 c | 12.6 a | 4.2 | 2.9 abc | 66.9 bc | 26.8 abc | 3.9 | 2.4 | 85.6 ab | 10.8 | 0.4 | 3.1 d |
| | Commando AR37 | 88.2 ab | 8.4 ab | 1.3 | 2.1 bc | 62.2 c | 31.0 a | 3.1 | 3.7 | 79.6 c | 13.8 | 0.4 | 6.1 ab |
| | Kamo AR37 | 85.6 b | 10.6 ab | 1.8 | 2.0 bc | 62.6 c | 30.0 ab | 2.0 | 5.4 | 79.9 c | 11.6 | 1.0 | 7.6 a |
| | One50 AR37 | 83.7 bc | 7.3 b | 4.7 | 4.3 a | 72.2 abc | 19.8 bcd | 3.5 | 4.6 | 81.6 bc | 10.9 | 0.3 | 7.2 a |
| | Prospect AR37 | 91.1 a | 3.2 c | 2.4 | 3.4 abc | 77.8 a | 16.3 d | 1.3 | 4.6 | 80.8 bc | 12.5 | 0.8 | 5.9 a |
| SED | | 2.5 | 2.1 | 1.7 | 0.7 | 5.1 | 4.6 | 2.0 | 1.3 | 2.6 | 2.4 | 0.5 | 1.3 |
| *P* value | N effect | 0.120 | < 0.05 | 0.263 | 0.948 | < 0.01 | < 0.001 | 0.369 | < 0.05 | < 0.001 | < 0.001 | 0.078 | < 0.05 |
| | Cultivar effect | < 0.01 | < 0.01 | 0.576 | < 0.05 | < 0.05 | < 0.05 | 0.538 | 0.144 | < 0.05 | 0.526 | 0.857 | < 0.001 |
| | N x cultivar interaction | 0.301 | 0.224 | 0.479 | 0.583 | 0.633 | 0.707 | 0.716 | 0.910 | 0.171 | 0.443 | 0.280 | 0.279 |

SED = standard error of the difference between means. Different letters within a column indicate statistical differences. In this table % and SED are from the analysis without angular transformation, but *P* value and letters are from the analysis with angular transformation.

### 3.4.5 Botanical composition: phenotypic contrast level

#### Morphological contrast

The white clover content of pastures was affected by the perennial ryegrass morphological contrast only during spring, when the average white clover percentage of pastures sown with open cultivars was greater than for pastures sown with dense cultivars in both years (Tables 3.13 and 3.14). However, the white clover percentage of Abermagic AR1, one of the two cultivars representing the dense type, was not significantly different from the percentage of the two open cultivars (Bealey NEA2 and Base AR37) during spring in both years (Tables 3.11 and 3.12). The clover content of pastures sown with Abermagic AR1 was significantly greater than its 'pair' in the dense contrast, Prospect AR37, in spring of both years (Tables 3.11 and 3.12). Thus, Abermagic AR1 and Prospect AR37 appeared to perform quite differently from each other; thereafter it is difficult to draw clear conclusions from this contrast.

The morphological contrast also had a significant effect on the percentage of dead matter in pastures in spring 2012 and autumn 2014, and there was a trend in dead matter content in summer in both years. In all cases, pastures based on dense cultivars had greater dead matter content than pastures based on open cultivars (Tables 3.13 and 3.14).

#### Heading date contrast

The heading date contrast significantly affected the white clover percentage of pastures during summer in both years and in autumn 2013. In all cases, mid heading date cultivars (Commando AR37 and Kamo AR37) had greater white clover content than late heading date cultivars (One50 AR37 and Alto AR37). There was no significant difference between the cultivars within heading date category.

There were no other effects of the heading date contrast on botanical composition, except for dead matter percentage in spring 2013, when pastures based on late heading cultivars had greater dead percentage than pastures based on mid heading cultivars (Table 3.14).

**Table 3.13 Botanical composition (% of DM) of pastures sown with perennial ryegrass with contrasting morphology and heading date (plus clover treatments only) during 2012 – 13.**

| | | Spring 2012 | | | | Summer 2012 - 13 | | | | Autumn 2013 | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Perennial ryegrass contrasts | | PRG% | WC% | Other% | Dead% | PRG% | WC% | Other% | Dead% | PRG% | WC% | Other% | Dead% |
| Morphology | Dense | 76.2 | 9.0 | 7.9 | 6.9 | 63.6 | 28.7 | 2.0 | 5.7 | 77.7 | 14.3 | 1.0 | 6.9 |
| | Open | 77.8 | 12.5 | 5.8 | 3.8 | 62.5 | 32.5 | 1.0 | 4.1 | 79.5 | 14.0 | 0.9 | 5.6 |
| Heading date | Mid | 83.5 | 7.2 | 2.8 | 6.5 | 53.6 | 38.8 | 1.4 | 6.2 | 73.8 | 17.2 | 0.9 | 8.1 |
| | Late | 80.3 | 6.7 | 6.4 | 6.6 | 61.9 | 30.1 | 1.9 | 6.0 | 80.5 | 10.9 | 0.9 | 7.7 |
| SED | | 2.8 | 2.0 | 2.0 | 1.1 | 3.7 | 3.4 | 0.8 | 0.9 | 2.9 | 2.5 | 0.4 | 1.2 |
| *P* value | Morphology | 0.579 | < 0.05 | 0.411 | < 0.01 | 0.789 | 0.173 | 0.349 | 0.057 | 0.449 | 0.836 | 0.996 | 0.316 |
| | Heading date | 0.242 | 0.779 | 0.078 | 0.828 | < 0.05 | < 0.05 | 0.782 | 0.715 | < 0.05 | < 0.05 | 0.251 | 0.802 |
| | Morphology x N | 0.282 | 0.142 | 0.876 | 0.594 | 0.077 | 0.193 | 0.590 | 0.440 | 0.236 | 0.367 | 0.996 | 0.659 |
| | Heading date x N | 0.659 | 0.199 | 0.932 | 0.453 | 0.318 | 0.292 | 0.748 | 0.822 | 0.166 | 0.527 | 0.945 | 0.352 |

SED = standard error of the difference between means. In this table % and SED are from the analysis without angular transformation, but *P* value is from the analysis with angular transformation.

**Table 3.14 Botanical composition (% of DM) of pastures sown with perennial ryegrass with contrasting morphology and heading date (plus clover treatments only) during 2013 – 14.**

| | | Spring 2013 | | | | Summer 2013 - 14 | | | | Autumn 2014 | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Perennial ryegrass contrasts | | PRG% | WC% | Other% | Dead% | PRG% | WC% | Other% | Dead% | PRG% | WC% | Other% | Dead% |
| Morphology | Dense | 88.4 | 5.9 | 3.1 | 2.6 | 72.2 | 20.3 | 3.0 | 4.5 | 82.9 | 11.7 | 0.6 | 4.8 |
| | Open | 82.6 | 10.3 | 4.3 | 2.8 | 71.0 | 22.7 | 3.1 | 3.1 | 85.9 | 10.4 | 0.4 | 3.3 |
| Heading date | Mid | 86.9 | 9.5 | 1.5 | 2.0 | 62.4 | 30.5 | 2.5 | 4.5 | 79.7 | 12.7 | 0.7 | 6.9 |
| | Late | 84.6 | 7.9 | 3.8 | 3.8 | 71.3 | 22.8 | 2.3 | 3.6 | 80.9 | 12.4 | 0.4 | 6.3 |
| SED | | 1.8 | 1.5 | 1.2 | 0.5 | 3.6 | 3.3 | 1.4 | 0.9 | 1.8 | 1.7 | 0.3 | 0.9 |
| *P* value | Morphology | < 0.001 | < 0.01 | 0.436 | 0.352 | 0.976 | 0.679 | 0.941 | 0.051 | 0.053 | 0.358 | 0.355 | < 0.05 |
| | Heading date | 0.122 | 0.457 | 0.144 | < 0.001 | < 0.05 | < 0.05 | 0.879 | 0.359 | 0.628 | 0.899 | 0.439 | 0.611 |
| | Morphology x N | 0.787 | 0.158 | < 0.05 | 0.535 | 0.108 | 0.372 | 0.515 | 0.311 | 0.568 | 0.950 | 0.132 | 0.133 |
| | Heading date x N | 0.147 | 0.395 | 0.819 | 0.221 | 0.441 | 0.229 | 0.104 | 0.960 | 0.576 | 0.512 | 0.050 | 0.611 |

SED = standard error of the difference between means. In this table % and SED are from the analysis without angular transformation, but *P* value is from the analysis with angular transformation.

### 3.4.6 Pasture nutritive value: cultivar level

**Crude protein (CP)**

There was a significant interaction between N and clover treatments in the CP content of pastures in all seasons of both years (Table 3.15 and 3.16).

In spring and summer, N fertiliser had no effect on CP % when clover was included in the mixture. However, when clover was not included (ryegrass monoculture), CP % was higher in the High N treatment than the Low N treatment (Tables 3.15 and 3.16). In autumn of both years, within clover treatments, the CP % of pasture was always higher under High N than Low N; however, the effect of N fertiliser was greater in the minus clover treatment (+ 3.9 – 4.9 % CP versus + 2.2 – 2.5 % for High N versus Low N in minus clover and plus clover respectively) (Tables 3.15 and 3.16).

Herbage from the Low N minus clover treatment had the lowest CP content throughout the experiment. The inclusion of clover in pastures grown under the Low N treatment always increased the CP % content; however, under the High N treatment, the inclusion of clover only increased the CP % of pastures in autumn 2013, spring 2014 and summer 2013 – 14.

During summer, the highest CP content occurred in pastures of the Low N plus clover treatment, although this treatment combination was not significantly different from pastures of the High N plus clover combination.

Except during the first spring of the experiment, cultivar had a significant effect on the CP content of pastures. Cultivar effects were inconsistent across seasons. Abermagic AR1, Kamo AR37 and Commando AR37 were amongst the cultivars with the highest CP during the first summer, but during autumn 2013 Abermagic was one of the cultivars with the lowest CP, while Kamo AR37 and Commando AR37 continued in the top group. In spring 2013, Abermagic AR1 had the highest CP content, while Commando AR37 had the lowest CP %. During summer 2013 - 14 and autumn 2014, Kamo AR37 and Commando AR37 had greater CP % than all other cultivars.

The only interaction involving cultivars for CP of pastures was with N treatment in autumn 2014 (Table 3.16). Although all cultivars had a higher CP content when grown under the High N treatment compared with Low N, this increase was greater for Base AR37, Abermagic AR1 and Bealey NEA2; these cultivars had the lowest CP % when grown under the Low N treatment but were amongst the cultivars with highest CP % under High N.

**Table 3.15 Nutritive value of pastures sown with or without white clover, and receiving high or low rates of N fertiliser during 2012 – 13.**

| | | Spring 2012 | | | Summer 2012 - 13 | | | Autumn 2013 | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | CP (%DM) | ME (MJ/kg DM) | NDF (%DM) | CP (%DM) | ME (MJ/kg DM) | NDF (%DM) | CP (%DM) | ME (MJ/kg DM) | NDF (%DM) |
| Nitrogen treatment | High | 12.8 | 12.5 | 46.8 | 15.1 | 11.7 | 53.0 | 21.6 | 12.0 | 46.8 |
| | Low | 10.9 | 12.7 | 45.5 | 14.3 | 11.2 | 52.6 | 18.6 | 12.2 | 45.5 |
| Clover treatment | + clover | 12.6 | 12.7 | 45.5 | 17.1 | 11.6 | 51.2 | 21.3 | 12.2 | 45.2 |
| | - clover | 11.1 | 12.5 | 46.9 | 12.3 | 11.3 | 54.4 | 18.9 | 12.1 | 47.1 |
| SED | | 0.31 | 0.07 | 0.52 | 0.71 | 0.09 | 0.61 | 0.38 | 0.07 | 0.53 |
| Perennial ryegrass cultivar | Abermagic AR1 | 11.4 | 13.0 bc | 43.1 cd | 15.8 a | 11.7 bc | 50.1 b | 19.6 c | 12.3 b | 42.4 c |
| | Alto AR37 | 11.6 | 12.5 d | 47.6 b | 13.8 d | 11.3 d | 54.5 a | 20.2 abc | 12.1 c | 47.0 a |
| | Base AR37 | 12.3 | 13.2 ab | 43.4 cd | 14.8 abcd | 11.9 ab | 51.6 b | 20.2 abc | 12.6 a | 44.9 b |
| | Bealey NEA2 | 12.2 | 13.3 a | 42.5 d | 14.2 cd | 12.0 a | 51.3 b | 19.4 c | 12.7 a | 44.9 b |
| | Commando AR37 | 11.0 | 11.9 e | 50.1 a | 15.2 abc | 11.0 e | 54.7 a | 20.6 ab | 11.8 de | 47.5 a |
| | Kamo AR37 | 11.6 | 11.8 e | 50.6 a | 15.6 ab | 10.9 e | 54.3 a | 20.8 a | 11.6 e | 47.1 a |
| | One50 AR37 | 12.3 | 12.8 c | 44.3 c | 14.6 bcd | 11.6 c | 51.3 b | 20.1 abc | 12.1 c | 47.5 a |
| | Prospect AR37 | 12.3 | 12.4 d | 47.8 b | 13.7 d | 11.1 de | 54.7 a | 19.9 bc | 11.8 d | 47.8 a |
| SED | | 0.65 | 0.09 | 0.68 | 0.62 | 0.10 | 0.77 | 0.44 | 0.08 | 0.75 |
| N x Clover treatment | High N + clover | 13.0 a | 12.6 | 46.4 | 16.1 ab | 11.7 a | 52.6 b | 22.4 a | 12.0 | 46.6 a |
| | High N - clover | 12.5 a | 12.5 | 47.3 | 14.2 b | 11.7 a | 53.4 b | 20.9 b | 12.1 | 47.0 a |
| | Low N + clover | 12.1 a | 12.7 | 44.6 | 18.1 a | 11.5 a | 49.7 c | 20.2 b | 12.3 | 43.8 b |
| | Low N - clover | 9.8 b | 12.6 | 46.5 | 10.5 c | 10.9 b | 55.5 a | 17.0 c | 12.1 | 47.2 a |
| SED | | 0.44 | 0.09 | 0.73 | 1.01 | 0.13 | 0.87 | 0.54 | 0.09 | 0.74 |
| *P* value | N effect | < 0.001 | 0.051 | < 0.05 | 0.250 | < 0.001 | 0.548 | < 0.001 | < 0.05 | < 0.05 |
| | Clover effect | < 0.001 | 0.069 | < 0.05 | < 0.001 | < 0.01 | < 0.001 | < 0.001 | 0.311 | < 0.01 |
| | Cultivar effect | 0.337 | < 0.001 | < 0.001 | < 0.01 | < 0.001 | < 0.001 | < 0.05 | < 0.001 | < 0.001 |
| | N x clover interaction | < 0.05 | 0.868 | 0.368 | < 0.01 | < 0.01 | < 0.01 | < 0.05 | 0.130 | < 0.05 |
| | N x cultivar interaction | 0.518 | 0.879 | 0.442 | 0.832 | 0.275 | 0.136 | 0.066 | < 0.05 | 0.266 |
| | Clover x cultivar interaction | 0.165 | 0.916 | 0.542 | 0.078 | < 0.01 | < 0.001 | 0.268 | 0.165 | 0.264 |

SED = standard error of the difference between means.

**Table 3.16 Nutritive value of pastures sown with or without white clover, and receiving high or low rates of N fertiliser during 2013 – 14.**

| | | Spring 2013 | | | Summer 2013 - 14 | | | Autumn 2014 | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | CP (%DM) | ME (MJ/kg DM) | NDF (%DM) | CP (%DM) | ME (MJ/kg DM) | NDF (%DM) | CP (%DM) | ME (MJ/kg DM) | NDF (%DM) |
| Nitrogen treatment | High | 19.3 | 12.3 | 49.8 | 16.6 | 12.3 | 47.3 | 27.6 | 12.1 | 47.8 |
| | Low | 16.8 | 12.8 | 46.4 | 16.0 | 12.3 | 48.2 | 23.9 | 12.3 | 48.6 |
| Clover treatment | + clover | 19.5 | 12.5 | 48.8 | 18.3 | 12.3 | 46.8 | 26.2 | 12.2 | 47.4 |
| | - clover | 16.6 | 12.7 | 47.4 | 14.3 | 12.2 | 48.7 | 25.3 | 12.2 | 49.0 |
| SED | | 0.36 | 0.10 | 0.98 | 0.67 | 0.07 | 0.44 | 0.55 | 0.08 | 0.46 |
| Perennial ryegrass cultivar | Abermagic AR1 | 18.9 a | 12.6 bc | 46.8 c | 16.2 b | 12.6 a | 44.8 e | 25.3 b | 12.3 b | 46.1 e |
| | Alto AR37 | 18.1 abc | 12.5 c | 48.6 b | 16.1 b | 12.4 b | 47.9 c | 25.6 b | 12.1 c | 49.0 abc |
| | Base AR37 | 18.2 ab | 12.9 a | 46.8 c | 15.4 b | 12.6 a | 47.6 c | 25.6 b | 12.5 a | 47.7 cd |
| | Bealey NEA2 | 17.4 bc | 12.9 a | 46.6 c | 15.9 b | 12.6 a | 46.0 d | 25.5 b | 12.6 a | 46.8 de |
| | Commando AR37 | 17.0 c | 12.3 d | 49.7 ab | 17.7 a | 12.0 c | 48.3 bc | 27.0 a | 12.1 c | 47.8 cd |
| | Kamo AR37 | 17.7 bc | 12.2 d | 50.3 a | 18.3 a | 11.7 d | 49.9 a | 26.6 a | 11.8 d | 48.5 bc |
| | One50 AR37 | 18.4 ab | 12.7 b | 47.2 c | 15.2 b | 12.3 b | 48.3 bc | 25.2 b | 12.1 c | 49.5 ab |
| | Prospect AR37 | 18.5 ab | 12.4 d | 49.1 ab | 15.6 b | 12.1 c | 49.2 ab | 25.3 b | 12.0 c | 50.0 a |
| SED | | 0.59 | 0.08 | 0.63 | 0.73 | 0.07 | 0.61 | 0.47 | 0.10 | 0.69 |
| N x Clover treatment | High N + clover | 19.9 a | 12.3 | 49.1 a | 17.7 a | 12.2 ab | 47.2 b | 27.4 a | 12.1 | 48.0 b |
| | High N - clover | 18.7 b | 12.3 | 50.5 a | 15.5 b | 12.3 ab | 47.5 b | 27.8 a | 12.2 | 47.6 b |
| | Low N + clover | 19.1 ab | 12.6 | 48.6 a | 18.9 a | 12.4 a | 46.5 b | 24.9 b | 12.4 | 46.8 b |
| | Low N - clover | 14.4 c | 13.0 | 44.3 b | 13.1 c | 12.2 b | 49.9 a | 22.9 c | 12.1 | 50.4 a |
| | | 0.51 | 0.14 | 1.39 | 0.95 | 0.09 | 0.62 | 0.78 | 0.12 | 0.65 |
| *P* value | N effect | < 0.001 | < 0.001 | < 0.01 | 0.416 | 0.923 | 0.074 | < 0.001 | 0.190 | 0.092 |
| | Clover effect | < 0.001 | 0.082 | 0.182 | < 0.001 | 0.387 | < 0.01 | 0.152 | 0.302 | < 0.01 |
| | Cultivar effect | < 0.05 | < 0.001 | < 0.001 | < 0.001 | < 0.001 | < 0.001 | < 0.001 | < 0.001 | < 0.001 |
| | N x clover interaction | < 0.001 | 0.114 | < 0.05 | < 0.05 | < 0.05 | < 0.01 | < 0.05 | 0.065 | < 0.001 |
| | N x cultivar interaction | 0.400 | < 0.05 | 0.063 | 0.118 | < 0.001 | 0.207 | < 0.05 | 0.920 | 0.839 |
| | Clover x cultivar interaction | 0.518 | 0.074 | 0.050 | 0.491 | < 0.001 | 0.169 | 0.631 | 0.759 | 0.667 |

SED = standard error of the difference between means.

### Metabolisable Energy (ME)

The effects of N and clover treatment on the ME density of pastures were inconsistent. In summer 2012 – 13, ME was greater in the High N treatment than the Low N treatment, however the reverse applied in autumn 2013 and spring 2013 (Tables 3.15 and 3.16). There was, however, a significant N × clover interaction in summer 2012 – 13; here, including clover in the mixture significantly increased ME under Low N, but not High N (Table 3.15). A similar, but smaller trend, was observed in summer 2013-14 (Table 3.16).

Cultivars differed in their ME density during all seasons in both years ($P < 0.001$). Bealey NEA2 and Base AR37 generally had the highest ME density and Prospect AR37, Commando AR37 and Kamo AR37 generally had lowest ME.

However, there were several interactions involving cultivar. The most consistent of these was between clover and cultivar, which was significant in summer in both years. In summer 2012 – 13, the ME density of pastures based on Alto AR37, Commando AR37 and Kamo AR37 increased when sown with clover whereas there was no difference between clover treatments for the other cultivars (Table 3.17). In summer 2013 – 14, pastures based on Bealey NEA2 and Commando AR37 had higher ME density when sown with clover compared with minus clover but there was no difference between plus and minus clover treatment for all other cultivars (Table 3.17).

No three-way interaction between N, clover and cultivar for the ME density of pastures was detected in any season.

**Table 3.17 ME density (MJ/kg DM) of pastures sown with or without white clover**

| | Summer 2012 - 13 | | | | Summer 2013 - 14 | | | |
|---|---|---|---|---|---|---|---|---|
| | + clover | | - clover | | + clover | | - clover | |
| Abermagic AR1 | 11.8 | ab | 11.6 | a | 12.5 | b | 12.6 | a |
| Alto AR37 | 11.5 | cd | 11.1 | b | 12.4 | bc | 12.3 | b |
| Base AR37 | 11.9 | ab | 11.8 | a | 12.5 | b | 12.7 | a |
| Bealey NEA2 | 12.1 | a | 11.8 | a | 12.8 | a | 12.5 | ab |
| Commando AR37 | 11.3 | de | 10.7 | c | 12.2 | cd | 11.8 | d |
| Kamo AR37 | 11.3 | de | 10.6 | c | 11.8 | e | 11.6 | d |
| One50 AR37 | 11.7 | bc | 11.6 | a | 12.2 | cd | 12.3 | b |
| Prospect AR37 | 11.1 | e | 11.1 | b | 12.1 | d | 12.1 | c |
| SED within clover treatment | 0.14 | | | | 0.10 | | | |
| SED between clover treatments | 0.16 | | | | 0.12 | | | |

Different letters within a column indicate statistical differences.

### Neutral detergent fibre (NDF)

During the first spring of the experiment, N and clover treatments both affected the NDF content of pastures, which was greater in the High N treatment than the Low N, and in the minus clover treatment than the plus clover treatment (Table 3.15).

From summer 2012 – 13 onwards, interactions between N and clover were present in every season. The presence of clover did not affect the NDF of pastures when grown under the High N treatment, but under Low N, the presence of clover decreased the NDF of the herbage, with the exception of spring 2013, when the NDF of the herbage was lower in the absence of clover (Tables 3.15 and 3.16).

Cultivar had significant effect on NDF in all seasons in both years. Bealey NEA2, Base AR37 and Abermagic AR1 were generally amongst the cultivars with the lowest NDF content in pastures. Kamo AR37 and Commando AR37 were the cultivars with highest NDF during spring in both years, while Prospect AR37 tended to be in the group with the highest NDF from the first summer of the experiment onwards.

There was an interaction between cultivar and clover treatment during summer 2012 – 13 (Table 3.15), when NDF was higher in the absence of clover than in the presence of clover for most of the cultivars with the exception of Prospect AR37 and Alto AR37. Abermagic AR1 was the cultivar with lowest NDF content in the presence or absence of clover (Table 3.18)

No three-way interactions between N, clover and cultivar for the NDF content were detected during any season in both years.

**Table 3.18 NDF (%DM) content of pastures sown with or without white clover.**

| | Summer 2012 - 13 | | | |
|---|---|---|---|---|
| | + clover | | - clover | |
| Abermagic AR1 | 48.6 | c | 51.7 | e |
| Alto AR37 | 54.0 | a | 54.9 | cd |
| Base AR37 | 50.4 | bc | 52.9 | de |
| Bealey NEA2 | 50.0 | bc | 52.5 | e |
| Commando AR37 | 52.1 | ab | 57.2 | ab |
| Kamo AR37 | 50.5 | bc | 58.1 | a |
| One50 AR37 | 49.6 | c | 52.9 | de |
| Prospect AR37 | 53.9 | a | 55.4 | bc |
| SED within clover treatment | | 1.09 | | |
| SED between clover treatment | | 1.19 | | |

Different letters within a column indicate statistical differences.

### 3.4.7 Pasture nutritive value: phenotypic contrast level

#### Morphological contrast

Nutritive value of pastures based on perennial ryegrass cultivars with contrasting morphologies is presented in Tables 3.19 and 3.20.

#### Crude protein (CP)

The morphological contrast affected the CP content of pastures only in spring 2013, when CP % was greater in pastures based on dense cultivars than open cultivars (Tables 3.19 and 3.20).

#### Metabolisable Energy (ME) and Neutral detergent fibre (NDF)

Open cultivars had greater ME density than dense cultivars in all seasons of both years (Tables 3.19 and 3.20). However there was also a significant difference in the ME density of the two cultivars representing the dense phenotype in all seasons; pastures based on Abermagic AR1 had greater ME than pastures based on Prospect AR37. Moreover, in spring 2012 and in summer in both years, the ME content of pastures based on Abermagic AR1 was not significantly different from the ME of pastures based on Base AR37 (one of the open cultivars) (Tables 3.15 and 3.16).

In addition, dense cultivars had greater NDF content than open cultivars in spring in both years (Tables 3.19 and 3.20). Nevertheless, there was also a significant difference in the NDF content of the two cultivars representing the dense phenotype in these seasons; pastures based on Abermagic AR1 had similar NDF content than pastures based on the two cultivars representing the open phenotype, but lower NDF content than pastures based on Prospect AR37 (the other dense cultivar) (Tables 3.15 and 3.16).

This overlap in ME density and NDF content between cultivars within the dense and open contrasts means that robust conclusions regarding the effect of morphology cannot be drawn.

**Table 3.19 Nutritive value of pastures based on perennial ryegrass cultivars with contrasting morphology and heading date during 2012 – 13.**

| | | Spring 2012 | | | Summer 2012 - 2013 | | | Autumn 2013 | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Perennial ryegrass contrasts | | CP (%DM) | ME (MJ/kg DM) | NDF (%DM) | CP (%DM) | ME (MJ/kg DM) | NDF (%DM) | CP (%DM) | ME (MJ/kg DM) | NDF (%DM) |
| Morphology | Dense | 11.8 | 12.7 | 45.5 | 14.8 | 11.4 | 52.4 | 19.8 | 12.1 | 45.1 |
| | Open | 12.2 | 13.2 | 42.9 | 14.5 | 11.9 | 51.4 | 19.8 | 12.6 | 44.9 |
| Heading date | Mid | 11.3 | 11.9 | 50.3 | 15.4 | 11.0 | 54.5 | 20.7 | 11.7 | 47.3 |
| | Late | 12.0 | 12.7 | 46.0 | 14.2 | 11.5 | 52.9 | 20.1 | 12.1 | 47.3 |
| SED | | 0.46 | 0.07 | 0.48 | 0.44 | 0.07 | 0.54 | 0.31 | 0.06 | 0.53 |
| *P* value | Morphology | 0.386 | < 0.001 | < 0.001 | 0.464 | < 0.001 | 0.085 | 0.888 | < 0.001 | 0.698 |
| | Heading date | 0.176 | < 0.001 | < 0.001 | < 0.01 | < 0.001 | < 0.01 | 0.053 | < 0.001 | 0.898 |
| | Morphology x N | 0.743 | 0.561 | 0.809 | 0.487 | 0.397 | 0.620 | 0.238 | 0.425 | 0.180 |
| | Morphology x clover | 0.707 | 0.152 | 0.202 | 0.394 | 0.508 | 0.859 | 0.051 | 0.279 | 0.309 |
| | Heading date x N | 0.609 | 0.669 | 0.539 | 0.904 | 0.309 | 0.510 | 0.993 | 0.494 | 0.291 |
| | Heading date x clover | 0.926 | 0.626 | 0.105 | 0.194 | < 0.01 | < 0.001 | 0.771 | 0.052 | 0.053 |

SED = standard error of the difference between means.

**Table 3.20 Nutritive value of pastures based on perennial ryegrass cultivars with contrasting morphology and heading date during 2013 – 14.**

| | | Spring 2013 | | | Summer 2013 - 2014 | | | Autumn 2014 | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Perennial ryegrass contrasts | | CP (%DM) | ME (MJ/kg DM) | NDF (%DM) | CP (%DM) | ME (MJ/kg DM) | NDF (%DM) | CP (%DM) | ME (MJ/kg DM) | NDF (%DM) |
| Morphology | Dense | 18.7 | 12.5 | 47.9 | 15.9 | 12.3 | 47.0 | 25.3 | 12.2 | 48.0 |
| | Open | 17.8 | 12.9 | 46.7 | 15.6 | 12.6 | 46.8 | 25.6 | 12.6 | 47.3 |
| Heading date | Mid | 17.4 | 12.3 | 50.0 | 18.0 | 11.8 | 49.1 | 26.8 | 11.9 | 48.2 |
| | Late | 18.3 | 12.6 | 47.9 | 15.6 | 12.3 | 48.1 | 25.4 | 12.1 | 49.2 |
| SED | | 0.41 | 0.05 | 0.45 | 0.52 | 0.05 | 0.43 | 0.33 | 0.07 | 0.49 |
| *P* value | Morphology | < 0.05 | < 0.001 | < 0.01 | 0.616 | < 0.001 | 0.702 | 0.375 | < 0.001 | 0.134 |
| | Heading date | < 0.05 | < 0.001 | < 0.001 | < 0.001 | < 0.001 | < 0.05 | < 0.001 | < 0.01 | < 0.05 |
| | Morphology x N | 0.279 | 0.861 | 0.948 | 0.244 | 0.064 | 0.402 | 0.216 | 0.492 | 0.691 |
| | Morphology x clover | 0.626 | 0.493 | 0.399 | 0.923 | 0.210 | 0.874 | 0.579 | 0.981 | 0.281 |
| | Heading date x N | 0.550 | 0.136 | 0.457 | 0.357 | 0.984 | 0.138 | 0.424 | 0.671 | 0.774 |
| | Heading date x clover | 0.655 | 0.554 | 0.127 | < 0.05 | < 0.01 | 0.077 | 0.836 | 0.556 | 0.756 |

SED = standard error of the difference between means.

### Heading date contrast

Nutritive value of pastures based on perennial ryegrass cultivars with contrasting heading dates is presented in Tables 3.19 and 3.20.

### Crude protein (CP)

During summer in both years, and in autumn 2014, pastures based on mid heading cultivars had greater CP% than pastures based on late heading cultivars, while the opposite occurred in spring 2013 (Tables 3.19 and 3.20). However, in summer 2013 – 14 there was a significant interaction ($P < 0.05$) between heading date and clover treatment. The CP content of pastures based on both heading dates were not significantly different when sown with clover, but it was greater for mid heading cultivars than for late heading cultivars in the absence of clover (Table 3.21).

**Table 3.21 CP (%DM) content of pastures based on cultivars with contrasting heading date and sown with or without clover.**

| | Summer 2013 - 14 | | | |
|---|---|---|---|---|
| | + clover | | - clover | |
| Mid heading | 19.5 | a | 16.6 | a |
| Late heading | 18.3 | a | 13.0 | b |
| SED within clover treatment | 0.73 | | | |
| SED between clover treatments | 0.83 | | | |

Different letters within a column indicate statistical differences.

### Metabolisable energy (ME)

Late heading cultivars had greater ME density than mid heading cultivars in all seasons of both years (Tables 3.19 and 3.20).

Significant interactions between heading date and clover treatment were detected in both summers. In summer 2012 – 13, the presence of clover increased significantly the ME density of both heading date contrasts, but this increment was greater for mid than for late heading cultivars. Meanwhile, in summer of the second year, ME density only increased in pastures based on mid heading date cultivars when grown in association with clover (Table 3.22).

**Table 3.22 ME density (MJ/kg DM) of pastures based on cultivars with contrasting heading date and sown with or without white clover**

| | Summer 2012 - 13 | | | | Summer 2013 - 14 | | | |
|---|---|---|---|---|---|---|---|---|
| | + clover | | - clover | | + clover | | - clover | |
| Mid heading | 11.3 | b | 10.6 | b | 12.0 | b | 11.7 | b |
| Late heading | 11.6 | a | 11.3 | a | 12.3 | a | 12.3 | a |
| SED within clover treatment | 0.10 | | | | 0.07 | | | |
| SED between clover treatments | 0.11 | | | | 0.08 | | | |

Different letters within a column indicate statistical differences.

### Neutral detergent fibre (NDF)

In spring in both years, pastures based on mid heading cultivars had greater NDF content than pastures based on late cultivars (Tables 3.19 and 3.20).

In summer in both years, mean NDF content of pastures based on mid heading cultivars (Commando AR37 and Kamo AR37) was also greater than for pastures based on late cultivars (Alto AR37 and One50 AR37). However, in summer 2012 – 13, the NDF content of pastures based on Alto AR37 was not significantly different from the NDF of pastures based on Commando AR37 and Kamo AR37, but it was greater than from pastures based on One50 AR37. Meanwhile, in summer 2013 – 14, the NDF content of pastures based on Kamo AR37 was greater than the NDF content of pastures based on Commando AR37, One50 AR37 and Alto AR37 (Tables 3.15 and 3.16). This confounding effect is also present when considering the interaction with clover treatment in summer 2012 – 13.

In autumn 2014, late cultivars had greater NDF content than mid cultivars ($P = 0.031$).

## 3.4.8 Perennial ryegrass and white clover population density: cultivar level

### Perennial ryegrass tiller density

In autumn 2013 there was a significant interaction between N and cultivar in ryegrass tiller density (Table 3.23 and Figure 3.6). Tiller density increased for all the cultivars when grown under the high N level compared with the low N level except for Bealey NEA2. The result of this interaction was a scaling effect whereby the difference between the most– and least– dense cultivars was greater under high N (91 %) than under low N (58 %).

Pastures based on Bealey NEA2 had lower tiller density than pastures based on all other cultivars in both years (Table 3.23).

**Table 3.23 Perennial ryegrass tiller density (tillers/m²) on pastures sown with or without clover, and receiving high (325 kg N/ha) or low (100 kg N/ha) N fertiliser annually.**

| | | Autumn 2013 | | Autumn 2014 | |
|---|---|---|---|---|---|
| Nitrogen treatment | High | 8872 | | 6806 | |
| | Low | 6646 | | 6568 | |
| Clover treatment | + clover | 7087 | | 5943 | |
| | – clover | 8431 | | 7431 | |
| SED | | 417.7 | | 275.2 | |
| Perennial ryegrass cultivar | Kamo AR37 | 9563 | a | 7553 | a |
| | Abermagic AR1 | 9057 | a | 7495 | a |
| | Commando AR37 | 8272 | b | 7420 | a |
| | Prospect AR37 | 7784 | bc | 6418 | bc |
| | Alto AR37 | 7581 | bc | 6493 | bc |
| | One50 AR37 | 7343 | c | 7102 | ab |
| | Base AR37 | 7035 | c | 5977 | c |
| | Bealey NEA2 | 5438 | d | 5037 | d |
| SED | | 386.3 | | 392.2 | |
| N x Clover treatment | High N + clover | 8390 | | 6329 | |
| | High N - clover | 9354 | | 7283 | |
| | Low N + clover | 5784 | | 5556 | |
| | Low N - clover | 7508 | | 7579 | |
| SED | | 590.7 | | 389.2 | |
| *P* value | N effect | < 0.001 | | 0.404 | |
| | Clover effect | < 0.01 | | < 0.001 | |
| | Cultivar effect | < 0.001 | | < 0.001 | |
| | N x Clover interaction | 0.381 | | 0.076 | |
| | N x Cultivar interaction | < 0.05 | | 0.889 | |
| | Clover x Cultivar interaction | 0.415 | | 0.853 | |

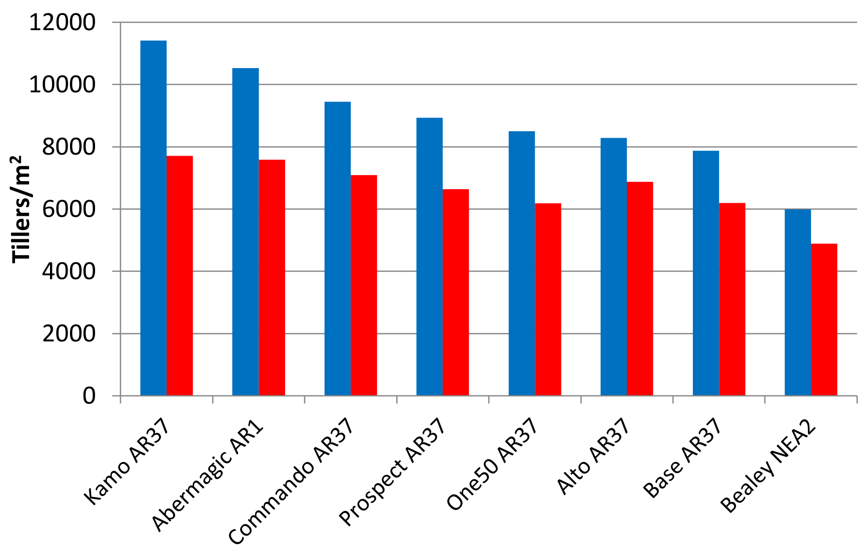


**Figure 3.6 Perennial ryegrass tiller density (tillers/m²) during autumn 2013. High (blue bar) or Low (red bar) rates of N fertiliser. SED between N treatments – 660.1 tillers/m²; SED within N treatment – 546.3 tillers/m².**

Tiller density was greater in the absence of clover (ryegrass monoculture) than in the presence of clover (mixed pastures) in both years (Table 3.23).

### White clover growing point density

Pastures in the Low N treatment had more white clover growing points per $m^2$ than pastures in the High N treatment in both years (Table 3.24)

**Table 3.24 White clover growing points (growing points/m2) on pastures from the with clover treatments only**

| | | Autumn 2013 | Autumn 2014 | |
|---|---|---|---|---|
| Nitrogen treatment | High | 904 | 469 | |
| | Low | 1674 | 1116 | |
| SED | | 85.2 | 198.1 | |
| Perennial ryegrass cultivar | Alto AR37 | 1404 | 1034 | a |
| | Bealey NEA2 | 1370 | 916 | ab |
| | Abermagic AR1 | 1388 | 814 | abc |
| | Base AR37 | 1320 | 804 | abc |
| | One50 AR37 | 1146 | 822 | bc |
| | Commando AR37 | 1350 | 634 | bc |
| | Prospect AR37 | 1036 | 682 | c |
| | Kamo AR37 | 1300 | 636 | c |
| SED | | 170.5 | 172.1 | |
| *P* value | N effect | < 0.001 | < 0.05 | |
| | Cultivar effect | 0.251 | < 0.05 | |
| | N x Cultivar interaction | 0.982 | 0.299 | |

Note: in this table, the number of growing points/$m^2$ and the SED are from the analysis without square root transformation but *P* values and letters are from the analysis with square root transformation.

Growing point density decreased between 2013 and 2014 ($P$ <0.001; repeated measures analysis of square root transformed data) and this decline was consistent across treatments.

There was an effect of ryegrass cultivar on the white clover growing point density in 2014, but not in 2013. In 2014, Alto AR37 had the highest density of growing points, although not significantly different from Bealey NEA2, Abermagic AR1 and Base AR37, while One50 AR37, Commando AR37, Prospect AR37 and Kamo AR37 had significantly lower growing point density than Alto AR37.

When possible associations between the number of tillers/$m^2$ and the number of growing points/$m^2$ was investigated, significant negative associations ($P$ value <0.001) were found for both seasons, either combined (N and Cultivar) or pooled within treatment, but this did not account for a high proportion of the variation in the data (values not shown; Autumn 2013, combined $r^2$ = 0.27; Autumn 2014, combined $r^2$ = 0.10).

### 3.4.9 Perennial ryegrass and white clover population density: phenotypic contrast level

#### Morphological contrast

#### Perennial ryegrass tiller density

Pastures based on dense cultivars had greater tiller density than pastures based on open cultivars in both years (Table 3.25).

**Table 3.25 Tiller density (tillers/m$^2$) of pastures sown with perennial ryegrass with contrasting morphology and heading date.**

| Perennial ryegrass contrasts | | Autumn 2013 | Autumn 2014 |
|---|---|---|---|
| Morphology | Dense | 8421 | 6957 |
| | Open | 6237 | 5507 |
| Heading date | Mid | 8918 | 7487 |
| | Late | 7462 | 6798 |
| SED | | 273.2 | 277.3 |
| *P* value | Morphology | < 0.001 | < 0.001 |
| | Heading date | < 0.001 | < 0.05 |
| | Morphology x N | < 0.05 | 0.174 |
| | Morphology x Clover | 0.396 | 0.370 |
| | Heading date x N | < 0.05 | 0.648 |
| | Heading date x Clover | 0.788 | 0.752 |

A significant interaction between morphology contrast and N treatment was detected in autumn 2013 (Figure 3.7). Pastures based on dense cultivars had 28 % more tillers than pastures based on open cultivars when grown under Low N treatment, but 40 % more tillers when grown under High N treatment.

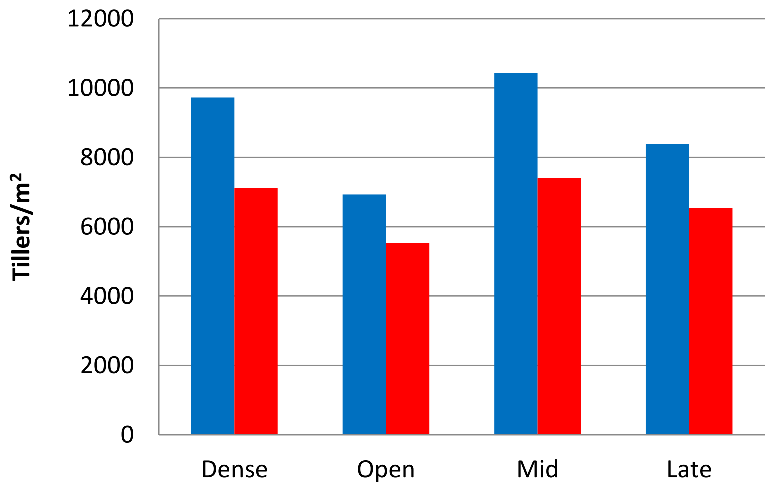


**Figure 3.7 Tiller density of perennial ryegrass cultivars with contrasting phenotypes – autumn 2013. High (blue bar) or Low (red bar) rates of N fertiliser. SED within N treatment 386.3 tillers/m$^2$.**

### White clover growing point density

Pastures based on dense and open cultivars did not differ significantly in the growing point density in any of the two years ($P$ = 0.191 and 0.394 for autumn 2013 and 2014 respectively).

### Heading date contrast

### Perennial ryegrass tiller density

Pastures based on mid heading date cultivars had greater tiller density than pasture based on late heading date cultivars in both years (Table 3.25).

A significant interaction between heading date contrast and N was detected in autumn 2013 (Figure 3.7). Pastures based on mid heading cultivars had 13 % more tillers than pastures based on late heading cultivars when grown under Low N treatment, but 24 % more tillers when grown under High N treatment.

### White clover growing point density

In autumn 2014 white clover growing point density was greater in pastures based on late heading cultivars than on pastures based on mid heading cultivars ($P < 0.05$; 928 growing points/m$^2$ in late cultivars versus 635 growing points/m$^2$; SED – 121.7 growing points/m$^2$). No effect of the heading date contrast on the white clover growing point density was detected in autumn 2013.

## 3.4.10 Endophyte infection frequency

Cultivars differed in their endophyte infection frequency in autumn 2013 and autumn 2014 (Table 3.26). Base AR37 and One50 AR37 had the greatest endophyte infection in 2013 while Commando AR37 had the lowest infection rate. In autumn 2014, Commando AR37 had the lowest endophyte infection frequency, and there was no difference among the other seven cultivars. Overall, there was a significant increase in infection frequency between 2013 and 2014 (mean 85.5 % versus 80.0 %, $P$ = 0.008, repeated measures analysis) which was consistent across cultivars.

**Table 3.26 Endophyte infection (% of tillers sampled) in pastures of the Low N plus clover treatment**

| | | Autumn 2013 | | Autumn 2014 | |
|---|---|---|---|---|---|
| Perennial ryegrass cultivar | Abermagic AR1 | 80.7 | ab | 92.0 | a |
| | Base AR37 | 89.3 | a | 90.0 | a |
| | One50 AR37 | 88.0 | a | 88.0 | a |
| | Bealey NEA2 | 76.0 | bc | 86.8 | a |
| | Kamo AR37 | 82.7 | ab | 86.0 | a |
| | Alto AR37 | 77.3 | b | 84.0 | a |
| | Prospect AR37 | 79.3 | ab | 84.0 | a |
| | Commando AR37 | 66.7 | c | 73.3 | b |
| SED | | 4.9 | | 3.8 | |
| *P* value | | < 0.01 | | < 0.01 | |

### 3.4.11 Reproductive development

Since the number of days between grazing and measurement were not the same in the High and the Low N treatments, adjusted MSC cannot be compared across N treatments. Hence, data are presented separately for the two N levels.

Cultivars differences in heading date were reflected in the stage of development of ryegrass tillers (adjusted MSC) in October and November 2013 (Tables 3.27 and 3.28). Kamo AR37 and Commando AR37 were significantly more advanced than other cultivars under both N levels.

No cultivar effect on adjusted MSC was detected in December 2013.

**Table 3.27 Adjusted MSC of tillers sampled in pastures of the High N minus clover treatment**

| Season 2013 - 2014 | | 15th October | | 5th November | | 17th December |
|---|---|---|---|---|---|---|
| Perennial ryegrass cultivars | Kamo AR37 | 9.8 | a | 31.2 | a | 16.9 |
| | Commando AR37 | 8.1 | ab | 29.3 | a | 18.6 |
| | Prospect AR37 | 6.8 | ab | 14.4 | b | 15.9 |
| | Alto AR37 | 3.9 | bc | 11.3 | bc | 18.9 |
| | One50 AR37 | 3.9 | bc | 7.9 | bc | 11.9 |
| | Abermagic AR1 | 1.6 | cd | 6.0 | c | 8.4 |
| | Bealey NEA2 | 1.5 | cd | 7.4 | bc | 8.3 |
| | Base AR37 | 0.7 | d | 8.0 | bc | 20.2 |
| SED | | 2.4 | | 4.1 | | 4.6 |
| *P* value | | 0.002 | | < 0.001 | | 0.100 |

In this table the adjusted MSC values are from the analysis without square root transformation, but *P* values and letters are from the results with transformation.

**Table 3.28 Adjusted MSC of tillers sampled in pastures of the Low N minus clover treatment**

| Season 2013 - 2014 | | 15th October | | 5th November | | 9th December |
|---|---|---|---|---|---|---|
| Perennial ryegrass cultivars | Kamo AR37 | 6.8 | ab | 53.4 | a | 49.9 |
| | Commando AR37 | 7.2 | a | 40.9 | a | 48.9 |
| | Prospect AR37 | 5.2 | abc | 19.7 | b | 32.4 |
| | One50 AR37 | 2.0 | cd | 15.0 | bc | 36.2 |
| | Alto AR37 | 3.8 | abcd | 12.0 | bcd | 39.6 |
| | Abermagic AR1 | 2.7 | bcd | 10.4 | cd | 41.3 |
| | Bealey NEA2 | 1.3 | d | 10.0 | cd | 42.0 |
| | Base AR37 | 3.7 | abcd | 5.9 | d | 37.8 |
| SED | | 1.8 | | 5.9 | | 8.8 |
| *P* value | | 0.027 | | < 0.001 | | 0.546 |

In this table the adjusted MSC values are from the analysis without square root transformation, but *P* values and letters are from the results with transformation.

In the High N treatments in December, the measurements were conducted 21 days after the plots were mown to bring all treatments back to a common residual herbage mass post-grazing; this could explain the sharp decrease in MSC of the mid heading cultivars (Kamo AR37 and Commando AR37) in December compared with November (Table 3.27).

The contribution of reproductive tillers to the total tiller sample (% of the total tiller number) varied between cultivars in November, in both N treatments (Tables 3.29 and 3.30). Kamo AR37 and Commando AR37 were the cultivars with highest presence of tillers in reproductive stages, expressed either as percentage of total tillers or % total sample dry weight (data not presented).

**Table 3.29 Reproductive tillers as a percentage of the tillers sampled in pastures of the High N minus clover treatment**

| | | 15th October | 5th November | | 17th December |
|---|---|---|---|---|---|
| Perennial ryegrass cultivars | Kamo AR37 | 0 | 22.2 | a | 13.3 |
| | Commando AR37 | 0 | 16.7 | a | 13.3 |
| | Alto AR37 | 0 | 1.1 | b | 14.4 |
| | Prospect AR37 | 0 | 1.1 | b | 10.0 |
| | Abermagic AR1 | 0 | 0.0 | b | 5.6 |
| | Base AR37 | 0 | 0.0 | b | 16.7 |
| | Bealey NEA2 | 0 | 0.0 | b | 5.6 |
| | One50 AR37 | 0 | 0.0 | b | 8.9 |
| SED | | | 2.3 | | 3.8 |
| *P* value | | | < 0.001 | | 0.078 |

In this table the % are from the analysis without angular transformation, but *P* values and letters are from the results with transformation.

**Table 3.30 Reproductive tillers as a % of the tillers sampled in pastures of the Low N minus clover treatment**

| | | 15th October | 5th November | | 9th December |
|---|---|---|---|---|---|
| Perennial ryegrass cultivars | Kamo AR37 | 0 | 45.6 | a | 36.7 |
| | Commando AR37 | 0 | 30.0 | a | 40.0 |
| | One50 AR37 | 0 | 3.3 | b | 31.1 |
| | Abermagic AR1 | 0 | 1.1 | b | 34.4 |
| | Alto AR37 | 0 | 1.1 | b | 32.2 |
| | Prospect AR37 | 0 | 1.1 | b | 25.6 |
| | Base AR37 | 0 | 0.0 | b | 30.0 |
| | Bealey NEA2 | 0 | 0.0 | b | 31.1 |
| SED | | | 5.7 | | 6.8 |
| *P* value | | | < 0.001 | | 0.576 |

In this table the % are from the analysis without angular transformation, but *P* values and letters are from the results with transformation.

Since no reproductive tillers (R ≥ 0, Moore & Moser, 1995; Moore et al., 1991) were observed until November, adjusted MSC values in October reflect stem elongation only.

Under both N treatments, the heading date contrast (mid cultivars Kamo AR37 and Commando AR37 versus late cultivars Alto AR37 and One50 AR37) had a significant effect on the adjusted MSC in October and November ($P$ = 0.010 and 0.005 in High and Low N treatment in October; $P$ < 0.001 for both High and Low N treatment in November; analysis of transformed data). Mid heading date cultivars had greater adjusted MSC than late heading date cultivars, but no effect of this contrast was detected in December.

Adjusted MSC of tillers followed the trend expected based on cultivar heading dates reported by the breeders of the cultivars included in the study (Figure 3.8, November 2013, $P$ < 0.001 for both N treatments).

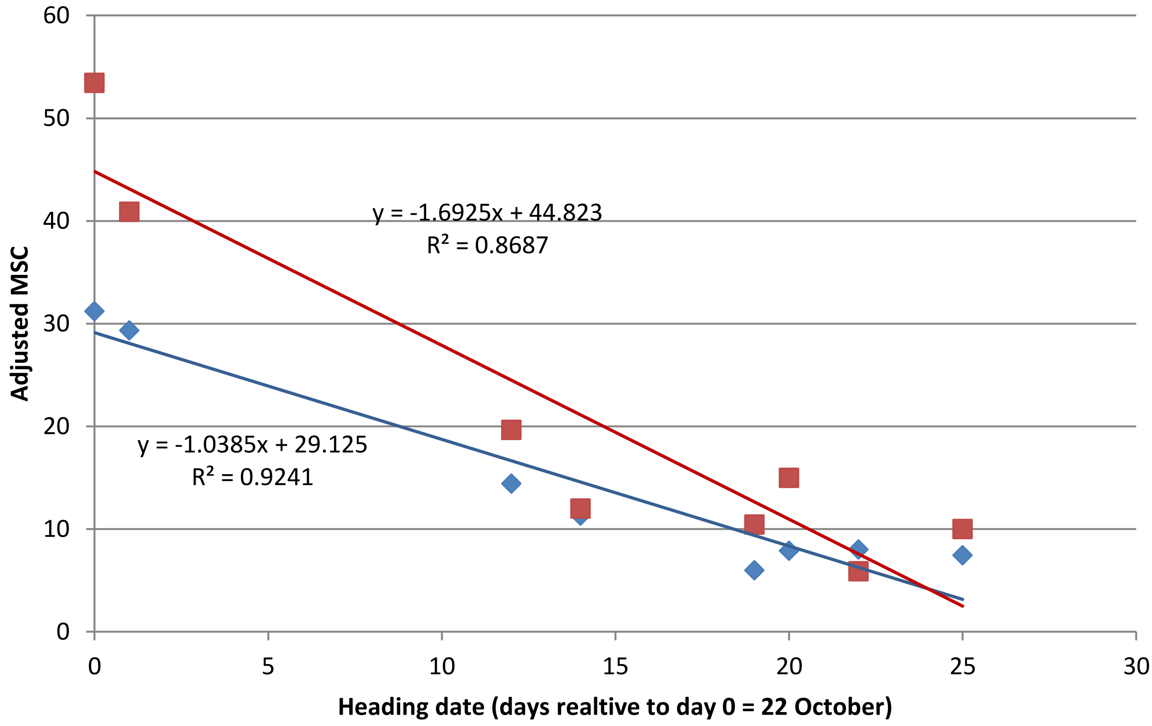


**Figure 3.8 Adjusted MSC of tillers from different cultivars on High or Low N minus clover treatments versus heading date of perennial ryegrass cultivar (November 2013). High (blue) or Low (red) rates of N fertiliser.**

## 3.4.12 Light interception and canopy height

Only results related to the cultivar effect will be considered here because the measurements for the different main treatments were not conducted on the same dates.

Pastures based on Prospect AR37 intercepted more light both during spring 2013 and summer 2013-14 (Table 3.31), while pastures based on Kamo AR37 intercepted the least (main effect of cultivar significant at $P$ = 0.01 and $P$ = 0.045 for spring and summer respectively).

Clover height, measured at the same time as light interception, was not affected by perennial ryegrass cultivar in spring or summer (*P* = 0.912 and 0.435 respectively).

Prospect AR37 had the highest herbage mass when these measurements were taken in spring and summer, while Bealey NEA2 had the lowest herbage mass (Table 3.31). The white clover content of herbage during spring was lower in the Prospect AR37 pastures than in pastures sown with Bealey NEA2 or Kamo AR37, resulting in a lower yield of white clover (kg DM/ha) (Table 3.31). However during summer, there was no cultivar effect on the white clover content or yield of the pastures (*P* = 0.168 and 0.734 for clover % and DM/ha respectively).

### 3.4.13 Leaf regrowth stage

During the season 2013 – 2014, samplings pre-grazing to determine leaf regrowth stage were conducted on pastures from the Block 2. The purpose of these samplings was to check the correct timing of grazing and to track seasonal trends for the different cultivars. The results (data not presented) show that leaf stage pre-grazing was in the range of 2.0 to 2.5 during most of the season.

**Table 3.31 Light interception, canopy height and kg DM/ha in subplots of cultivars Bealey NEA2, Kamo AR37 and Prospect AR37 (means for all four treatment combinations).**

| | Spring 2013 | | | | | | | | | | Summer 2013-14 | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Cultivar | % PAR intercepted | | Perennial ryegrass height (cm) | White clover heigth (cm) • | Total kg DM/ha all treatments | | White clover % • | | WC kg DM/ha • | | % PAR Intercepted | | Perennial ryegrass height (cm) | | White clover heigth (cm) • | Total kg DM/ha all treatments | | White clover % • | WC kg DM/ha • |
| Prospect AR37 | 61.4 | a | 15.6 | 9.3 | 2760 | a | 6.8 | b | 169 | b | 53.9 | a | 15.3 | a | 10.6 | 2523 | a | 14.9 | 338 |
| Bealey NEA2 | 53.6 | b | 14.8 | 9.2 | 2013 | b | 20.0 | a | 411 | a | 45.4 | ab | 14.1 | a | 9.3 | 1887 | b | 19.9 | 368 |
| Kamo AR37 | 49.9 | b | 14.9 | 8.9 | 2442 | a | 13.6 | a | 317 | ab | 42.3 | b | 12.2 | b | 10.3 | 2305 | a | 19.5 | 397 |
| SED | 3.6 | | 0.8 | 1.0 | 156 | | 3.1 | | 70.4 | | 4.6 | | 0.6 | | 1.0 | 138.2 | | 2.8 | 74.5 |
| *P* value | 0.010 | | 0.533 | 0.912 | < 0.001 | | < 0.01 | | < 0.05 | | < 0.05 | | < 0.001 | | 0.435 | < 0.001 | | 0.168 | 0.734 |

• Results of analyses conducted in the with clover treatments only. Mean days since grazing in spring – 28; mean days since grazing in summer 21.

## 3.5 Discussion

### 3.5.1 Do cultivars re-rank?

The dynamic relationships between perennial ryegrass and white clover in a mixed sward are influenced by environmental and management factors and by intrinsic characteristics of both species (W. Harris, 1990; Schwinning & Parsons, 1996a, 1996b, 1996c). Differences in their seasonal growth rates due to different optimum temperatures for growth (Brougham, 1959; W. Harris & Hoglund, 1977; Mitchell, 1956a; Turkington & Harper, 1979a, 1979b) as well as the ability of white clover to fix atmospheric $N_2$ (Ledgard, 1991) facilitate the development of systems in which both species compete for 'different space' according to the de Wit (1960) definition. Their proportion in the sward as well as their dry matter yields are affected by the level of N fertiliser applied and the cultivars used (Camlin, 1981; Collins & Rhodes, 1989; Frame & Boyd, 1986a, 1986b; Gilliland, 1996; S. L. Harris & Clark, 1996; S. L. Harris, Clark, et al., 1996; S. L. Harris, Thom, et al., 1996; Whitehead, 1970) amongst other factors. However, despite the effects of these sources of variation on sward composition and production, under moderately high levels of N, total yield of mixed pastures sown with different perennial ryegrass cultivars tend to reflect the yield of the grass component (Camlin, 1981). Therefore, the working hypothesis was that the relative ranking of the perennial ryegrass cultivars for total dry matter yield would not change when sown with white clover under high and low N fertiliser application rates.

The combination of N and clover treatments created markedly different environments for plant growth as shown by the large range in annual yield (Table 3.4). White clover was well established in the swards; its content (expressed as % DM) was always greater in pastures of the Low N treatments than in the High N treatments. The perennial ryegrass cultivars provided contrasting phenotypes for two traits that may influence competition between grass and clover: morphology (dense versus open) and heading date (mid-season versus late-season). Thus, the environments and contrasts created by the different treatments and cultivars provided a fair test of the hypothesis.

During the two years of the experiment, significant interactions between cultivar and N and cultivar and clover were detected in winter 2013, but not during the other seasons nor in the total annual yields. As a consequence, no evidence of re-ranking emerged and therefore the hypothesis was supported by the results. Although the white clover content of the swards, expressed as % DM, was significantly different across the perennial ryegrass cultivars in three of the six sampling seasons, these differences were insufficient to cause re-ranking on a total DM yield basis. Moreover, during summer in both years and autumn in the first year, mid heading cultivars supported greater clover content than late heading cultivars, similar to the findings of Camlin (1981), Gooding, Frame, and Thomas (1996) and Hoen (1970), but this difference was not reflected in changes in relative ranking positions.

Therefore, performance values in the Forage Value Index (Chapman et al., 2016; DairyNZ), which are calculated using DM yield data from cultivar evaluation trials conducted using perennial ryegrass monocultures (Easton et al., 2001), do not need adjustment to account for grass-clover interactions over time and their effects on total pasture DM yield. These results have important implications for the breeding industry and the pastoral sector in New Zealand because they support the notion that improvements achieved by breeding programs should be reflected in increments in DM yield in mixed pastures at a farm scale.

### 3.5.2 Total DM yield

**N effects**

N had a significant effect on DM yield throughout the duration of the experiment (Table 3.4); annual total DM yield in the High N treatment was 28 % greater than in the Low N treatment during 2012 - 13 and 31 % greater during 2013 - 14. However, this response was not uniform amongst seasons and clover treatments as shown by the presence of an interaction between N and clover on DM yield in summer of the first year, autumn in both years and the annual total in both years (Table 3.4). As a result, DM yields from the Low N with clover treatment were similar to those from the High N treatments and significantly greater than from the Low N without clover treatment. Variation in the response to N is common (Ball & Field, 1982; Ball et al., 1978; Feyter, O'Connor, & Addison, 1985; S. L. Harris & Clark, 1996; S. L. Harris, Clark, et al., 1996; Hennessy et al., 2012; C. W. Holmes, 1982; Laidlaw, 1980; Moir, Cameron, Di, Roberts, & Kuperus, 2003; Shepherd & Lucci, 2011; Whitehead, 1995) and is related to variation in factors such as soil temperature, N supply by the soil, season, pasture composition, and N application rate.

For the entire grazing period (spring to autumn), the average N fertiliser response for the two years was 16.6 kg DM/kg N in the minus clover treatment and 5.9 kg DM/kg N when ryegrass was grown with clover. This considerable difference in response was mainly attributable to the contribution of the white clover to the DM yield in summer and autumn, but other factors such as an increased N supply by the soil in the white clover treatments (not measured) are likely to have also contributed.

In agreement with previous work (Feyter et al., 1985; Martin, 1960; Moir et al., 2003; O'Connor & Cumberland, 1973) the yield response to N was greater during spring than in other seasons in both years, with an average across the two springs of 21.8 kg DM/kg N in the minus clover treatments and 15.6 kg DM/kg N in the plus clover treatments. The dominant component of the pastures in the plus clover treatments during spring was perennial ryegrass, comprising approximately 80 and 86 % of the herbage during the first and second spring respectively (average of the High plus clover and Low plus clover treatments). Thus a strong response to N was not surprising given the well-known effect of this nutrient on promoting perennial ryegrass growth (S. L. Harris, Thom, et al., 1996; Whitehead, 1970; Woledge & Pearse, 1985). The contribution of clover to herbage mass (expressed as % DM) was low during spring due to its requirement for a higher temperature for optimum growth compared with ryegrass. This intrinsic relative competitive disadvantage of white clover in spring was accentuated when more N was available, especially in the spring of the establishment year, when $N_2$ fixation could have been insufficient to sustain clover growth, and the legume may have been 'competing' with grass for the 'same space' (same N) according to the de Wit definition (W. Harris, 2001). Meanwhile, the contribution of clover (expressed as % DM) in the Low N treatment during the first spring was greater, probably due to less competition for light (W. Harris, 2001) from the grass which was limited by N availability. In this environment of less N available in the soil and greater clover content, $N_2$ fixation by the legume should have been greater than under higher N availability (Ledgard et al., 2001), creating the basis for the development of an 'exploitation' interaction (Chapman et al., 1996; Schwinning & Parsons, 1996a) between both species. Average yield responses to N in the absence of clover continued at a high level in summer (17.6 kg DM/kg N; average of two summers), indicating a considerable limitation in the N supply from the soil. In the presence of clover, however, a very low response of 2.5 kg DM/kg N (average of the two years) was observed. During this time of the year higher temperatures gave a relative advantage to white clover and greater contributions to the DM yield were expected from the legume. In the Low N treatment, clover comprised 50.5 % and 36.4 % of the DM during the first and second summer respectively and a consequent increase in $N_2$ fixed (not measured) compared with the High N treatments pastures (which had 14.6 and 11.7 % DM of clover in the same periods) would be expected. Thus, the important contribution to the total DM by the clover, as well as the increased N availability for grass growth in the system due to $N_2$ fixation, created a smaller gap in yield between High and Low N treatments in the presence of clover during summer, and the consequent lower response to additional N applied as fertiliser. Average response to N during autumn was lower than in spring and it was higher in the absence than in the presence of white clover (11.8 versus 1.7 kg DM/kg N, average of the two years; Table 3.5). This lower response in autumn compared with spring is likely a consequence of less favourable environment conditions for growth as temperature, day length and radiation intensity decrease (Frame & Boyd, 1986a). Variable responses to N at this time of the year have been observed in previous studies (Feyter et al., 1985; O'Connor, 1982), and one of the possible explanations is the difference in soil N mineralisation rates during this season. A high response to N in mixed swards was reported by Moir et al. (2003) in Canterbury during autumn when N was applied in the form of urea to mixed pastures (between 10 and 15 kg DM/kg N). However, pastures in their study contained less clover during this time of the year than the Low N with clover treatment in this experiment.

### Clover effects

White clover had a significant effect on the DM yield during every season, with the exception of the first spring of the experiment, when the swards were still establishing. Mean annual total DM yield in the with clover treatments was 23 % greater than in the without clover treatments at the end of the first year and 28 % greater at the end of the second year. Most of the increase in DM due to white clover occurred in summer and autumn, which together accounted for 99 % and 72 % of the total increase during the first and second year respectively. However, due to the interactions between N and clover treatments mentioned above, the significance of clover inclusion on DM yield differed under High or Low N treatment (Figure 3.9).

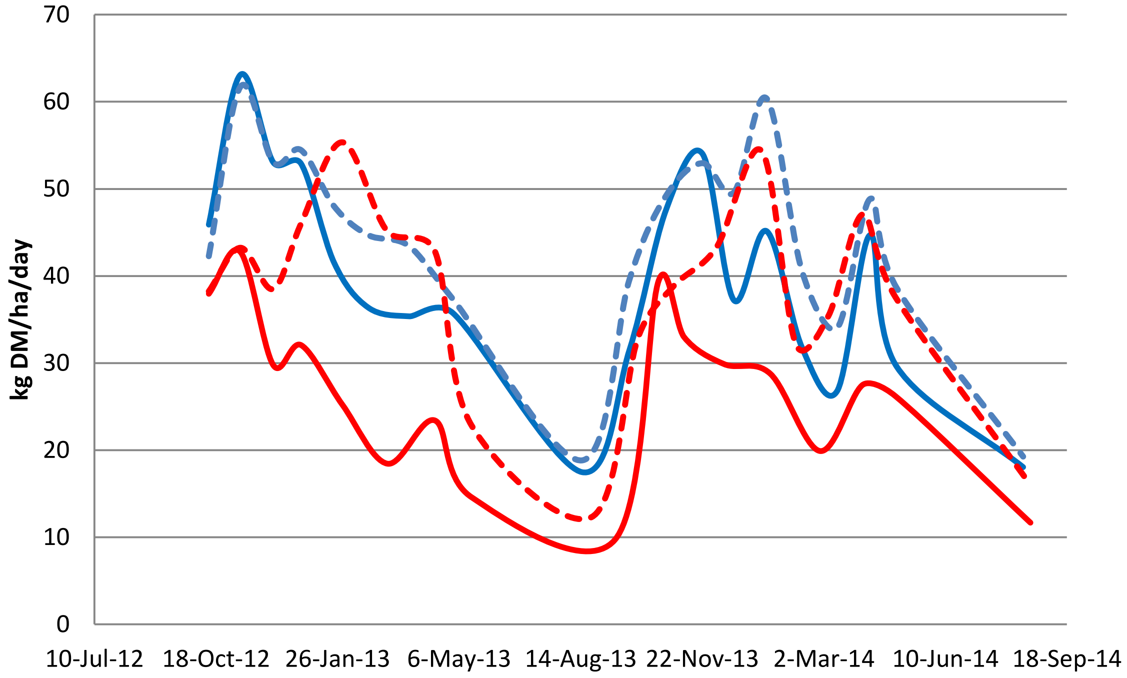


**Figure 3.9 Growth rate (kg DM/ha/day) of pastures sown plus or minus clover and receiving High or Low N fertiliser annually. High N minus clover (solid blue line), High N plus clover (dashed blue line), Low N minus clover (solid red line), Low N plus clover (dashed red line).**

DM yield gains due to the inclusion of clover in pastures have been reported previously (Enriquez-Hidalgo et al., 2016; Ledgard et al., 1990; Reid, 1983) and they are a consequence of several factors. The different seasonal growth patterns of grass and clover in New Zealand determined by their temperature requirements for optimal growth (Brougham, 1959; W. Harris & Thomas, 1973; Mitchell, 1956b; Turkington & Harper, 1979a) allow the clover to contribute to yield during warmer times of the year, especially when the ryegrass growth is depressed post-flowering (Anslow, 1965). In this way, the 'resource space' (W. Harris, 2001) is used more efficiently in a mixture than in a monoculture. The ability of clover to fix $N_2$ also contributes to the yield gain through the reduced competition for the available N in the soil, and through the increase in the pool of this nutrient in the system. Thus, when both grass monoculture and grass/clover mixture are grown under the same N regime the increased yield of the mixtures is possible because amongst other factors, the species are operating in 'different N spaces'. The more effective use of resources by perennial ryegrass and white clover mixtures than by the corresponding monocultures has also been indicated by Turkington and Jolliffe (1996), using the index relative resource total.

When the grass/clover system is operated under a low N fertiliser rate, the clover uses its ability to fix $N_2$, and becomes independent of N supply from the soil, creating the conditions for the development of an 'exploitation' interaction (W. Harris, 1990; Schwinning & Parsons, 1996a, 1996b). This type of interaction is characterised by the occurrence of cycles in which the increased N in the soil as a consequence of greater $N_2$ fixation will favour grass dominance since it benefits more per unit increase in mineral N than the legume does (Schwinning & Parsons, 1996c; Thornley et al., 1995). The increased grass growth will diminish the pool of this nutrient in the soil, promoting the development of another cycle of legume dominance. This type of interaction allows the coexistence and self-regulation of both species in the community (Schwinning & Parsons, 1996a, 1996b). In this environment of low N fertiliser, the increased yield due to clover inclusion will be not only the consequence of different seasonal growth patterns (Figure 3.9) and better use of the 'light space', but importantly due to the increased N supply through $N_2$ fixation and the contribution of the clover itself. The duration of this study limited the ability to detect the development of exploitation interaction; observations during a longer period of time would have been needed to overcome this limitation.

If the grass/clover system is managed under a high N fertiliser application rate, clover plants may substitute part of their N needs previously met from $N_2$ fixation with mineral N uptake from the soil, which has lower metabolic cost for each unit of N assimilated compared with biologically-fixed N (Ryle et al., 1979). However N fixation will continue when N is freely available in the soil, albeit at a lower rate (Ledgard, Penno, & Sprosen, 1999) and some benefit from the return of N through breakdown of dead clover material could be expected. Gains due to different seasonal growth patterns are expected in this situation as well, although at a reduced scale (Figure 3.9) due to lower clover content in the sward. The interaction between the components in the mixtures in this high N environment moves more towards competition for the 'same light space', a process that is mediated by the fact that both species have different types of leaves (plagiophile leaves for the grass and planophile leaves for the clover).

**Cultivar effects**

DM yield differed among perennial ryegrass cultivars in spring and autumn in both years, winter 2013 and in the total annual 2013 – 14. Prospect AR37, Bealey NEA2 and One50 AR37 were generally the highest yielding cultivars while Kamo AR37, Abermagic AR1 and Commando AR37 were generally among the lowest yielding cultivars from autumn 2013 onwards (Table 3.4). Differences in the structural characteristics of the swards associated with the different cultivars, such as leaf size, tiller density, as well as in the phenotypic plasticity of the different genotypes, may result in different herbage accumulation rates (Bahmani, 1999; Lee et al., 2012; Lemaire & Chapman, 1996; Sartie, Matthew, Easton, & Faville, 2011; van Loo et al., 1992).

In the experiment, tiller density was the only grass sward characteristic measured that could be used to explain differences in yield among cultivars. However, despite the key role of tillering in the productivity of pastures, the size-density compensation response of grass to environmental and management factors (Matthew, Assuero, Black, & Hamilton, 2000; Yoda, 1963), limits the utility of the tiller density alone as an indicator of productivity. Regression analysis of the relationships between tiller density and autumn yield revealed significant negative associations in the High N – clover treatment in autumn 2013 ($P$ <0.001), autumn 2014 ($P$ = 0.047) and in the Low N + clover treatment in autumn 2014 ($P$ = 0.002). However, these relationships accounted for a low proportion of the variation in the yield ($R^2$ between 0.10 and 0.27). Therefore, the effect of tiller density on DM yield has to be considered in conjunction with tiller size. For the same eight perennial ryegrass cultivars used in this experiment, lamina width, length and area, pseudo-stem length and diameter, tiller shape index, leaf : non leaf ratio, and tiller dry weight and density were assessed by Griffiths, Matthew, Lee, and Chapman (2016) when grown in monoculture. Significant cultivar differences were observed for all traits, with the exception of the pseudo-stem length. Principal component analysis in their study revealed that tiller morphology and DM yield were independent. Griffiths et al. (2016) also observed a lower slope in the relationship between logarithmic tiller dry weight and tiller density (- 1.0) compared to the theoretical (- 1.5). They concluded that the constant yield compensatory relationship observed could be the consequence of breeding and selection programmes.

Therefore, in this experiment, those cultivars with greater yields may have combined in a more effective way a collection of attributes that promoted DM accumulation under the management and environmental conditions of the experiment, and this condition should have held under both N treatments and in monocultures or mixtures with white clover.

Phenotypic contrasts were included in this study with the purpose of creating different environments for clover growth and to identify if grass phenotype characteristics, more than cultivar characteristics, could be linked to herbage yield and to interactions with white clover. Thus, cultivars were selected to provide contrasts for two traits that may influence competition between grass and clover: morphology (dense versus open) and heading date (mid-season versus late-season) (Frame & Boyd, 1986a; M. A. Sanderson & Elwinger, 1999). The morphological contrast did not work as expected (see Results 3.4.2 and section 3.6 Limitations of this study) and lacks the internal consistency required to draw robust conclusions. The performance of cultivars within the heading date contrast was internally consistent (see Results 3.4.2), allowing more confidence to be placed in the conclusions drawn from this comparison. Although in spring in both years and in summer of the first year there was no difference in yield between cultivars representing the mid or the late-heading date cultivars, the main effect of heading date strengthened over time, with late cultivars yielding more than mid cultivars during the second year (with the exception of spring 2013). However, the difference in yield does not necessarily mean an advantage due to heading date per se, and may be the results of different yield potential of the cultivars included in the contrast, management or year related factors. As an example, Gowen et al. (2003), using intermediate and late-heading date diploid and tetraploid cultivars, found that late-heading date cultivars had significantly higher herbage mass in year 1 of their study, but not in year 2. Adjusted MSC data from October and November 2013 confirmed that mid heading date cultivars matured earlier than late heading cultivars (Tables 3.27, 3.28. 3.29 and 3.30), in agreement with Wims et al. (2014a); Wims, Lee, Rossi, and Chapman (2014b) findings, confirming that the cultivars included in this contrast were appropriate.

### 3.5.3 Botanical composition

#### N effect on white clover content

The white clover content of pastures (expressed as %DM) decreased with increasing N fertiliser application rate, as expected (Caradus et al., 1993; A. Davies & Evans, 1990; Egan et al., 2015; Enriquez-Hidalgo et al., 2015; Frame & Boyd, 1986a, 1987b; Ledgard, 2001; Ledgard et al., 1995; Nassiri & Elgersma, 2002). Average content for the two years was 25.2 % DM in the Low N treatment and 8.1 % DM in the High N treatment. As a result, every 13.2 kg/ha of additional N applied (from 100 to 325 kg N/ha/year), decreased the clover content of the sward by 1 % of DM. An increase of 100 kg N/ha/year in N fertiliser application rate (in the range mentioned) resulted in a 7.6 % reduction in the clover content, similar to the 6 % (from 18 % in treatments receiving no N to 12 % in treatments receiving 100 kg N/ha) reported by O'Connor (1982) in a review of 158 trials conducted throughout New Zealand. Compared with studies conducted in Europe, it is greater than the 4.1 % reported by Egan (2015) when N fertiliser increased from 150 to 250 kg N/ha/year, but smaller than the reduction reported by Frame and Boyd (1987b) after spring applications in the range of 0 to 75 kg N/ha (17 %).

Critically, grazing management plays an important role in the manipulation of botanical composition of the sward. S. L. Harris and Clark (1996) found that it was possible to maintain a reasonable level of clover content in the sward (14.9 % DM), when the extra feed grown due to the increased N supply (200 kg N/ha/year) was used more efficiently in a farmlet with a higher stocking rate (4.5 cows/ha).

The reduction in clover content with increasing N fertiliser application is explained more by the effects of this nutrient on the grass component of the pasture, than on the clover itself. Increased leaf elongation rate, leaf size, site filling and shoot:root ratio (Ball & Field, 1982; Donald, 1963; Lemaire & Chapman, 1996; O'Connor, 1982; Robson & Deacon, 1978; Whitehead, 1995; Wilman & Asiegbu, 1982a; Wilman & Wright, 1983a) contribute to an increase in leaf area of the grass and therefore greater canopy gross photosynthesis (Robson & Parsons, 1978) when more N is available. In this way, the grass has the ability to translate the N uptake into morphological changes that favour competition for light and negatively affect the associated clover plants (Collins et al., 2003) through modification of the quantity and quality of light (red : far-red ratio) (Thompson & Harper, 1988). As the grass canopy accumulates, the red : far-red ratio of light reaching lower layers of the canopy decreases, inducing modifications in the resource allocation within and morphology of plants (M. G. Holmes & Smith, 1977). In white clover, the consequences of this alteration of the light environment are an increase in petiole length and internode length, at the expense of resources for branching and development of growing points (Caradus et al., 1993; Dennis & Woledge, 1987; S. L. Harris, Clark, et al., 1996; Hoglind & Frankow-Lindberg, 1998; Pinxterhuis, 2000; Thompson, 1995; Wilman & Asiegbu, 1982b). Also, Pinxterhuis (2000) observed a decrease in the number of rooted nodes with N fertiliser application, and as a consequence, clover plants could become more vulnerable to soil moisture stress or damage caused by root-feeding insects such as grass grub (*Costelytra zealandica* White). The decrease in rooting may impact $N_2$ fixation in the long term, through a reduction in the number of potential sites for nodule establishment (Pinxterhuis, 2000). Nitrogen fixation and the weight of nodules are reduced by the addition of N fertiliser (Burchill et al., 2014; Cowling, 1961; Crush et al., 1982; Enriquez-Hidalgo et al., 2016; S. L. Harris & Clark, 1996; Ledgard, 2001), and white clover growing point density also declines when high rates of N fertiliser are used (Caradus et al., 1993; A. Davies & Evans, 1990; Dennis & Woledge, 1985, 1987; Hoglind & Frankow-Lindberg, 1998). In the High N treatment in this study, clover growing point density was almost half that in the Low N treatment when measured in autumn in both years (Table 3.24).

In this experiment, the average white clover content in the Low N treatment (25.2 % DM), was above the minimum estimated through simulation by R. J. Thomas (1992) to provide "the N requirements for a productive and sustainable pasture" (20 – 45% of the herbage DM) and by J. R. Simpson and Stobbs (1981) to be "optimal for animal production" (20 – 30% legume DM content). However it was under the minimum of the range that Martin (1960) proposed for maximum DM and protein yield (30 – 50%). Ettema and Ledgard (1992) proposed a clover content of about 30 % to maintain high total pasture production, noting that it was "*uncommon to find more than 20 % clover content (on an annual pasture production basis)*" on most farms. By comparison, the white clover content of pastures from the High N treatment plots was below all these recommended minima in every season (Tables 3.11 and 3.12).

### Perennial ryegrass cultivar effect on white clover content

Perennial ryegrass cultivar affected the white clover content of pastures during spring in both years and summer of the second year, and the effect was similar under both high and low N fertiliser application rates (Tables 3.11 and 3.12). As mentioned earlier, the relationships between the grass and clover components of mixed pastures can be classified as 'exploitation' (Chapman et al., 1996; W. Harris, 1990; Schwinning & Parsons, 1996a) or 'competition' for the same or different 'spaces' (W. Harris, 2001). The 'exploitation' interaction is linked to the dynamics of N in the system, but not to other resources. Under the grazing management of the experiment no differences in N return from excreta among the different cultivars would be expected, and no interaction between N treatment and cultivar on clover content was detected. Therefore, the interactions between different perennial ryegrass cultivars and clover should be looked upon as competition for resources (mainly light).

The inclusion of grass monoculture treatments (minus clover) allows the effects of high-level grass traits on clover percentage in mixture to be analysed. Two key traits are grass DM yield, and tiller density. Additionally the presence of cultivars representing the heading date contrast permits the analysis of the effect of the time of reproductive development on clover content. Moreover, it was also possible to examine the effect of post-grazing residual on clover percentage, due to the preference showed by cows for some of the cultivars over others. The following sections consider these four factors and their influence in the competition between grass and clover in the sward.

*Ryegrass yield in monoculture*

When regression analyses were conducted between the seasonal yield for each cultivar in the minus clover treatments and the seasonal white clover content (%DM) in pastures sown with the same ryegrass cultivar, only two significant associations (out of a total of twelve analyses) were observed. The first occurred in autumn 2013 under the Low N treatment when a moderate negative association was present ($R^2 = 0.682$, $P = 0.012$), and the second occurred during summer 2013 – 14 also under the Low N treatment when another moderate negative association was detected ($R^2 = 0.564$, $P = 0.032$). Thus, differences in white clover content of pastures were not generally explained by the DM yield of the cultivars when grown in monoculture. A limitation of this analysis is the assumption that the clover percentage in each season will be uniform and equal to the percentage in only one sampling conducted per season. Botanical composition data for every sampling would have given more accurate information to use in this analysis.

*Tiller density*

Tiller density has been proposed to affect the white clover content of pastures through its effect on the light intercepted by the grass canopy (Brereton, Carton, & Conway, 1985; Frame & Boyd, 1986a; Gilliland, 1996). Furthermore, it has been indicated that tetraploids are more compatible with clover due to their more open habit, and their lower bulk density at the base of the canopy, compared with diploids (Frame & Laidlaw, 1998; Swift et al., 1993). This lower tiller density would allow more light to reach lower levels of the canopy and as a consequence maintain a more favourable ratio of red : far-red light compared with denser cultivars (Frame & Laidlaw, 1998; Heraut-Bron, Robin, Varlet-Grancher, Afif, & Guckert, 1999; Swift et al., 1993); changes in the morphology of clover plants with a reduction in this ratio have been observed by Thompson and Harper (1988) and Héraut-Bron, Robin, Varlet-Grancher, and Guckert (2001). Nevertheless, other research has reached different conclusions regarding the importance of tiller density as a determinant of clover compatibility; similar clover content has been observed in tetraploid and diploid cultivars, and other factors such as growth habit have been proposed to play an important role in botanical composition (Cougnon, Baert, Waes, & Reheul, 2012; Elgersma & Li, 1997; Elgersma & Schlepers, 1997a, 1997b; Rhodes & Ngah, 1983). In the present study, when mean tiller density for each cultivar in the minus clover treatment was regressed against the white clover percentage in the plus clover treatment in autumn, no significant associations were found. Similarly, when the analyses were conducted between mean tiller density for each cultivar in the plus clover treatment and the white clover percentage, within N treatment, no significant associations were found. Thus, there was no evidence to suggest that tiller density, either in monoculture of mixture explained differences among cultivars in clover content in mixed swards. There were significant negative associations (*P* value <0.001) between tillers/m$^2$ and growing points/m$^2$ in autumn 2013 and autumn 2014 but these accounted for a low proportion of the variation in the data (autumn 2013, combined $R^2$ = 0.27; autumn 2014, combined $R^2$ = 0.10). When analysis of variance was conducted on the white clover content of swards based on cultivars representing the dense and open phenotypes, the results also showed that this contrast lacked the internal consistency required to draw robust conclusions (see Results 3.4.5 and section 3.6 Limitations of this study).

Therefore other cultivar characteristics or management factors could have played a role in determining sward clover content.

*Post-grazing residual*

One of these factors is the post-grazing residual; pastures sown with the two tetraploids and Abermagic AR1, were in general grazed lower than most of the other cultivars during the two years of the experiment (Figure 3.5). During spring 2012 they also had the lowest percentage of dead material, indicating a more efficient grazing. As a result, the quantity and quality of light reaching the base of the canopy post-grazing was probably greater than in pastures sown with other cultivars. Supporting this theory is the fact that pastures sown with Abermagic AR1 and Bealey NEA2 had the greatest content of other species during spring 2012, indicating weaker competition from grass immediately after grazing during this season. The low post-grazing height in pastures sown with tetraploids may be attributed to the preference shown by cows for these cultivars, as shown by the Chesson-Manly index (Tables 3.9 and 3.10). Therefore, a low post-grazing residual during spring appears to have favoured clover growth. O'Donovan and Delaby (2005) also found lower post-grazing height in tetraploid cultivars compared with diploid cultivars; however they also found an interaction between ploidy and heading date on post grazing residual.

*Heading date contrast*

Thus, other factors such as temporal separation due to seasonal growth patterns and heading date may have also contributed to the creation of different environments for clover growth. Camlin (1981) and Gooding et al. (1996) have previously found that mid heading cultivars support a greater clover content than late cultivars, and the first author suggested that a lower ryegrass competitive ability of the mid heading cultivars at the same time of the start of clover growth appears to facilitate the growth of the legume (Camlin, 1981). In agreement with this previous research, M. A. Sanderson and Elwinger (1999) found that early –maturing cultivars were more compatible with white clover during the establishment phase. The findings that Commando AR37 and Kamo AR37, two mid-heading cultivars, recorded high clover percentage during summer 2013 – 14 agree with these results. By contrast, Tozer et al. (2014) found in autumn/winter 2011 in Canterbury that clover proportion was higher in pastures sown with late-season diploids than with mid-season diploids.

Therefore analysis of variance was conducted on the white clover content of swards based on cultivars representing the mid and late heading date contrast, and the results showed that this contrast affected the white clover content during summer in both years and autumn 2013. Pastures based on cultivars representing the mid-heading date had greater clover content than pastures based on cultivars representing the late-heading date. Thus, the results of this experiment agree with previous studies (Camlin, 1981; Gooding et al., 1996).

In an attempt to explain these results, seasonal perennial ryegrass yield and white clover yield in mixture were calculated based on the seasonal yield and the grass and clover content (in the sampling conducted in each season) for each heading date contrast, and the data was analysed statistically. In summer 2013 – 14 and in autumn in both years, perennial ryegrass seasonal yield was greater in late than in mid-heading cultivars ($P = 0.042$, autumn 2013; $P = 0.002$, summer 2013 – 2014; $P = 0.002$, autumn 2014), confirming that, during these seasons, late cultivars were competing more aggressively with white clover for resources, such as light. A similar trend was observed in summer 2012 – 13 ($P = 0.054$). The greater competition resulted in lower clover yield in pastures based on late than in pastures based on mid-heading cultivars in summer 2012 – 13 ($P = 0.015$) and autumn 2013 ($P = 0.020$), seasons in which their clover content was also lower. However, in summer 2013 – 14, although late-heading cultivars had greater ryegrass yield than mid-heading cultivars and lower clover percentage, the clover yields were not significantly different, because of the greater total DM yield of the late-heading cultivars that compensated for the lower clover content.

Therefore it is the combination of a series of phenotype and management factors that contribute to a greater clover content in mixed swards. A lower post-grazing residual in spring, when the competition from grass is stronger, seems to favour the development of the legume in the sward during this season. Mid heading date also appears to favour the contribution of clover to the sward by avoiding competition with clover in the warmer months of the year, when the legume has the advantage due to its adaptation to higher temperatures.

### 3.5.4 Reasons for the limited number of cultivar by treatment interactions on DM yield

The first aspect to consider to explain this limited number of interactions between cultivar and treatments on DM yield is the fact that, despite certain N limitations, the mixtures were dominated by grass during the two years of the experiment (Tables 3.11 and 3.12), with the exception of pastures in the Low N plus clover treatment in Summer 2012 – 13. Camlin (1981) found that in perennial ryegrass/white clover pastures fertilised with 200 – 240 kg N/ha/year, the mixture yield tended to reflect the yield of the grass component. The results of this experiment agree with Camlin's findings (Camlin, 1981). In this experiment, ryegrass persistence, one of the factors that Camlin (1981) indicated as influencing the interaction between ryegrass and clover cultivars, did not seem compromised in the two years of the experiment. No indications of great risks from pests or diseases were detected through the duration of the experiment (A.J. Popay et al., 2015). The tiller density of the sward during both autumns (Table 3.23) was high compared with tiller density on Canterbury farms (mean 3252 tillers/m2) recorded by Tozer et al. (2014). This good persistence could have been facilitated by the presence of fungal endophyte *Epichloë festucae* var. *lolii*, formerly *Neotyphodium lolii* (Leuchtmann et al., 2014) that was detected in 85.5 % of the tillers collected in the field in autumn 2014 versus 80.0 % in autumn 2013 ($P < 0.01$) (A. J. Popay & Hume, 2011). Although the potential evapotranspiration and irrigation data suggest the presence of soil water deficit during spring and summer which may have limited growth and therefore the expression of the potential yield of the cultivars, it did not create a critical environment for perennial ryegrass survival. This soil water stress could have had greater impact on white clover growth, due to its smaller root system compared to ryegrass (H. Thomas, 1984).

#### Cultivar x clover interactions

Regardless of this grass dominance, the contribution of clover to DM yield was significant in every season except in the first spring. Despite the different characteristics of the perennial ryegrass cultivars selected, a significant cultivar effect on clover content (% DM) was observed in only three of the six seasons, two of which were in spring when overall clover percentage was low. These differences in content were not large enough to create an interaction between cultivar and clover treatment (presence or absence) on DM yield during these seasons. To explain why no interaction was detected, seasonal perennial ryegrass yield in monoculture or mixtures and white clover yield in mixture were calculated based on the seasonal yield and the grass and clover content (in the sampling conducted in each season), and analyses of variance were performed (Table 3.32).

**Table 3.32 Clover and cultivar effects on seasonal perennial ryegrass and white clover yields.**

| | | *P* value | |
|---|---|---|---|
| | | Perennial ryegrass seasonal yield | White clover seasonal yield |
| Spring 2012 | Clover effect | 0.366 | |
| | Cultivar effect | < 0.01 | < 0.05 |
| Summer 2012 - 13 | Clover effect | 0.541 | |
| | Cultivar effect | < 0.001 | 0.063 |
| Autumn 2013 | Clover effect[1] | < 0.01 | |
| | Cultivar effect | < 0.01 | 0.269 |
| Spring 2013 | Clover effect | 0.528 | |
| | Cultivar effect | < 0.01 | < 0.05 |
| Summer 2013 - 14 | Clover effect | 0.097 | |
| | Cultivar effect | < 0.001 | 0.121 |
| Autumn 2014 | Clover effect[1] | < 0.001 | |
| | Cultivar effect | < 0.001 | 0.734 |

Note – No N x Cultivar interaction was detected on perennial ryegrass or white clover seasonal yield. [1] N x clover interaction P < 0.05.

Cultivar effect on white clover yield was detected in spring 2012 and spring 2013 as was the case for white clover content. Meanwhile a cultivar effect on perennial ryegrass yield was detected in every season. Thus a combination of a low white clover percentage and yield during spring, in conjunction with an apparent substitution between grass and clover, explain why in two of the three seasons in which a ryegrass cultivar effect on white clover content of pasture was observed, this did not result in an interaction between cultivar and clover on total pasture DM yield (Figure 3.10).

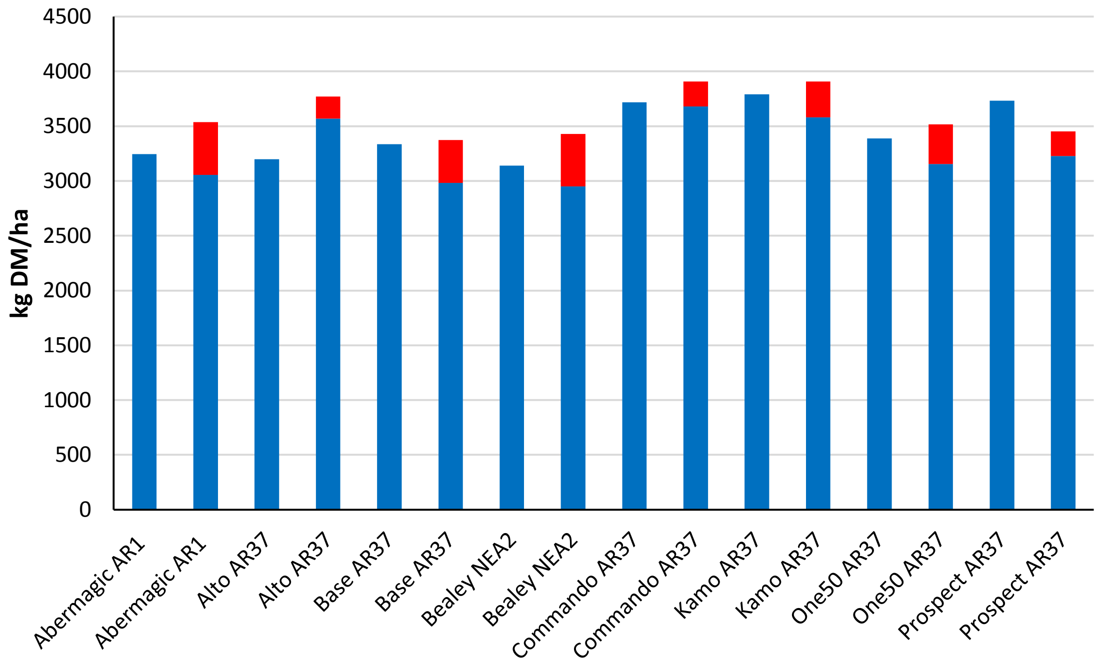


**Figure 3.10 Seasonal perennial ryegrass and white clover yield in minus clover (left) and plus clover (right) treatments in spring 2012. Perennial ryegrass (kg DM/ha – blue bar), white clover (kg DM/ha – red bar).**

The clover content of pastures increased from spring to summer, when it reached the highest % of the year (13.2 % on pastures of the High N treatment and 43.5 % on pastures of the Low N treatment, average for the two years), before decreasing again during autumn. This pattern conforms with the expected seasonal cycle of clover content in grazed pastures (Caradus, Harris, et al., 1996; Caradus, Woodfield, et al., 1996; Frame & Laidlaw, 1998; W. Harris, 1987; W. Harris & Hoglund, 1977), confirming that the study captured the competitive interaction between grass and clover that typically operates in grass/clover mixtures growing in temperate environments. Thus, this is the time of the year when a different contribution of clover to herbage mass could have greater implications for the relative ranking of cultivars based on DM yield. However, in summer 2013 – 14, although there was cultivar effect on clover content, the effect on clover yield was not significant (Table 3.32), and the relative ranking of cultivars based on DM yield was similar in monoculture or mixture (Figure 3.12). The same effect was seen in summer 2012 – 13 (Figure 3.11). Therefore, all ryegrass cultivars allowed clover to grow equally well during the period of active clover growth.

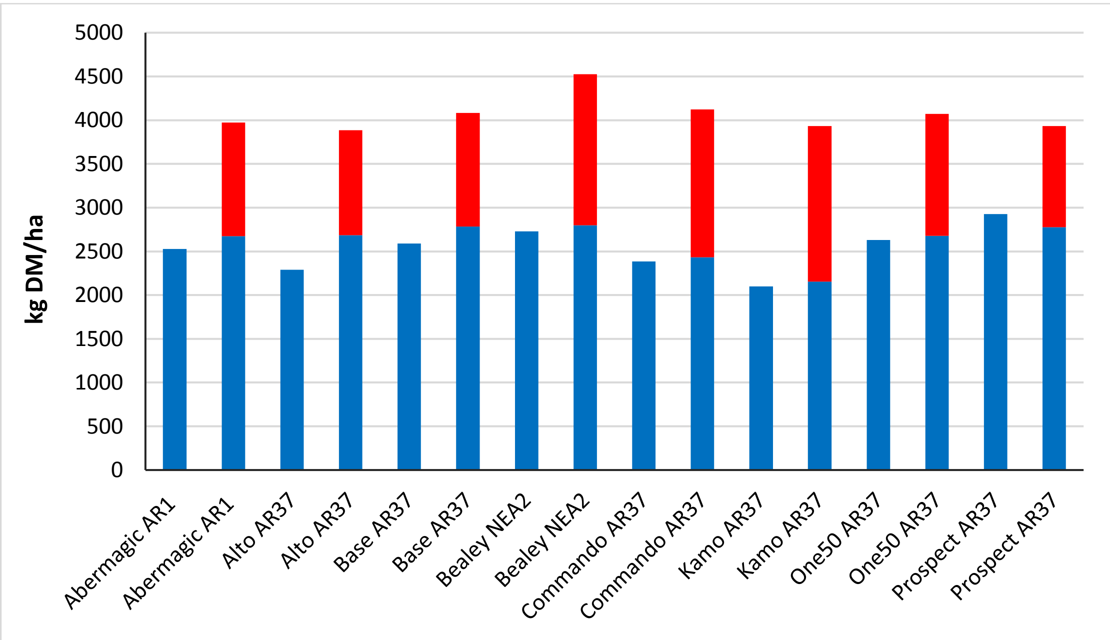


**Figure 3.11 Seasonal perennial ryegrass and white clover yield in minus clover (left) and plus clover (right) treatments in summer 2012 - 13. Perennial ryegrass (kg DM/ha – blue bar), white clover (kg DM/ha – red bar).**

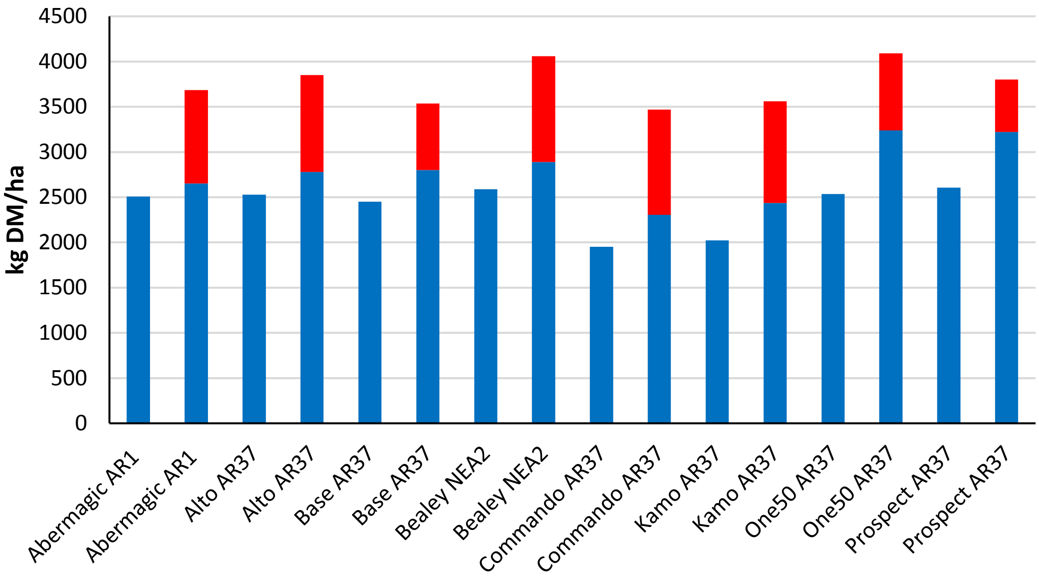


**Figure 3.12 Seasonal perennial ryegrass and white clover yield in minus clover (left) and plus clover (right) treatments in summer 2013 - 14. Perennial ryegrass (kg DM/ha – blue bar), white clover (kg DM/ha – red bar).**

From the Table 3.32 it is also possible to observe that in autumn in both years, there was an effect of the presence of clover on ryegrass yield that was accompanied by an interaction between N and clover. Under the Low N treatment the clover effect was positive, indicating a possible increase in N supply by the soil due to the contribution of the legume; however, under the High N treatment, there was no effect of clover presence on ryegrass yield (Table 3.32).

Only in winter 2013 interactions between cultivar and N treatment and cultivar and clover treatment on total DM yield were present. These interactions were probably the result of the stronger growth of some of the cultivars in winter (Objective description of variety, Plant Variety Rights Office of New Zealand, personal communication, July 2013, April 2015; DairyNZ, evaluation dates December 2014 and December 2015) and consequently their increased ability to use the extra N available in the plus clover treatments after one year in pasture, or from the fertiliser applied in late autumn.

### 3.5.5 Metabolisable energy density (ME, MJ/kg DM) of swards

The effects of N and clover treatments on the ME density of swards were inconsistent. In both summers, the inclusion of clover in swards grown under the Low N treatment increased the ME density of the herbage but the same did not happen under the High N treatment, probably due to the lower clover content of these swards and the increase in ME in the grass due to the greater N application (McKenzie, Jacobs, & Kearney, 2003). Previous research has also found increments in the energy density of pastures due to the inclusion of clover (S. L. Harris et al., 1998; S. L. Harris et al., 1997).

Differences between cultivars in ME density occurred in both years. The greater ME density of the two tetraploids compared with other cultivars agrees with findings from previous studies (Beecher et al., 2015; Burns, Gilliland, Grogan, Watson, & O'Kiely, 2013; Gilliland, Barrett, Mann, Agnew, & Fearon, 2002; Salama et al., 2012). When the analysis of variance was conducted for the phenotypic contrasts, the morphological contrast did not work for traits related to pasture nutritive value either; the overlap in ME density between cultivars within the dense and open contrasts means that robust conclusions regarding the effect of morphology cannot be drawn. Moreover, ME density of pasture apparently followed a trend not related to their seasonal reproductive development, as it was always greater in the late than in the mid-heading cultivars.

There was some re-ranking of cultivars for ME density when sown with clover compared to ryegrass monoculture (e.g. summer 2012 – 13 and summer 2013 – 14, Tables 3.15, 3.16 and 3.17). However, the magnitude of change was too small to warrant adjustment of ryegrass cultivar performance values in the FVI (Chapman et al., 2016; DairyNZ).

## 3.6 Limitations of this study

The design of this type of experiment is complicated by the difficulty of selecting treatments that represent discretely different plant traits, when in nature these traits overlap. Moreover, it is often impossible to balance for phenotype and endophyte strain, for example, and for any other 'unseen' traits such as physiology- or root-related traits.

Phenotypic contrasts were included in this study with the purpose of identifying if grass phenotype characteristics, more than cultivar characteristics, could be linked to interactions with white clover. This proved to be possible for the heading date contrast, but it was not possible for the morphology contrast, that did not work as expected (see Results 3.4.2). Significant differences were observed in yield of the two cultivars used to represent the dense phenotype, as well as differences in yield of the two cultivars used to represent the open phenotype during some seasons. Moreover, the white clover content of pastures was affected by the morphological contrast only in spring in both years (see Results 3.4.5), but in both seasons, the clover percentage of one of the cultivars representing the dense phenotype (Abermagic AR1) was not significantly different from the percentage of the two open cultivars (Bealey NEA2 and Base AR37) and greater than the clover content in the other dense cultivar (Prospect AR37). In addition, although mean tiller density of pastures sown with cultivars representing the dense phenotype was greater than tiller density of pastures sown with cultivars representing the open phenotype, pastures sown with Prospect AR37 did not differ significantly in their tiller density from pastures sown with Base AR37 (Table 3.23). For these reasons, the morphological contrast lacked the internal consistency required to draw robust conclusions. In an experiment including the same eight perennial ryegrass cultivars in Waikato, New Zealand, Griffiths et al. (2016) found that the cultivars representing the morphological contrast differed in traits associated with tiller size. The two tetraploids had greater lamina area and width (as well as greater dry weight per tiller) than the two cultivars representing the dense phenotype, in agreement with their expected broad leaf morphology. These traits were not measured in this experiment, but Griffiths et al. (2016) results suggest that the cultivars were properly selected based on their leaf characteristics.

Other methodological and management issues also complicate this type of experiment. Grazing of cultivars with different morphology and heading date, at the same time and in the same main plot, and achieving similar post-grazing mass residuals is a challenge in itself. Preference for tetraploid cultivars by grazing animals and lower post-grazing mass residuals compared to diploid cultivars have been observed in previous research (O'Donovan & Delaby, 2005) and also occurred in the present experiment. Additionally, the onset of reproductive development and changes in the stem content of pastures affect intake (Waghorn & Clark, 2004) and preference by grazing animals, creating different residuals. In the experiment, the extra time required to force the cows to graze lower and to spatially uniform residuals carried the risk of overgrazing of the preferred cultivars and white clover, excessive deposition of urine and dung and the possibility of damage to the plots by pugging. As a consequence, in general, the post-grazing mass achieved was higher than the target (1500 - 1750 kg DM/ha), especially in summer. Occasionally the plots were mown to bring all treatments back to a common residual herbage mass post-grazing. The variation amongst cultivars in the post-grazing herbage mass confirmed the need for adjustment of the DM yield from the harvester, to avoid biasing the results in favour of some cultivars over others.

Total annual DM yields for the different treatments were, in general, lower than yields from similar pastures in the area, especially during the first year. As a comparison, the Lincoln University Dairy Farm (LUDF) reported 16.8 t DM/ha of pasture eaten for the season 2012 – 2013, and 14.9 t DM/ha for the season 2013 – 2014, with the use of 350 and 250 kg N/ha/year for each farming season respectively (South Island Dairying Development Centre, 2015). Moreover, at the same farm (Lincoln University Research Dairy Farm – LURDF), but on different soils and with different management history, pastures under the Higher Input system receiving 400 kg N/ha/year (Clement, Dalley, Chapman, Edwards, & Bryant, 2016), grew 18.0 t DM/ha, and under the Lower Input system (150 kg N/ha/year) 16.5 t DM/ha (average for the seasons 2011 – 12 to 2014 – 15). One of the reasons for these lower than expected yields, could be low soil organic matter and N availability due to the area being sown to pasture only one year before the establishment of the experiment, following a history of crop production. Therefore, soils at LUDF which had been under long term sheep pastures until conversion to dairy pastures in March 2001, could have more N available than soils used in this study at a similar level of N fertiliser application. Another reason for the lower yield than expected could be that, although maintenance fertilisers were applied consistently and following technical recommendations, soil nutrient availability did not increase (at least in the first 7.5 cm which was the sampling depth) as promptly as expected, and it took more than two years to return to the levels of P, K and S that were present in the soil prior to cultivation. If the experiment was conducted under a more-developed soil, as it is the case in an important proportion of dairy farms, ryegrass could have had a stronger relative advantage compared to white clover, diminishing the possibility of detecting interactions and tipping the balance of competition towards the ryegrass component of the pasture.

Botanical composition at every sampling would have added valuable information to confirm if perennial ryegrass and white clover seasonal yields were positively associated to their correspondent content (%DM) in pastures, to explain the limited number of interactions present in this experiment.

## 3.7 Conclusions

Significant interactions between cultivar and N and cultivar and clover on total DM yield were only detected in winter 2013, but not during the rest of the seasons nor in the total annual yield. As a consequence, no evidence of re-ranking emerged and therefore performance values in the Forage Value Index (DairyNZ), which are calculated using dry matter yield data from cultivar evaluation trials conducted using perennial ryegrass monocultures do not need adjustment to account for grass-clover interactions over time and their effects on total pasture dry matter yield.

Although the white clover content of the swards, expressed as % DM, was significantly different across the perennial ryegrass cultivars in three of the six sampling seasons, these differences were insufficient to cause re-ranking on a total DM yield basis. Moreover, during summer in both years and autumn in the first year, mid heading cultivars supported greater clover content than late heading cultivars, but this difference was not reflected in a change of the relative ranking position.

N had a significant effect on the DM yield throughout the duration of the experiment; annual total DM yield in the High N treatment was 28 % greater than in the Low N treatment during 2012 - 13 and 31 % during 2013 - 14. However, this response was not uniform amongst seasons and clover treatments. For the grazing period (spring to autumn), the average N fertiliser response for the two years was 16.6 kg DM/kg N in the minus clover treatment and 5.9 kg DM/kg N when ryegrass was grown with clover.

Mean annual total DM yield in the with clover treatments was 23 % greater than in the without clover treatments at the end of the first year and 28 % greater at the end of the second year. Most of the increase in DM due to white clover occurred in summer and autumn, which together accounted for 99 % and 72 % of the total increase during the first and second year respectively.

The white clover content of pastures (expressed as % DM) decreased with increasing N fertiliser application rate. Average content for the two years was 25.2 % DM in the Low N treatment and 8.1 %

DM in the High N treatment. Every 13.2 kg/ha of additional N applied (from 100 to 325 kg N/ha/year), decreased the clover content of the sward by 1 % of DM.

The effects of N and clover treatments on the ME density of pastures were inconsistent. Variations in the ME density of cultivars occurred in both years. The greater ME density of the two tetraploids compared with other cultivars agrees with findings from previous studies. There was some re-ranking of cultivars for ME density when sown with clover compared to ryegrass monoculture. However, the magnitude of change was too small to warrant adjustment of ryegrass cultivar performance values in the FVI (Chapman et al., 2016; DairyNZ).

# Chapter 4

# Influence of perennial ryegrass and white clover phenotypes on DM yield and botanical composition of mixed swards receiving either low or high rates of N fertiliser application.

## 4.1 Introduction

Increasing DM production and quality of pastures is an important objective for breeders, scientists, agronomists and farmers in New Zealand. Management factors as well as cultivar selection play an important role in achieving these objectives.

Perennial ryegrass (*Lolium perenne* L.) and white clover (*Trifolium repens* L.) are the two main components of grazed pastures in New Zealand. They have the potential to influence each other when in association (Camlin, 1981); however, previous research have shown that, in general, when perennial ryegrass and white clover cultivars with different phenotypes are grown in mixture, the total annual yield of the pasture is similar (Camlin, 1981; Connolly, 1968; Ledgard et al., 1990; Reid, 1961; Widdup & Turner, 1983), although differences in botanical composition could emerge (Connolly, 1968; Rhodes & Harris, 1979; Widdup & Turner, 1983).

Due to the multiple benefits that the inclusion of clover brings to the production system, such as the ability to fix $N_2$, high nutritive and feeding value, and its seasonal growth complementary to grass growth (W. Harris & Hoglund, 1977; Ledgard & Steele, 1992; Nicol & Edwards, 2011; Ulyatt, 1970; Walker et al., 1954; Whitehead, 1970), research has also emphasised in finding grass and clover plant characteristics that result in an improved white clover content in the sward (Collins & Rhodes, 1989; Elgersma et al., 1998; Elgersma & Schlepers, 1997a; Frame & Boyd, 1986a; Gilliland, 1996).

However in New Zealand, the clover content of dairy pastures is typically low (less than 20 % on an annual basis) (Caradus, Woodfield, et al., 1996; Chapman et al., 1996; Ettema & Ledgard, 1992; Tozer et al., 2014), limiting the possibilities for exploiting the advantages of the grass/legume system (Chapman et al., 1996). Defoliation regime and N fertiliser application play an important role in determining the balance between these components of the sward. Nevertheless, the availability of grass and clover cultivars with a range of phenotypes, plus the possibility of using irrigation on Canterbury farms, raises the question whether interactions between cultivars with different phenotypes could affect pasture yield and botanical composition and result in more productive mixtures.

The experiment reported in this Chapter was conducted to address this question and to analyse how phenotypic characteristics of perennial ryegrass and white clover may affect their competitive ability in the sward. Based in previous research (Camlin, 1981; Connolly, 1968; Elgersma et al., 1998; Hoen, 1970; Widdup & Turner, 1983; Williams et al., 2000), the working hypothesis was that the DM production of the sward will not differ when modern perennial ryegrass and white clover cultivars with different phenotypes are grown in mixture. The factors used to test this hypothesis were: different perennial ryegrass and white clover cultivars grown in mixtures or in monocultures; and different N fertilizer levels.

## 4.2 Objectives

The objectives of this study were:

- To compare the total DM yield (kg DM/ha) of swards based on perennial ryegrass and white clover cultivars with different phenotypes grown in association and receiving either low or high rates of N fertiliser application.

- To compare the yield of the perennial ryegrass and white clover components of these mixed swards.

- To compare the total DM yield of mixed swards with the yield of perennial ryegrass and white clover monocultures at low and high rates of N application.

- To analyse the role of the perennial ryegrass and white clover phenotypes in determining the botanical composition of the sward (white clover content expressed as % DM).

- To determine which factors were affecting the competitive ability of the different perennial ryegrass and white clover phenotypes when grown in mixtures at low and high rates of N application.

## 4.3 Materials and Methods

### 4.3.1 Site description and preparation

The experiment was conducted at the Lincoln University Research Dairy Farm (LURDF), Lincoln, Canterbury, New Zealand (latitude 43°38’10.26”S; longitude 172°27’42.91”E; altitude 12 m a.s.l.) from the 1$^{st}$ June 2014 to 31$^{st}$ May 2015.

The soils at the site are Paparua sandy loam and Wakanui sandy loam. They are typic immature pallic soils and mottled immature pallic soils respectively, according to the New Zealand soil classification (Hewitt, 2010). According to the USDA classification (Soil survey staff, 1998) they are Udic Haplustept and Aquic Haplustept fine silty, mixed, mesic soils respectively.

Prior to 2009, the area used for the experiment (approximately 0.3 ha) had been in a perennial ryegrass and white clover pasture used for young dairy cattle. In 2009 the pasture was re-sown also with perennial ryegrass and white clover, and thereafter grazed by the milking herd. Preparation for the establishment of the experiment started in July 2013, when the area was cultivated with a rota-crumbler. In August 2013, the area was sprayed with Roundup Transorb® (540 g/litre glyphosate) applied at 2 litres/ha. Three more cultivations with a rota-crumbler followed on 4$^{th}$ October, 24th October and 1$^{st}$ November, the last one including use of a Cambridge roller. The experiment was sown on the 4$^{th}$ November 2013.

### 4.3.2 Meteorological conditions

Historical data from the Broadfield meteorological station located 1 km north of the site show an average annual rainfall of 599 mm and an average mean air temperature of 11.7°C for the period 1981 to 2010 (National Institute of Water and Atmospheric Research, 2015). Total rainfall for the season 2014 – 2015 (376 mm) was 223 mm lower than the historical average with only two months (November 2014 and April 2015) receiving more rain than the corresponding monthly historical average (Figure 4.1 and Table A.2 in Appendix A). Meanwhile, the mean temperature for the season was 0.7°C higher than the historical average (Figure 4.1)

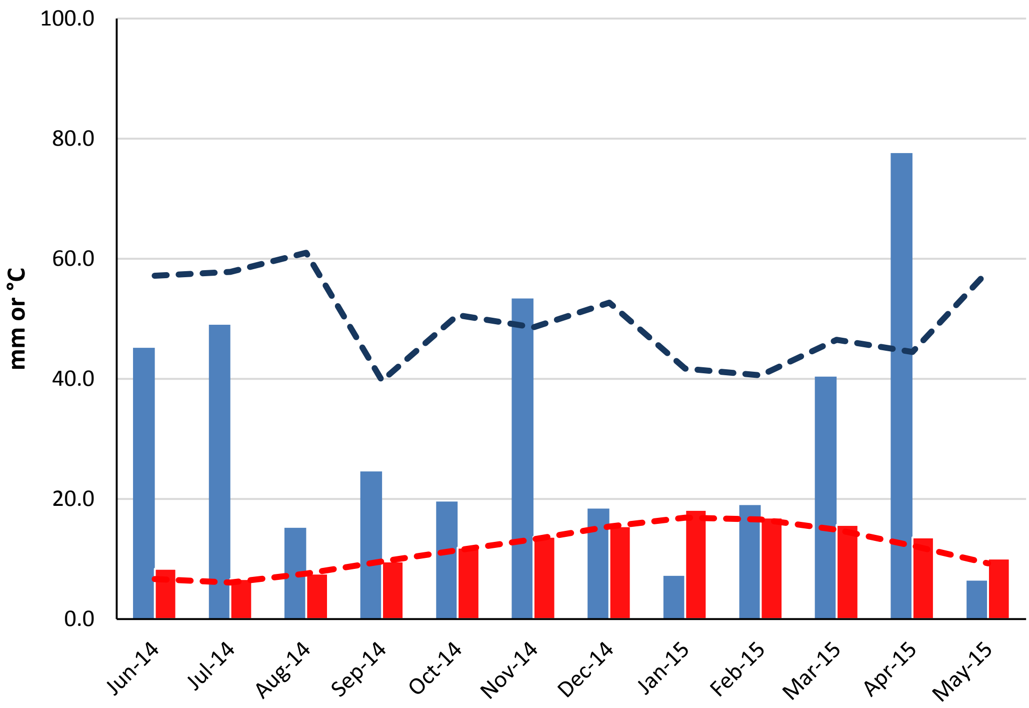


**Figure 4.1 Monthly total rainfall (mm) and mean air temperature (°C) during the seasons 2014 – 15 and historical data (1981 to 2010). Monthly total rainfall (blue bar), Mean air temperature (red bar), Mean monthly rainfall historical data (dashed blue line), Mean monthly temperature historical data (dashed red line).**

Total Penman potential evapo-transpiration (mm) (National Institute of Water and Atmospheric Research, 2015) during spring and summer exceeded total rainfall and irrigation, creating an accumulated soil water deficit of 230 mm between September 2014 and February 2015 (Figure 4.2).

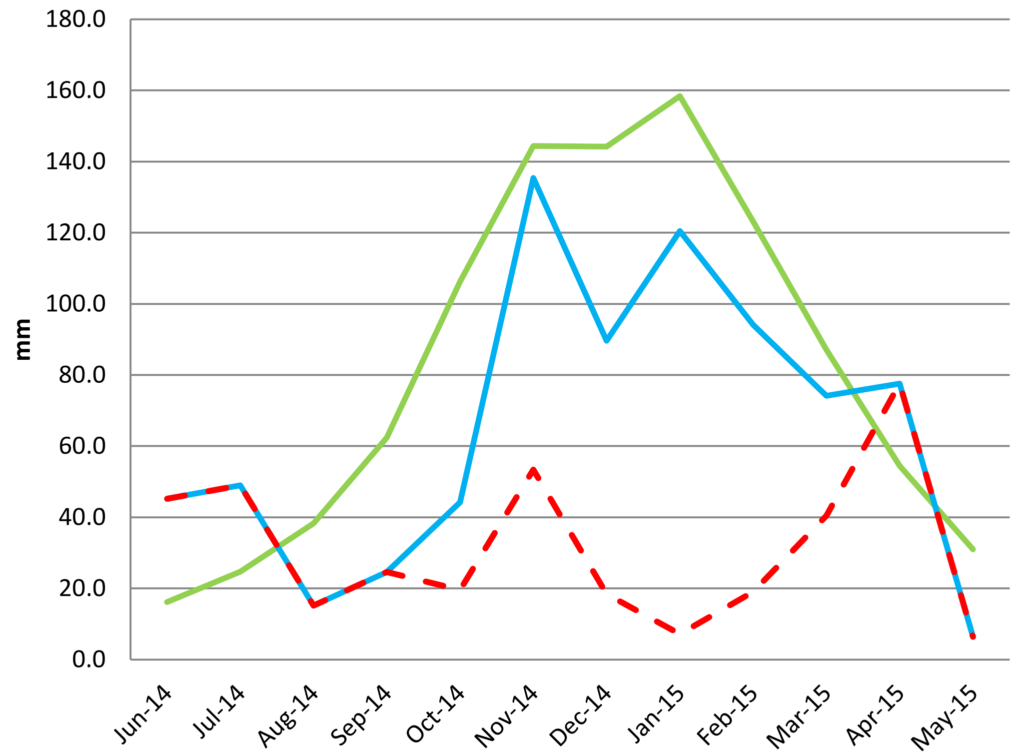


**Figure 4.2 Rainfall, irrigation and EVT potential during the period June 2014 – May 2015 (mm). Total rainfall (dashed red line), Total rainfall + irrigation (solid blue line) and Total Penman potential evapo-transpiration (solid green line).**

### 4.3.3 Design of the experiment

The experiment used a split plot design with four blocks (Figure A.2 in the Appendix A shows the layout of the experiment). Main plots were two N levels (100 and 325 kg N/ha/year), randomised within blocks. Subplots were the pasture types (24), made up of a 4 × 4 factorial of 4 perennial ryegrass cultivars and 4 white clover cultivars (16 subplots), plus monocultures of each cultivar (8 subplots), randomised within main plots. Blocks were separated by a 6 m buffer areas.

Each subplot was 3 m long by 1.8 m wide; from within that area, the 10 central drill lines (1.5 m width) were harvested, resulting in a measurement area of 4.5 $m^2$.

The rates of N fertiliser applied annually were either low (100 kg N/ha) or high (325 kg N/ha). The high N level is above the average of the N applied in the Canterbury region during the farming season 2011 – 12 (229 kg N/ha/year, DairyBase® personal communication, January 2016) while the low N is below this average, and low enough to create a large difference between N treatments.

Four perennial ryegrass cultivars were selected to create a range from fine to broader leaved material and from open to denser cultivars. The cultivars selected were Abermagic AR1 (fine leaf, high sugar grass, diploid), Arrow AR1 (medium to broad leaf, diploid), Prospect AR37 (medium to wide leaf, diploid) and Bealey NEA2/6 (open, medium leaf, tetraploid) (Table 4.1).

**Table 4.1 Description of the perennial ryegrass cultivars used in the experiment**

| Cultivar | Endophyte[1] | Ploidy | Heading date | Leaf: width (vegetative stage) | Leaf: length (vegetative stage) |
|---|---|---|---|---|---|
| Abermagic AR1 | AR1 | Diploid | Late (+19) | Narrow | Short |
| Bealey NEA2 | NEA2/6 | Tetraploid | Very late (+25) | Medium | Medium to long |
| Arrow AR1 | AR1 | Diploid | Late (+7) | Medium to broad | Medium to long |
| Prospect AR37 | AR37 | Diploid | Late (+12) | Medium to wide | Medium to long |

Note to Table: Heading date - time when 50 % of plants have emerged seedhead in a typical year and it is defined relative to cultivar Nui (heading at date zero, 22 October each year). Maturity groups used for classification (after Lee et al., 2012) were: mid-season maturing (day 0 to +6), late-season maturing (day +7 to +21), very late-season maturing (day +22 to +25). Information about ploidy, leaf width and length is based on the Objective Description of Variety (Plant Variety Rights Office of New Zealand, personal communication, July 2013, April 2015). Heading dates in this Table are based on commercial information (PGG Wrightson Seeds, 2015).
*Epichloë festucae* var. *lolii;* formerly *Neotyphodium lolii* (Leuchtmann et al., 2014).

Four white clover cultivars were selected to represent a range in leaf size: Nomad (small leaved), Bounty (medium leaved), Tribute (medium large leaved) and Kopu II (large leaved) (Table 4.2).

**Table 4.2 Description of the white clover cultivars used in the experiment (Plant Variety Rights Office of New Zealand, personal communication, December 2015)**

| Cultivar | Length of central leaflet* | Width of central leaflet* | Length of petiole | Thickness of petiole | Thickness of stolon |
|---|---|---|---|---|---|
| Grasslands Nomad | Short | Narrow | Short | Thin | Thin to Medium |
| Grasslands Bounty | Short | Medium | Short to Medium | Medium | Medium |
| Grasslands Tribute | Medium | Medium | Short to Medium | Medium | Medium |
| Grasslands Kopu II | Long | Broad | Long | Thick | Thick |

Note to Table - * 3rd to 4th leaf from end of tip of rapidly growing stolon; within 1-2 weeks after mean date of flowering.

### 4.3.4 Baseline site data

Soil sampling to determine fertility status was conducted during August 2013. Forty cores (ten per replicate) were collected at regular intervals with a soil corer (2.5 cm in diameter) to 7.5 cm depth. These forty cores were combined to form a composite sample that was then dried at 25°C prior to analysis. The results show that the level of nutrients was in general below biological optimum level for pasture growth (Table 4.3).

**Table 4.3 Soil fertility status on samples collected on the 13th August 2013.**

| | Soil test results | Target soil test |
|---|---|---|
| pH [1] | 6.0 | 5.8 – 6 [5] |
| Ca - Calcium MAF QT [1] | 9.0 | > 1.5 [6] |
| P - Olsen Phosphate µg/mL [2] | 15.0 | 20 – 30 [5] |
| K - Potassium MAF QT [1] | 3.0 | 5 – 8 [5] |
| S(SO4) - Sulphate Sulphur ppm [3] | 3.0 | 10 – 12 [5] |
| Mg - Magnesium MAF QT [1] | 6.0 | 8 – 10 [5] |
| Na - Sodium MAF QT [1] | 5.0 | > 5 [7] |
| Organic Matter (%) [4] | 4.0 | - |

[1] (Blakemore et al., 1987; Cornforth, 1980); [2] (Ammerman, 2003; Cornforth, 1980); [3] (Watkinson & Perrott, 1990); [4] (Rayment & Lyons, 2011); [5] (Roberts & Morton, 2009); [6] (Edmeades & Perrott, 2004); [7] (Edmeades & O'Connor, 2003).

On 14th October 2013 two soil samples were collected to determine the numbers and species identity of buried seeds. Samples were collected using a soil corer (2.5 cm in diameter) to 7.5 cm in depth. Each sample consisted of 25 cores processed using a minor modification of the method described in Rahman, James, Grbavac, and Mellsop (1995). Due to the need for a final cultivation prior to the sowing of the trial, another sample was conducted on the 1st of November following the same procedure, because the mixing of the soil could have altered the proportion of seeds present in the upper profile of the soil. The first count and identification of the seedlings present occurred one month after sampling, after which the soil was mixed and replaced in trays. One month later, the second seedling count was conducted. On average, 59 % of these seeds were from grasses other than perennial ryegrass (mostly *Poa annua* L.) and 41 % were broadleaf weeds (mostly *Capsella bursa-pastoris* L., *Fumaria* sp.and *Lepidium didymium* L.). No ryegrass or white clover seeds germinated during the time of the test. However, a few months after the end of the test, some white clover plants appeared in the soil that was still in the trays, indicating the presence of seeds of this legume, although in very small numbers.

### 4.3.5 Establishment of the experiment

On 30th October 2013, two days before the final cultivation, sulphur-enriched superphosphate (Sulphur Super 30; 0-7-0 + 16 Ca + 30.1 S) was applied at 1 ton/ha to the area to correct nutrients deficiencies shown by the soil test results.

On 4th November 2013, the trial was sown using a Flexiseeder plot drill with 14 coulters spaced 15 cm apart. Seed was sown at a depth of 1.5 cm.

**Figure 4.3 General view of the paddock after sowing – 4th November 2013**

The perennial ryegrass seed was treated either with Gaucho® or Superstrike®, to protect seedling plants against black beetle and grass grub larvae during the establishment period. The white clover seed was treated with Superstrike® coating containing *Rhizobia* bacteria, molybdenum, lime and a nematicide.

Sowing rates were equivalent to 20 kg/ha of seed for the diploid ryegrasses and 28 kg/ha for the tetraploid ryegrass to account for differences in seed weight between the ploidy levels. The white clover was sown at a rate equivalent to 4 kg/ha of bare seed (correction of this sowing rate was applied to account for coating of the seed). Both perennial ryegrass and white clover seeds were sown together in the same drill rows.

Details of the seed quality are presented in Table 4.4.

**Table 4.4 Seed analysis**

| Cultivar | Purity (%) | Germination (%) | Endophyte infection frequency |
|---|---|---|---|
| Abermagic AR1 | 99.8 | 93 | 72 |
| Arrow AR1 | 99.9 | 98 | 94 |
| Bealey NEA2 | 99.7 | 90 | 76 |
| Prospect AR37 | 99.8 | 93 | 76 |
| | | | |
| Grasslands Bounty | 99.9 | 90 | Not applicable |
| Grasslands Kopu II | 99.6 | 92 | Not applicable |
| Grasslands Nomad | 100.0 | 82 | Not applicable |
| Grasslands Tribute | 100.0 | 92 | Not applicable |

### 4.3.6 Management

Defoliation management was cutting only, and irrigation was applied from October 2014 to March 2015. Cutting management was dictated by the protocol for measurements of dry matter yield, since the entire measurement area (4.5 $m^2$) was harvested for the latter.

#### N fertilizer

The annual rates of N fertiliser applied were: for the low N treatments 100 kg N/ha/year and for the high N treatments 325 kg N/ha/year, applied manually as urea (46-0-0). In the Low N treatment, urea was applied at rates of 25 kg N/ha on four occasions (October, January, March and April). In the High N treatment, it was applied initially at a rate of 35.2 kg N/ha in October, November, early December, late December, January and February, and then at a rate of 57 kg N/ha for the last two applications in March and May.

To replace the N removed by cutting, estimated at 3 % of mean dry matter harvested in each N treatment, extra urea was applied in two occasions (October and November). However, this practice was discontinued because it did not permit the creation of contrasting N treatments.

#### Herbicide application

Throughout the season, mowing and herbicide application (Buster®, 200 g/L glufosinate-ammonium or Roundup ULTRA® MAX, 570 g/L glyphosate) was used to keep the area between plots free of weeds and to avoid the spread of with white clover between neighbouring subplots.

On 3rd January 2015, T-Max™ (30 g/L aminopyralid) at 60 ml/10 L water was applied with a knapsack to the perennial ryegrass monoculture plots to control white clover and other legumes; the same day Gallant™ Ultra (520 g/L haloxyfop-P) at 12 ml/10 L water with Uptake™ spraying oil (582 g/L paraffinic oil and 240 g/L alkoxylated alcohol non-ionic surfactants) at 15 ml/10 L water were applied with a knapsack to the white clover monoculture plots to control perennial ryegrass and other grasses.

#### Maintenance fertilizer

On 9th October 2014, and following the same procedure used in August 2013, soil was sampled to determine nutrient status.

**Table 4.5 Soil fertility status measured in October 2014 (NZLABS).**

| | Soil test results | Biological optimum |
|---|---|---|
| pH [1] | 5.4 | 5.8 – 6 [5] |
| Ca - Calcium MAF QT [1] | 12.0 | > 1.5 [6] |
| P - Olsen Phosphate µg/mL [2] | 34.0 | 20 – 30 [5] |
| K - Potassium MAF QT [1] | 4.0 | 5 – 8 [5] |
| S(SO4) - Sulphate Sulphur ppm [3] | 37.0 | 10 – 12 [5] |
| Mg - Magnesium MAF QT [1] | 8.0 | 8 – 10 [5] |
| Na - Sodium MAF QT [1] | 5.0 | > 5 [7] |
| Organic Matter (%) [4] | 3.9 | - |

[1] (Blakemore et al., 1987; Cornforth, 1980); [2] (Ammerman, 2003; Cornforth, 1980); [3] (Watkinson & Perrott, 1990); [4] (Rayment & Lyons, 2011); [5] (Roberts & Morton, 2009); [6] (Edmeades & Perrott, 2004); [7] (Edmeades & O'Connor, 2003).

Based on the test results (Table 4.5), the equivalent of 4.1 ton/ha of lime and 1 ton/ha of 50 % Potash Super (0-4.5-25 + 10 Ca + 5.5 S) was applied during February and March 2015.

### Irrigation

The experiment was irrigated according to the schedule organized for the farm by the LURDF management team. In the period October 2014 to March 2015, 400 mm of water was applied using a centre pivot irrigator (Table A.2 in Appendix A).

## 4.3.7 Measurements

### Total DM yield

Total DM yield was measured on 9 occasions by harvesting the entire 4.5 m$^2$ measurement area to 5.5 cm above ground level, using a Haldrup forage harvester (Haldrup F-55, Denmark). The fresh weight of the harvested herbage was recorded and subsamples were collected to determine DM content (DM %) and botanical composition. The subsample for DM content (approximately 80-100 g) was weighed before and after being oven-dried for not less than 72 hours at 60 – 65°C. Based on the fresh weight of the harvested herbage and the DM %, yield per hectare (kg DM/ha) was determined. The first harvest occurred on 22$^{nd}$ August 2014 and the last on 12$^{th}$ May 2015; both High and Low N treatments were harvested on the same dates.

### Pasture biomass estimation using rising plate meter (RPM)

Herbage mass was estimated using a rising plate meter (Jenquip, Feilding, New Zealand) pre and post-cutting at every harvest (L'Huillier & Thomson, 1988; Litherland et al., 2008). The procedure consisted of walking in a “W” pattern across each subplot taking 9 readings to estimate pasture height (measured in units of 0.5 cm of compressed pasture height); these measurements were taken within one day of harvest. The general calibration equation (Equation 4) was used to provide an estimate of the biomass available per hectare.

**Equation 4**

Herbage mass (kg DM/ha) = RPM units x 140 + 500

## Botanical composition

Botanical composition was determined by dissecting the subsample collected from the harvested herbage at every harvest. This subsample, of approximately 10-15 g fresh weight was dissected into: live perennial ryegrass, live white clover, live other species and dead material of all species. Herbage was then oven dried for not less than 72 hours at 60 – 65°C before weighing, to determine the percentage contribution of each component to the total DM of the sample.

## Perennial ryegrass and white clover population density

The perennial ryegrass and white clover population density was measured during June 2014, November 2014, January 2015 and May 2015. For this purpose a 5 cm × 20 cm (100 $cm^2$) frame was randomly position at three locations in each subplot and the number of perennial ryegrass tillers and white clover growing points within each frame was counted.

## Light interception and canopy height

In December 2014, January 2015 and April 2015, photosynthetically active radiation (PAR, 400-700 nm) received above and below the canopy was measured in all subplots 4 – 5 days before harvest using a SunScan canopy analysis system (Delta-T Devices Ltd.). For this purpose the Bean Fraction sensor was connected to a radio transmitter which was linked to the radio receiver included in the SunScan probe. This Bean Fraction sensor was located at a maximum distance of approximately 30 m from the subplots. At each sampling, three measurements per subplot were conducted locating the SunScan probe underneath the canopy and positioned perpendicular to the drill lines. Readings were collected from the central 1.0 m width of the subplot. The first of these measurement was at approximately 0.5 m from one end of the subplot, the next was at the centre of the subplot (approximately 1.0 m from the previous measurement), and the last was taken at approximately 0.5 m from the other end of the 3.0 m long subplot. These three measurements were averaged to calculate the PAR  interceptance (fraction of incident radiation intercepted by the canopy, Russell et al., 1989) for each pasture type. Measurements were conducted between 9:30 and 13:15; during this time calibration measurements were taken periodically to ensure the accuracy of the data collected.

After light interception measurements were conducted and before the harvest, the undisturbed height of perennial ryegrass and white clover was measured using an automated sward stick (Jenquip, Feilding, New Zealand) similar to the method described by Bluett and Macdonald (2002). Ten measurements for the grass height and ten measurements for the clover height were conducted in each subplot following a zig-zag pattern, to calculate the average height for each species.

### White clover leaf size

In spring 2015 (November 2015), sampling was conducted to estimate white clover leaf size. Twenty randomly selected leaves were collected per subplot in the white clover monocultures grown under the Low N treatment, and the centre leaflet length (cm) and width (cm) were measured, to calculate leaflet size, by multiplying these two dimensions.

### Perennial ryegrass leaf regrowth stage

Leaf regrowth stage was measured on 10 randomly selected perennial ryegrass tillers per subplot in one block before every harvest using the method of Donaghy (1998). These measurements were conducted to track seasonal trends and to help in deciding on the timing of each harvest.

## 4.3.8 Data analysis

The data were analysed using ANOVA in GenStat 17 (VSN International, 2014) with perennial ryegrass cultivar, white clover cultivar and nitrogen treatment and their interactions as fixed effects, and block, main plot within block and subplot as random effects. Least significant differences (LSD) at the 5 % level were used to declare differences among means. Analyses were conducted for mixtures only, monocultures of perennial ryegrass, monocultures of white clover, monocultures of perennial ryegrass and mixtures, monocultures of white clover and mixtures, or for all twenty four pasture combinations.

Repeated measurements analyses were conducted on the total DM yield using the AREPMEASURES procedure in GenStat 17 (VSN International, 2014); since there were significant interactions between N treatment, perennial ryegrass cultivar, white clover cultivar and harvest time, results of the analysis of variance of the individual harvests will be presented.

Perennial ryegrass and white clover yields (kg DM/ha) within the mixture treatments were also analysed by ANOVA (VSN International, 2014). These yields were calculated based on the total DM and botanical composition data for each harvest.

For the white clover %, the repeated measurements through time for the mixtures were analysed using spline models within the linear mixed model framework as described by Verbyla et al. (1999). N treatment, perennial ryegrass cultivar, white clover cultivar, the linear trend of harvest date and the interactions of these were included in the model as fixed effects and block, main plot within block, subplot, linear trend of harvest date within subplot, the interaction of subplot with spline and the interactions of the treatment factors with spline were included as random effects. Results of the analyses of variance for each harvest are reported since the interactions between N treatment and harvest time, perennial ryegrass cultivar and harvest time and white clover cultivar and harvest time were significant.

White clover population density data were analysed before and after square root transformation. Visual assessment of residual plots was conducted; when a transformation was necessary *P* values and letters (to indicate significant differences) presented in the Tables are from the analysis of transformed data, but the values for number of growing points/m$^2$ and the SED are from the analysis of untransformed data for ease of interpretation.

The PAR interceptance data were analysed before and after angular transformation. Visual assessment of residual plots was conducted; the *P* values and letters (to indicate significant differences) presented in the Tables are from the analysis of transformed data, but the percentages and SED are from the analysis of untransformed data for ease of interpretation.

Regression analyses were conducted between perennial ryegrass and white clover population density, population density and DM yield and between DM yields, for monocultures and mixture.

## 4.4 Results

### 4.4.1 Total DM yield (kg DM/ha) of the perennial ryegrass and white clover mixtures

Total DM yield (kg DM/ha) of mixed pastures sown with different perennial ryegrass and white clover cultivars and receiving high (325 kg N/ha) or low (100 kg N/ha) rates of N fertiliser annually, are presented in Table 4.6.

Total DM yield for the season was 20.5 % greater in the High (19.9 t/ha) than in the Low N treatment (16.5 t/ha) (*P* = 0.001). This result was driven largely by significant differences between the N treatments in August, October, December and May.

**Table 4.6 Total DM yield (kg DM/ha) from pastures sown with mixtures of perennial ryegrass and white clover cultivars, and receiving high (325 kg N/ha) or low (100 kg N/ha) N fertiliser annually.**

| | | Aug-14 | Oct-14 | Nov-14 | Dec-14 | Jan-15 | Feb-15 | Mar-15 | Apr-15 | May-15 | Total season 2014 - 15 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| N treatment | High N | 2490 | 2790 | 1700 | 3410 | 2130 | 1850 | 1560 | 2970 | 1020 | 19920 |
| | Low N | 1340 | 1860 | 1715 | 2750 | 1945 | 1790 | 1470 | 2745 | 920 | 16540 |
| SED | | 145.7 | 190.6 | 60.8 | 82.9 | 89.2 | 47.5 | 64.6 | 77.5 | 16.4 | 290.2 |
| Perennial ryegrass cultivar | Abermagic AR1 | 1170 c | 2275 b | 2165 a | 2985 | 2095 a | 1885 | 1565 | 2900 a | 1080 a | 18120 |
| | Arrow AR1 | 2035 b | 2610 a | 1455 c | 3040 | 2005 ab | 1840 | 1485 | 2840 ab | 970 b | 18290 |
| | Bealey NEA2 | 2260 a | 2215 b | 1715 b | 3150 | 2150 a | 1770 | 1545 | 2965 a | 945 b | 18710 |
| | Prospect AR37 | 2190 a | 2210 b | 1500 c | 3145 | 1905 b | 1785 | 1465 | 2720 b | 880 c | 17795 |
| White clover cultivar | Bounty | 1860 | 2105 b | 1710 | 3025 | 2120 | 1830 ab | 1515 | 2880 | 915 b | 17960 |
| | Kopu II | 1870 | 2430 a | 1715 | 3085 | 2080 | 1880 a | 1535 | 2815 | 965 ab | 18375 |
| | Nomad | 1920 | 2340 a | 1715 | 3125 | 1990 | 1870 a | 1480 | 2860 | 975 ab | 18275 |
| | Tribute | 2005 | 2425 a | 1700 | 3085 | 1960 | 1700 b | 1530 | 2880 | 1025 a | 18305 |
| SED | Perennial ryegrass (White clover) | 65.1 | 113.1 | 45.6 | 93.8 | 74.9 | 67.2 | 66.5 | 70.3 | 32.0 | 361.1 |
| *P* value | N | < 0.01 | < 0.05 | 0.841 | < 0.01 | 0.128 | 0.299 | 0.247 | 0.063 | < 0.05 | < 0.01 |
| | Perennial ryegrass | < 0.001 | < 0.05 | < 0.001 | 0.232 | < 0.01 | 0.299 | 0.374 | < 0.01 | < 0.001 | 0.089 |
| | White clover | 0.113 | < 0.05 | 0.980 | 0.753 | 0.125 | < 0.05 | 0.847 | 0.757 | < 0.05 | 0.670 |
| | N x Perennial ryegrass interaction | < 0.01 | 0.823 | < 0.001 | 0.164 | 0.913 | 0.156 | 0.807 | 0.087 | 0.140 | 0.692 |
| | N x White clover interaction | < 0.05 | 0.299 | 0.747 | 0.629 | 0.651 | < 0.01 | 0.436 | 0.400 | 0.683 | 0.326 |
| | Perennial ryegrass x White clover interaction | 0.095 | 0.584 | < 0.05 | 0.189 | 0.363 | 0.155 | 0.858 | 0.577 | 0.508 | 0.459 |
| | N x Perennial ryegrass x White clover interaction | 0.434 | 0.251 | < 0.05 | 0.216 | 0.238 | 0.609 | 0.646 | 0.827 | 0.913 | 0.290 |

The effect of perennial ryegrass cultivar on the DM yield of the mixture was significant for six of the nine harvests (Table 4.6). Mixtures sown with different ryegrass cultivars were variable in their production, and the highest yielding mixture was not the same at every harvest. Thus, total annual DM yield was similar irrespective of the perennial ryegrass cultivar included ($P = 0.089$).

Similarly, the effect of white clover cultivar on the DM yield of the mixture was significant on only three occasions, resulting in a similar annual DM yield for the mixtures with different white clover cultivars ($P = 0.670$).

Interactions between N treatment and perennial ryegrass or white clover cultivars were present in only three of the nine harvests. In the first harvest (August 2014), when grown under the Low N treatment, the mixtures including Abermagic AR1 yielded significantly less than the mixtures based on other grass cultivars, all of which have similar yields. However, when grown under the High N treatment, although all the mixtures increased their DM yield, Bealey NEA2 mixtures yielded significantly more than mixtures with Arrow AR1, while Prospect AR37 mixtures were intermediate, and mixtures with Abermagic AR1 yielded the least ($P = 0.007$). At the same harvest, mixtures including the white clover cultivar Bounty were the lowest yielding when grown under the Low N treatment, but were the highest yielding when grown under the High N treatment ($P = 0.022$).

In November 2014, the mixtures including Abermagic AR1 increased in DM yield when grown under the High N treatment compared with the Low N treatment, while mixtures involving the other perennial ryegrass cultivars yielded similarly at both levels of N ($P$ value $< 0.001$).

Finally, in February 2015 mixtures with Nomad were the highest yielding under the High N treatment, while under the Low N treatment the highest yielding mixtures included Kopu II; at this harvest, mixtures with Kopu II yielded more under the Low than under the High N treatment.

During November 2014, when the only interaction between perennial ryegrass cultivar and white clover cultivar on DM yield occurred ($P = 0.046$), as well as the only interaction between N treatment, perennial ryegrass cultivar and white clover cultivar ($P = 0.045$), mixtures including Abermagic AR1 were the highest yielding irrespective of the N treatment and white clover cultivar included. Meanwhile, mixtures including Arrow AR1 and Kopu II grown under High N treatment yielded less than the same mixture under Low N treatment.

No other interactions between perennial ryegrass and white clover cultivars on DM yield were detected.

### 4.4.2 Perennial ryegrass and white clover yield (kg DM/ha) in mixed swards

Perennial ryegrass and white clover yields (kg DM/ha) from mixed pastures sown with different cultivars and receiving high (325 kg N/ha) or low (100 kg N/ha) rates of N fertiliser annually, are presented in Tables 4.7 and 4.8.

**Perennial ryegrass yield (kg DM/ha) in mixed swards**

Total perennial ryegrass yield was 54 % greater under the High (16.6 t/ha) than under the Low N treatment (10.8 t/ha) (Table 4.7). This increase in yield was evident at every harvest and was reasonably consistent throughout the season.

**Table 4.7 Perennial ryegrass yield (kg DM/ha) from pastures sown with mixtures of perennial ryegrass and white clover cultivars, and receiving high (325 kg N/ha) or low (100 kg N/ha) N fertiliser annually.**

| | | Aug-14 | Oct-14 | Nov-14 | Dec-14 | Jan-15 | Feb-15 | Mar-15 | Apr-15 | May-15 | Total season 2014 - 15 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| N treatment | High N | 2215 | 2455 | 1570 | 2940 | 1730 | 1455 | 1185 | 2210 | 880 | 16635 |
| | Low N | 1140 | 1480 | 1350 | 1990 | 965 | 905 | 715 | 1535 | 710 | 10795 |
| SED | | 128.4 | 140.0 | 64.2 | 82.3 | 47.8 | 75.0 | 42.2 | 68.1 | 9.8 | 68.7 |
| Perennial ryegrass cultivar | Abermagic AR1 | 995 c | 1870 b | 1830 a | 2375 b | 1365 | 1270 | 995 | 1900 ab | 905 a | 13500 b |
| | Arrow AR1 | 1785 b | 2225 a | 1240 c | 2360 b | 1260 | 1100 | 900 | 1800 b | 745 c | 13420 b |
| | Bealey NEA2 | 2005 a | 1865 b | 1440 b | 2505 ab | 1460 | 1210 | 1020 | 2015 a | 810 b | 14330 a |
| | Prospect AR37 | 1925 a | 1910 b | 1335 c | 2620 a | 1300 | 1145 | 880 | 1770 b | 725 c | 13610 b |
| White clover cultivar | Bounty | 1660 ab | 1760 b | 1435 | 2360 | 1345 | 1105 b | 865 b | 1825 b | 740 b | 13095 c |
| | Kopu II | 1605 b | 2010 a | 1440 | 2415 | 1265 | 1210 ab | 920 b | 1705 b | 735 b | 13295 c |
| | Nomad | 1675 ab | 2040 a | 1525 | 2550 | 1405 | 1325 a | 1065 a | 2095 a | 880 a | 14560 a |
| | Tribute | 1770 a | 2065 a | 1440 | 2535 | 1375 | 1085 b | 950 ab | 1865 b | 820 a | 13910 b |
| SED | Perennial ryegrass (White clover) | 58.9 | 111.5 | 50.0 | 97.2 | 76.9 | 69.0 | 59.5 | 87.3 | 33.0 | 298.8 |
| *P* value | N | < 0.01 | < 0.01 | < 0.05 | < 0.01 | < 0.001 | < 0.01 | < 0.01 | < 0.01 | < 0.001 | < 0.001 |
| | Perennial ryegrass | < 0.001 | < 0.01 | < 0.001 | < 0.05 | 0.058 | 0.088 | 0.052 | < 0.05 | < 0.001 | < 0.05 |
| | White clover | < 0.05 | < 0.05 | 0.222 | 0.153 | 0.314 | < 0.01 | < 0.05 | < 0.001 | < 0.001 | < 0.001 |
| | N x Perennial ryegrass interaction | < 0.01 | 0.885 | < 0.001 | 0.741 | 0.064 | 0.370 | 0.308 | 0.806 | 0.110 | 0.694 |
| | N x White clover interaction | < 0.05 | 0.424 | 0.565 | 0.342 | 0.121 | 0.181 | 0.321 | 0.475 | 0.278 | 0.394 |
| | Perennial ryegrass x White clover interaction | 0.218 | 0.700 | 0.073 | 0.206 | 0.301 | 0.083 | 0.859 | 0.441 | 0.413 | 0.203 |
| | N x Perennial ryegrass x White clover interaction | 0.525 | 0.863 | < 0.01 | 0.754 | 0.515 | 0.143 | 0.987 | 0.987 | 0.913 | 0.801 |

The effect of perennial ryegrass cultivar on grass yield was significant at six of the nine harvests (Table 4.7). Apart from December, a significant effect of perennial ryegrass cultivar on the total mixture yield was also observed at the same harvests (Table 4.6). The ranking order among cultivars was the same for perennial ryegrass yield and total mixture yield in August, October, November and very similar on April and May. During December, there was a significant effect of grass cultivar on perennial ryegrass yield ($P$ = 0.029), but there was no effect of grass cultivar on total mixture yield. For the entire season, the effect of the perennial ryegrass cultivar on the yield of the grass component was significant ($P$ = 0.011) resulting in mixtures with Bealey NEA2 yielding more grass herbage than mixtures with the other three cultivars, but not more total mixture herbage (Table 4.6).

The legume cultivar affected perennial ryegrass yield at six of the harvests, and also affected total grass yield for the season. At the first harvest in August 2014, mixtures including Kopu II had lower perennial ryegrass yield than mixtures with Tribute, while in October, mixtures based on Bounty yielded less grass than mixtures based on all other white clover cultivars. In late summer and autumn (February, March, April and May) mixtures including Nomad white clover had the highest grass yield at all harvests; a trend that was reflected in the total annual grass yield, where mixtures including Nomad had the highest grass yield, followed by mixtures including Tribute, while mixtures including Kopu II and Bounty had the lowest grass yield.

Interactions between N and perennial ryegrass cultivar and between N and white clover cultivar on grass yield were present in the first harvest of the season. In August, the interaction between perennial cultivar and N on grass yield mimicked the interaction described above for total mixture yield. Abermagic AR1 mixtures yielded less grass than mixtures of the other cultivars when grown under the Low N treatment, while under the High N treatment Bealey NEA2 mixtures yielded more grass than Prospect AR37 and Arrow AR1 mixtures, with Abermagic AR1 mixtures having the lowest grass yield ($P$ = 0.002). Meanwhile, mixtures including the white clover cultivar Bounty, had the lowest grass yield when grown under the Low N treatment, but the highest grass yield when grown under the High N treatment ($P$ = 0.033).

During November, there were significant interactions between N and perennial ryegrass cultivar on grass yield ($P$ < 0.001), as well as between N, perennial ryegrass cultivar and white clover cultivar on grass yield ($P$ = 0.005). While for Abermagic AR1 the grass yield increased under the High N when sown with Bounty, Kopu II and Tribute, but not with Nomad, for Bealey NEA2 the grass yield was similar irrespective of the clover cultivar included in the mixture or the N treatment. For others, like Arrow AR1, the grass yield increased when associated with Tribute under High N treatment compared with Low N treatment, while the opposite occurred when sown with Kopu II. Prospect

AR37, on the other hand, increased its grass yield under the High N treatment only when grown in association with Tribute.

No other interactions involving perennial ryegrass and white clover cultivars on perennial ryegrass yield were detected.

### White clover yield (kg DM/ha) in mixed swards

White clover yield (kg DM/ha) was greater under the Low than under the High N treatment at all harvests from November to the end of the season (Table 4.8). As a result the annual clover yield when less N was available was more than double the yield when a high rate of N fertiliser was applied ($P = 0.002$).

**Table 4.8 White clover yield (kg DM/ha) of pastures sown with mixtures of perennial ryegrass and white clover cultivars, and receiving high (325 kg N/ha) or low (100 kg N/ha) N fertiliser annually.**

| | | Aug-14 | Oct-14 | Nov-14 | Dec-14 | Jan-15 | Feb-15 | Mar-15 | Apr-15 | May-15 | Total season 2014 - 15 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| N treatment | High N | 25 | 135 | 110 | 355 | 305 | 305 | 320 | 480 | 95 | 2135 |
| | Low N | 35 | 275 | 335 | 670 | 905 | 815 | 710 | 955 | 175 | 4880 |
| SED | | 8.3 | 57.3 | 45.5 | 61.6 | 49.1 | 77.1 | 41.1 | 71.5 | 12.8 | 278.3 |
| Perennial ryegrass cultivar | Abermagic AR1 | 50 a | 290 a | 310 a | 530 | 665 | 550 | 515 | 750 | 135 b | 3790 |
| | Arrow AR1 | 30 ab | 200 bc | 200 bc | 575 | 630 | 640 | 540 | 750 | 185 a | 3755 |
| | Bealey NEA2 | 30 ab | 215 ab | 240 ab | 525 | 605 | 500 | 475 | 710 | 105 b | 3400 |
| | Prospect AR37 | 15 b | 110 c | 135 c | 425 | 515 | 555 | 530 | 665 | 120 b | 3080 |
| White clover cultivar | Bounty | 20 bc | 195 | 235 | 540 | 705 a | 650 a | 605 a | 790 a | 135 b | 3880 ab |
| | Kopu II | 55 a | 275 | 250 | 590 | 715 a | 595 ab | 560 a | 870 a | 185 a | 4100 a |
| | Nomad | 15 c | 155 | 165 | 460 | 495 b | 455 b | 360 b | 475 b | 65 c | 2640 c |
| | Tribute | 35 ab | 195 | 235 | 465 | 505 b | 545 ab | 535 a | 735 a | 155 ab | 3410 b |
| SED | Perennial ryegrass (White clover) | 9.7 | 44.7 | 43.4 | 79.9 | 72.7 | 72.0 | 55.5 | 84.2 | 19.4 | 292.9 |
| *P* value | N | 0.270 | 0.096 | < 0.05 | < 0.05 | < 0.01 | < 0.01 | < 0.01 | < 0.01 | < 0.01 | < 0.01 |
| | Perennial ryegrass | < 0.05 | < 0.01 | < 0.01 | 0.302 | 0.211 | 0.295 | 0.636 | 0.717 | < 0.001 | 0.056 |
| | White clover | < 0.001 | 0.052 | 0.189 | 0.318 | < 0.01 | < 0.05 | < 0.001 | < 0.001 | < 0.001 | < 0.001 |
| | N x Perennial ryegrass interaction | 0.956 | 0.585 | 0.251 | < 0.05 | < 0.05 | 0.702 | 0.860 | 0.329 | 0.792 | 0.249 |
| | N x White clover interaction | 0.070 | 0.542 | 0.932 | < 0.05 | 0.063 | 0.269 | 0.409 | 0.207 | 0.347 | 0.057 |
| | Perennial ryegrass x White clover interaction | 0.847 | 0.781 | 0.869 | 0.061 | 0.111 | 0.817 | 0.387 | 0.912 | < 0.05 | 0.352 |
| | N x Perennial ryegrass x White clover interaction | 0.863 | 0.159 | 0.882 | 0.474 | 0.941 | 0.082 | 0.504 | 0.888 | 0.697 | 0.360 |

Perennial ryegrass cultivar affected clover yield at four of the nine harvests but did not affect annual clover yield ($P = 0.056$) (Table 4.8). In the winter and spring, mixtures with Abermagic AR1 and Bealey NEA2 had the highest clover yield followed by Arrow AR1, while the mixtures with Prospect had the lowest clover yield. Meanwhile, during May 2015, mixtures including Arrow AR1 perennial ryegrass had greater clover yield than mixtures with the rest of the grass cultivars. In summer 2014 – 15 and early autumn 2015, no effect of perennial ryegrass cultivar on clover yield was detected.

In contrast, white clover cultivar effects on clover yield were evident from January to May 2015, as well as in the first harvest (August 2014) and in the annual clover yield for the season. During the first harvest, mixtures including Kopu II had the highest clover yield, while mixtures with Nomad had the lowest ($P < 0.001$). From January onwards, and for total annual clover yield, mixtures including Nomad were amongst the lowest yielding for clover. Meanwhile Kopu II was amongst the cultivars with highest clover yield during summer and autumn, and also had the highest clover yield for the season, although it was not significantly different than Bounty (Table 4.8).

During December 2014, even though there was no effect of perennial ryegrass or white clover cultivar on clover yield, interactions between N and grass cultivar ($P = 0.016$) and N and clover cultivar ($P = 0.014$) on the legume yield were present. Under the High N treatment mixtures with all the grass cultivars had similar clover yield, but when grown under the Low N treatment, the clover yield increased significantly in all the mixtures, except when Prospect AR37 was the grass cultivar included, resulting in mixtures with lower clover yield than the rest (Figure 4.4).

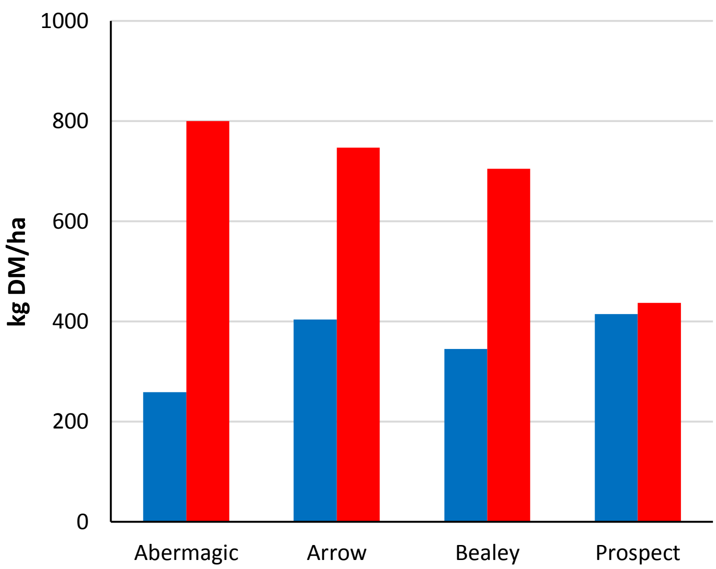


**Figure 4.4 White clover yield (kg DM/ha) of mixtures receiving high or low N fertiliser annually – December 2014. High N (blue bar), Low N (red bar). SED between N treatments – 115.6 kg DM/ha.**

Meanwhile, legume yield of mixtures including different white clover cultivars increased when grown under the Low N treatment, except when Bounty was the clover included; with this cultivar the mixtures under high N application rate had the highest clover yield, but under the Low N treatment had the lowest clover yield.

In January 2015, again an interaction between N and grass cultivar on clover yield was present (*P* = 0.027). Under the High N treatment mixtures with all the grass cultivars had similar clover yield; under the Low N treatment, clover yield increased in all mixtures but again mixtures including Prospect AR37 had the lowest clover yield, although not significantly different than mixtures with Arrow AR1.

The only significant interaction between perennial ryegrass cultivar and white clover cultivar on clover yield was present in the last harvest of the season, May 2015 (*P* = 0.011). Pastures including Nomad had the lowest clover yield irrespective of the grass cultivar included in the mixture (Figure 4.5). Meanwhile, pastures including Tribute yielded more clover when the perennial ryegrass associated was Prospect AR37 than when it was Bealey NEA2; on the other hand, mixtures including Bounty yielded more clover when associated with Arrow AR1 than when associated with Prospect AR37 or Bealey NEA2. When Kopu II was included in the mixture, the clover yield was greater when Arrow AR1 was the grass cultivar included; this combination of Arrow AR1 and Kopu II yielded more clover than any other mixture during May 2015.

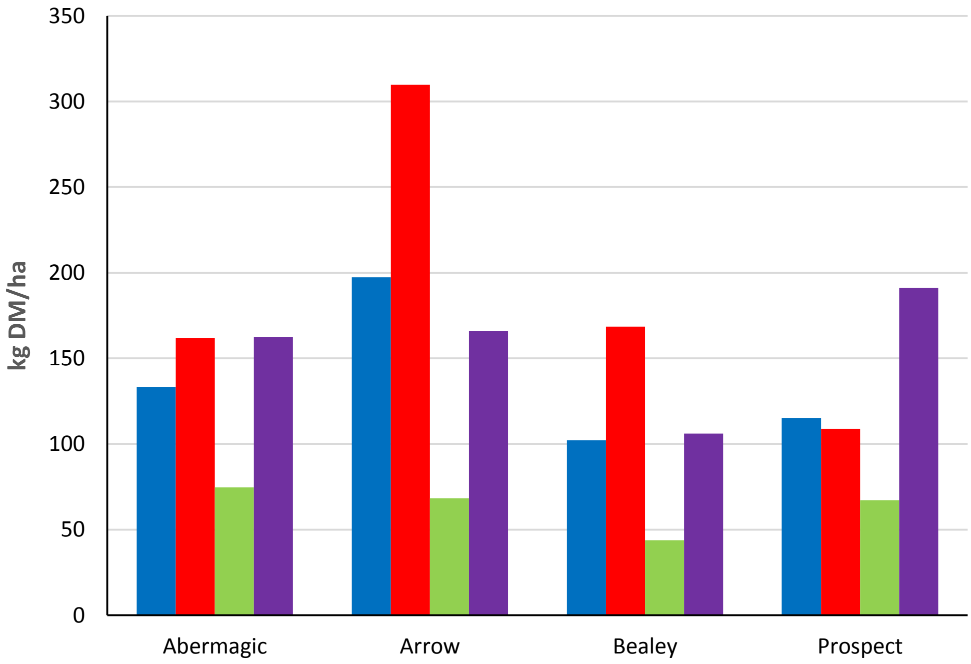


**Figure 4.5 White clover yield (kg DM/ha) of mixtures – May 2015. Bounty (blue bar), Kopu II (red bar), Nomad (green bar), Tribute (purple bar). SED – 38.8 kg DM/ha.**

### 4.4.3 White clover content (% DM) in mixed swards

The white clover content (% DM) of mixtures was greater under Low than under High N treatment (Figure 4.6), with the exception of the first harvest of the season (August 2014).

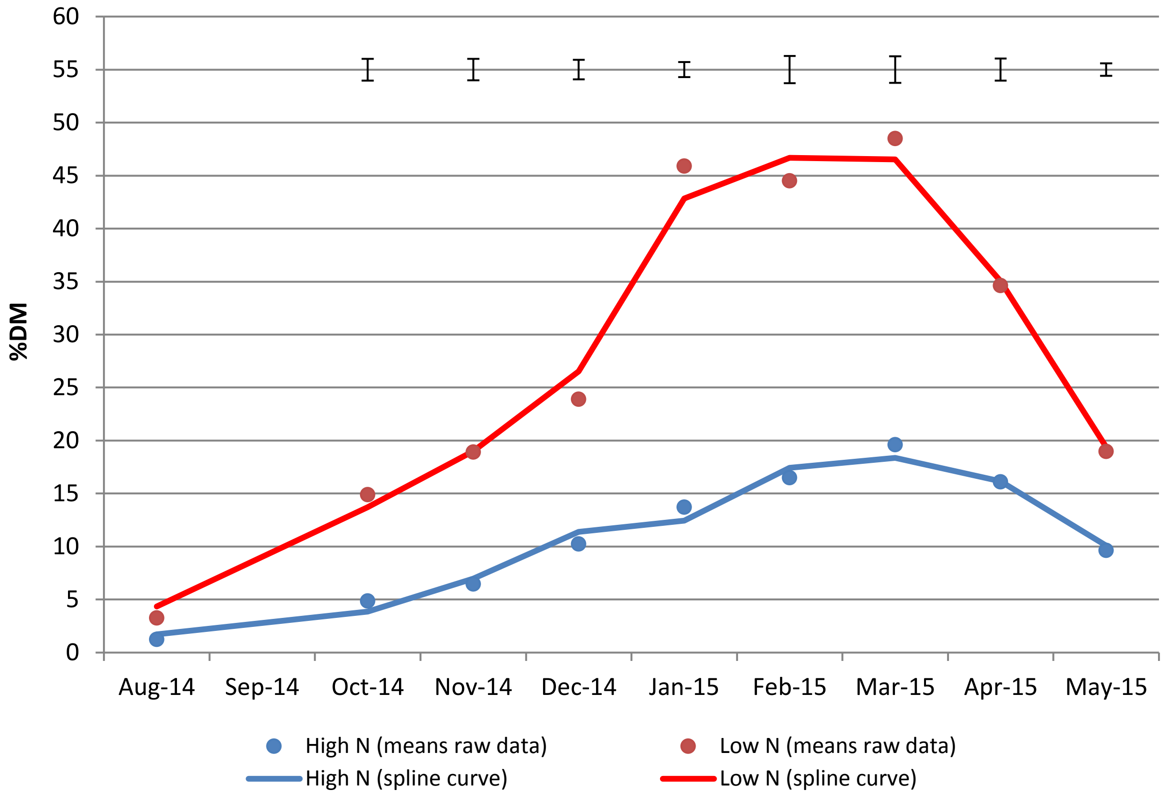


**Figure 4.6 White clover content (% DM) of mixtures under High or Low N treatment (bars indicate SED – standard error of differences between means).**

The effect of the perennial ryegrass cultivar on the white clover content of pastures was significant in only three of the nine harvests (Figure 4.7). In the first harvest of the season, pastures including Abermagic AR1 had greater clover content than mixtures with the other three cultivars ($P < 0.001$). In the following harvest (October 2014), again mixtures including Abermagic AR1 had the greatest clover content (13.9 %), but were not significantly different in clover content from mixtures including Bealey NEA2 (10.7 %); mixtures with Arrow AR1 followed (8.7 %), while mixtures including Prospect AR37 had the lowest clover content (6.2 %) ($P = 0.003$; SED – 2.05). Finally, at the last harvest of the season (May 2015), mixtures including Arrow AR1 had greater white clover percentage than mixtures based on all other cultivars. The interaction between N and perennial ryegrass cultivar as it affected clover % was significant on only one occasion, December 2014 ($P = 0.021$), when the clover % was similar in all mixtures at high N but significantly lower in mixtures based on Prospect AR37 than in mixtures based on all other cultivars, when grown under the Low N treatment.

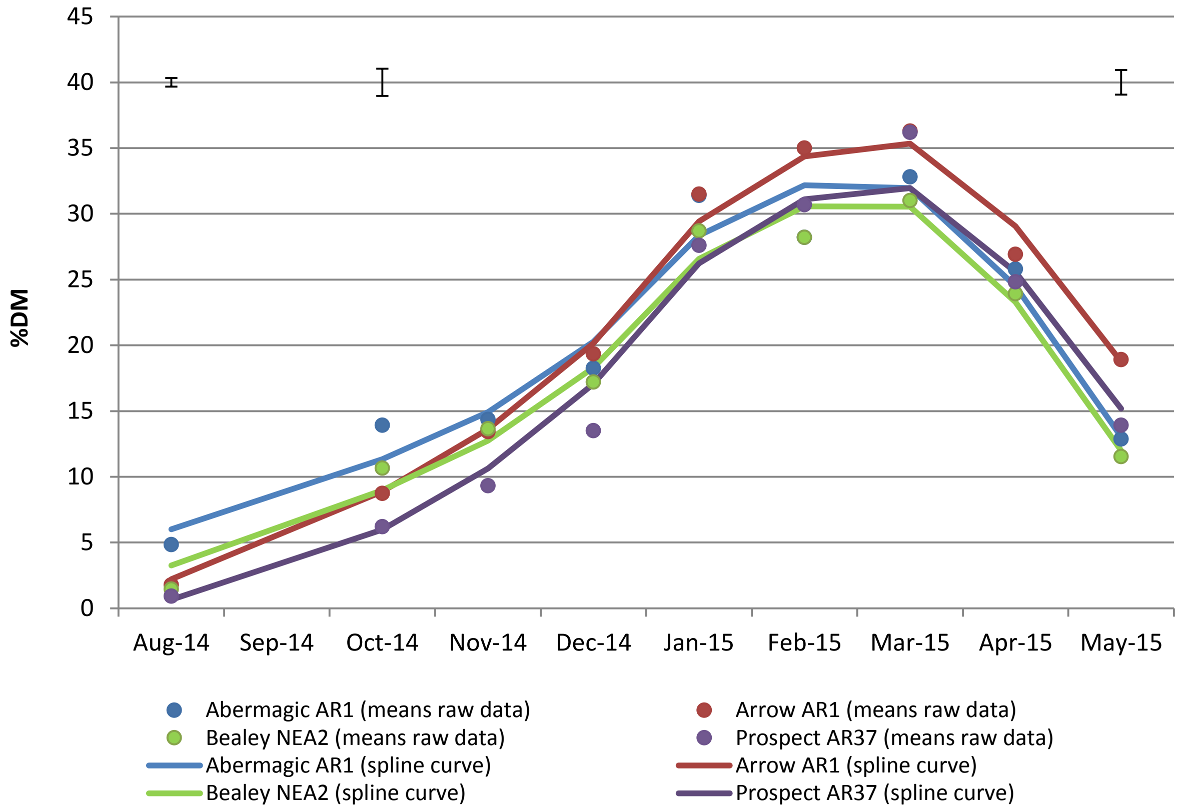


**Figure 4.7 White clover content (%DM) of mixtures sown with different perennial ryegrass cultivars (bars indicate SED – standard error of differences between means).**

By contrast, the effect of white clover cultivar on clover % was significant at seven of the nine harvests (Figure 4.8). In the first harvest of the season (August 2014), this effect was accompanied by an interaction between N treatment and clover cultivar ($P = 0.017$); as a result, when the mixtures were grown under the High N treatment, the clover content was similar, irrespective of the clover cultivar included, but when less N was applied, the mixtures including Kopu II had the greatest legume content (6.3 %), while mixtures including Nomad and Bounty had the lowest clover % (1.6 % both), and Tribute was intermediate (3.6 %). At the second harvest (October 2014), mixtures including Kopu II had higher clover content than mixtures including Tribute and Nomad ($P = 0.033$) while mixtures with Bounty were intermediate. In December 2014, an interaction between N and clover cultivar was present ($P = 0.019$); mixtures including Kopu II, Tribute and Nomad increased their clover content when less N was applied, but this did not happen when Bounty was the clover included. Mixtures including this cultivar had the highest clover content under the High N treatment, but the lowest content under the Low N treatment. From January 2015 onwards, mixtures with Kopu II, Bounty and Tribute had the highest clover content while mixtures with Nomad had lower clover %. During January, an interaction between N treatment and clover cultivar was present ($P = 0.040$); under the High N treatment all the mixtures had similar clover content, but under the Low N treatment mixtures including Kopu II and Bounty resulted in greater clover % than mixtures including Nomad and Tribute.

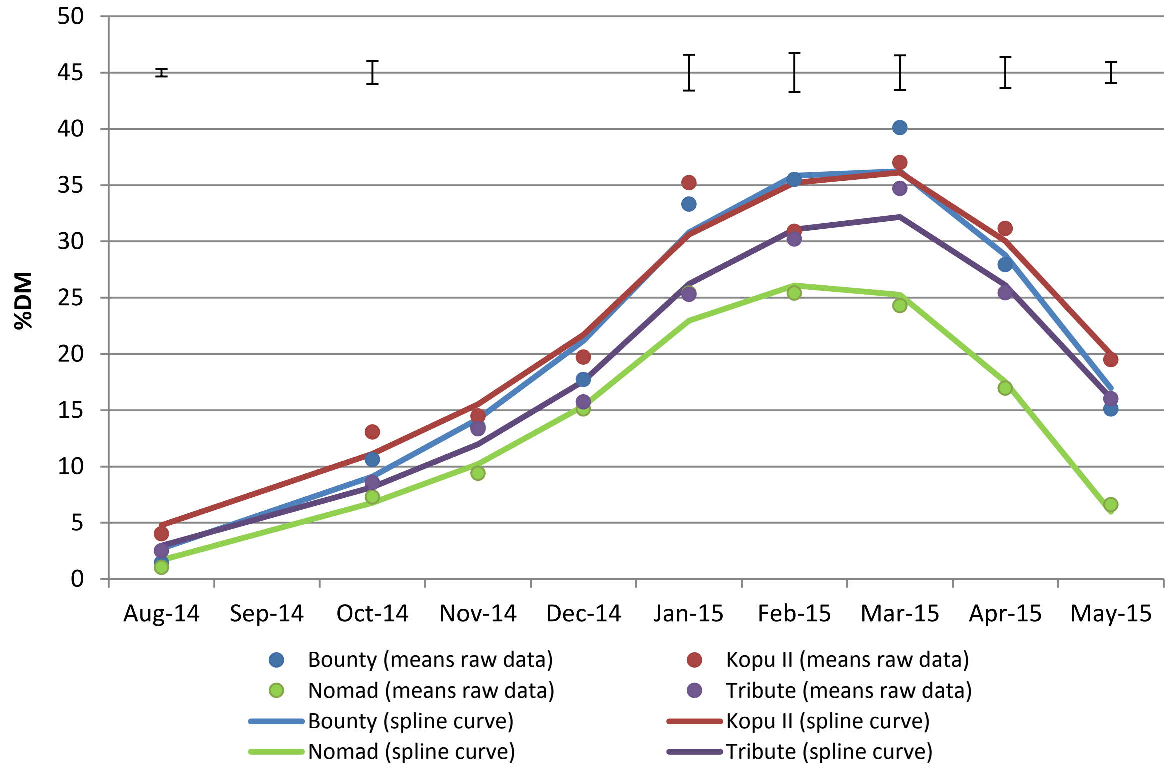


**Figure 4.8 White clover content (%DM) of mixtures sown with different white clover cultivars (bars indicate SED – standard error of differences between means).**

In the last harvest of the season (May 2015), an interaction between perennial ryegrass cultivar and white clover cultivar was detected ($P = 0.005$). Mixtures including Arrow AR1 and Bealey NEA2 had higher clover % when the cultivar included in the mixture was Kopu II (31.1 and 19.0 % respectively), but with Prospect AR37, Tribute was the cultivar with highest white clover % (20.9 %). With Abermagic AR1, mixtures including Tribute, Kopu II and Bounty had similar clover content, ranging from 16.2 to 13.9 %. Mixtures including Nomad had similar clover content irrespective of the perennial ryegrass cultivar included and varied from 4.9 to 7.8 % DM.

### 4.4.4 Total DM yield (kg DM/ha) of perennial ryegrass monocultures

The DM yield of perennial ryegrass monocultures was, on average, 47 % higher under the high N fertilisation rate than the low N fertilisation rate, and this effect was evident at most of the harvests (Table 4.9). On the other hand, the grass cultivar effect was present on only two occasions (August and November 2014). In the first harvest, the mean yield of pastures sown with Abermagic AR1 was lower than for pastures sown with the other cultivars ($P < 0.001$). In contrast, in November, Abermagic AR1 pastures had the highest yield ($P < 0.001$), and Abermagic AR1 was the only cultivar that increased production when more N was applied ($P = 0.001$). Total annual DM yield did not differ among cultivars ($P = 0.211$, range 15.1 to 16.6 t DM/ha).

**Table 4.9 Total DM yield (kg DM/ha) of perennial ryegrass monocultures receiving high (325 kg N/ha) or low (100 kg N/ha) rates of N fertiliser annually.**

| | | Aug-14 | | Oct-14 | Nov-14 | | Dec-14 | Jan-15 | Feb-15 | Mar-15 | Apr-15 | May-15 | Total season 2014 - 15 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| N treatment | High N | 2460 | | 2730 | 1710 | | 3200 | 1755 | 1650 | 1410 | 2710 | 1030 | 18650 |
| | Low N | 1315 | | 1440 | 1595 | | 2110 | 1110 | 1170 | 995 | 2115 | 815 | 12665 |
| SED | | 152.8 | | 349.9 | 80.6 | | 170.8 | 43.1 | 32.7 | 130.3 | 169.9 | 16.9 | 741.5 |
| Perennial ryegrass | Abermagic AR1 | 1020 | b | 2085 | 2140 | a | 2570 | 1595 | 1370 | 1325 | 2475 | 970 | 15555 |
| | Arrow AR1 | 2015 | a | 2125 | 1350 | d | 2625 | 1355 | 1425 | 1190 | 2325 | 960 | 15370 |
| | Bealey NEA2 | 2400 | a | 2200 | 1635 | b | 2765 | 1485 | 1450 | 1215 | 2525 | 915 | 16590 |
| | Prospect AR37 | 2110 | a | 1925 | 1480 | c | 2660 | 1305 | 1395 | 1075 | 2330 | 840 | 15125 |
| SED | | 184.5 | | 236.8 | 59.9 | | 142.2 | 105.4 | 98.3 | 109.0 | 104.8 | 63.7 | 708.5 |
| *P* value | N | < 0.01 | | < 0.05 | 0.255 | | < 0.01 | < 0.001 | < 0.001 | 0.050 | < 0.05 | < 0.01 | < 0.01 |
| | Perennial ryegrass | < 0.001 | | 0.698 | < 0.001 | | 0.579 | 0.056 | 0.868 | 0.193 | 0.162 | 0.185 | 0.211 |
| | N x Perennial ryegrass | 0.683 | | 0.274 | < 0.01 | | 0.173 | 0.974 | 0.928 | 0.314 | 0.326 | 0.374 | 0.906 |

### 4.4.5 Total DM yield (kg DM/ha) of white clover monocultures

In the first harvest of the season (August 2014), the white clover monocultures were not harvested due to the forage being mostly under the cutting height of the Haldrup harvester.

From October 2014 onwards, white clover monocultures yielded similarly under the High and the Low N treatments at every harvest (Table 4.10). As a result there was no effect of N on annual DM yield ($P$ = 0.567). During spring and summer, all white clover cultivars yielded similarly when grown in monoculture, but during autumn, monocultures of Tribute and Kopu II yielded more than monocultures of Nomad and Bounty. However, the annual DM yield of the four monocultures was similar ($P$ = 0.054), ranging from 13.7 to 15 t DM/ha.

**Table 4.10 Total DM yield (kg DM/ha) of with white clover monocultures receiving high (325 kg N/ha) or low (100 kg N/ha) rates of N fertiliser annually.**

| | | Oct-14 | Nov-14 | Dec-14 | Jan-15 | Feb-15 | Mar-15 | | Apr-15 | | May-15 | | Total season 2014 - 15 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| N treatment | High N | 3160 | 1655 | 2555 | 2020 | 1650 | 1225 | | 1825 | | 485 | | 14565 |
| | Low N | 3160 | 1635 | 2645 | 1955 | 1610 | 1200 | | 1735 | | 480 | | 14420 |
| SED | | 105.4 | 78.1 | 142.0 | 63.1 | 69.7 | 69.5 | | 102.6 | | 32.8 | | 231.2 |
| White clover | Bounty | 3190 | 1635 | 2525 | 2055 | 1680 | 1125 | b | 1760 | b | 385 | b | 14355 |
| | Kopu II | 3230 | 1715 | 2535 | 2050 | 1670 | 1330 | a | 1725 | b | 580 | a | 14840 |
| | Nomad | 2890 | 1565 | 2755 | 1900 | 1540 | 1015 | b | 1665 | b | 400 | b | 13730 |
| | Tribute | 3320 | 1670 | 2585 | 1935 | 1625 | 1375 | a | 1965 | a | 570 | a | 15045 |
| SED | | 171.6 | 92.9 | 143.1 | 104.3 | 110.4 | 88.7 | | 68.1 | | 35.6 | | 472.4 |
| *P* value | N | 0.993 | 0.853 | 0.590 | 0.376 | 0.614 | 0.744 | | 0.455 | | 0.966 | | 0.567 |
| | White clover | 0.106 | 0.438 | 0.378 | 0.351 | 0.580 | < 0.01 | | < 0.01 | | < 0.001 | | 0.054 |
| | N x White clover | 0.208 | 0.495 | 0.234 | 0.397 | 0.859 | 0.919 | | 0.058 | | 0.669 | | 0.609 |

### 4.4.6 DM yield (kg DM/ha) of pastures sown with or without white clover

In the first harvest of the season the inclusion of clover did not increase the DM yield of the pasture, irrespective of N treatment (Table 4.11). Then in October, mixtures yielded more than perennial ryegrass monoculture and the increase in pasture yield when the clover was present tended to be greater under Low than under High N treatment (*P* value of the N x White clover presence interaction – 0.051). In November, grass monocultures and mixtures yielded similarly, although the same trend was present, and the increase in yield due to clover seemed greater when less N was available (*P* value of the N x White clover presence interaction – 0.074). This was certainly the case in December, when the inclusion of clover significantly increased the DM yield of the pasture under the Low N treatment, but not under the High N treatment. From January to April, mixed pastures yielded more than grass monocultures when grown under either high or low N application rate, but the increment due to the inclusion of clover was always greater when less N fertiliser was available. In the last harvest of the season (May 2015), the presence of clover increased the DM yield under the Low N treatment, but not under the High N treatment. When considering the annual total yield, the results show that mixed pastures yielded 16.4 % more than perennial ryegrass monoculture (2.6 t DM/ha more, average of both N treatments); however, the increment due to the inclusion of clover varied when the pastures were grown under different N treatment. Mixtures that received low N application rate annually yielded 30.6 % more than perennial ryegrass monocultures under the same level of N (3.9 t DM/ha more), while under the high N application rate, mixed pastures yielded only 6.8 % more than perennial ryegrass monocultures (1.3 t DM/ha).

**Table 4.11 Total DM yield (kg DM/ha) of pastures sown with (mixtures) or without the presence of white clover (perennial ryegrass monoculture) and receiving high (325 kg N/ha) or low (100 kg N/ha) rates of N fertiliser annually.**

| | | Aug-14 | Oct-14 | Nov-14 | Dec-14 | Jan-15 | Feb-15 | Mar-15 | Apr-15 | May-15 | Total season 2014 - 15 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| White clover presence | Mixture | 1915 | 2325 | 1710 | 3080 | 2040 | 1820 | 1515 | 2855 | 970 | 18230 |
| | Perennial ryegrass monoculture | 1885 | 2085 | 1650 | 2655 | 1435 | 1410 | 1200 | 2415 | 920 | 15660 |
| SED | | 55.6 | 90.6 | 35.1 | 75.1 | 56.4 | 50.5 | 51.9 | 56.7 | 25.2 | 284.8 |
| N x White clover presence | High N mixture | 2490 | 2790 | 1700 | 3410 | 2130 | 1850 | 1560 | 2970 | 1020 | 19920 |
| | High N perennial ryegrass monoculture | 2460 | 2730 | 1710 | 3200 | 1755 | 1650 | 1410 | 2710 | 1030 | 18650 |
| | Low N mixture | 1340 | 1860 | 1715 | 2750 | 1945 | 1790 | 1470 | 2745 | 920 | 16540 |
| | Low N perennial ryegrass monoculture | 1315 | 1440 | 1595 | 2110 | 1110 | 1170 | 995 | 2115 | 815 | 12665 |
| SED (within N treatment) | | 78.6 | 128.1 | 49.6 | 106.2 | 79.8 | 71.5 | 73.4 | 80.2 | 35.7 | 402.8 |
| *P* value | White clover presence | 0.636 | < 0.01 | 0.110 | < 0.001 | < 0.001 | < 0.001 | < 0.001 | < 0.001 | 0.056 | < 0.001 |
| | N x White clover presence interaction | 0.969 | 0.051 | 0.074 | < 0.01 | < 0.001 | < 0.001 | < 0.01 | < 0.01 | < 0.05 | < 0.001 |

To examine if the inclusion of different white clover cultivars in mixtures with different perennial ryegrass cultivars affected pasture production, ANOVA was conducted on the DM yield of the mixtures and the perennial ryegrass monocultures, considering the latter to represent a zero white clover (No white clover) treatment. In this way, the analyses were conducted as a factorial of 4 perennial ryegrass cultivars × 5 white clover "cultivars". No interaction was detected between perennial ryegrass and white clover cultivar, or between N, perennial ryegrass and white clover cultivars, on DM yield, at any harvest or in the annual total for the season (Figure 4.9).

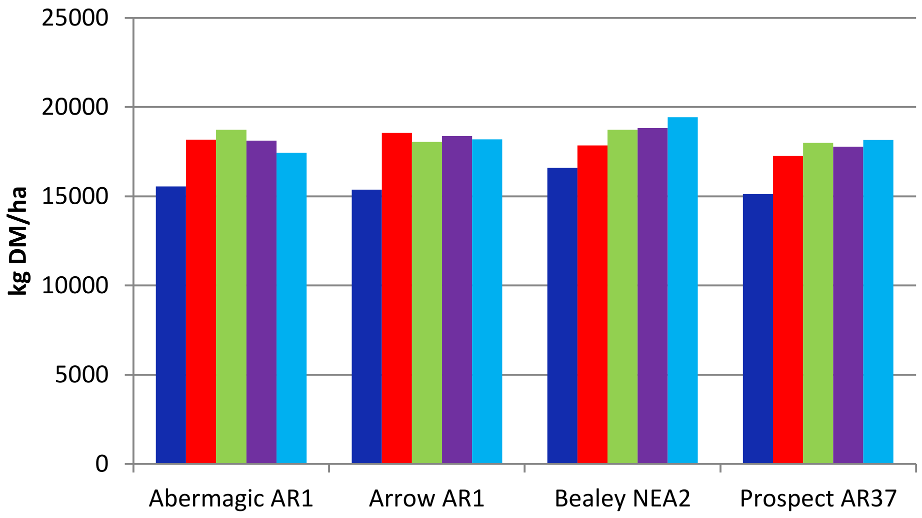


**Figure 4.9 Annual total DM yield (kg DM/ha) of pastures sown with or without white clover. No white clover (dark blue bar), Bounty (red bar), Kopu II (green bar), Nomad (purple bar), Tribute (light blue bar). SED – 720.5 kg DM/ha.**

### 4.4.7 DM yield (kg DM/ha) of pastures sown with or without the presence of perennial ryegrass

In the first harvest of the season (August 2014), the white clover monocultures were not harvested due to the forage being mostly under the cutting height of the Haldrup harvester (Table 4.12). In October 2014, the monocultures yielded more than the mixtures, probably as a consequence of the advantage of not having forage removed in the previous harvest, and the interaction with N is the result of the mixtures yielding less under Low than under High N treatment. In November, there was no effect of grass inclusion in the pasture, and mixtures and monocultures yielded similarly under both levels of N. Then in December, mixtures and white clover monocultures yielded similarly under Low N treatment, but under High N treatment, the mixtures yielded 33.3 % more than the white clover monocultures. In January, again mixtures and monocultures yielded similarly, but from February to May, mixed pastures yielded more than clover monocultures. In the last sampling (May 2015), the increase due to the inclusion of perennial ryegrass in the mixture was greater under High than under Low N treatment. As a result, the total yield of mixtures for the season was 36.8 %

greater than the yield of white clover monoculture when grown under the High N treatment, and 14.7 % greater under the Low N treatment.

**Table 4.12 Total DM yield (kg DM/ha) of pastures sown with (mixtures) or without the presence of perennial ryegrass (white clover monocultures) and receiving high (325 kg N/ha) or low (100 kg N/ha) rates of N fertiliser annually.**

| | | Aug-16 | Oct-16 | Nov-16 | Dec-16 | Jan-16 | Feb-16 | Mar-16 | Apr-16 | May-16 | Total season 2014 - 15 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Perennial ryegrass presence | Mixture | 1915 | 2325 | 1710 | 3080 | 2040 | 1820 | 1515 | 2855 | 970 | 18230 |
| | White clover monoculture | 0 | 3160 | 1645 | 2600 | 1985 | 1630 | 1210 | 1780 | 485 | 14495 |
| SED | | 47.6 | 87.3 | 37.3 | 73.9 | 55.8 | 50.8 | 49.6 | 52.0 | 23.4 | 268.8 |
| N x Perennial ryegrass presence | High N mixture | 2490 | 2790 | 1700 | 3410 | 2130 | 1850 | 1560 | 2970 | 1020 | 19920 |
| | High N white clover monoculture | 0 | 3160 | 1655 | 2555 | 2020 | 1650 | 1225 | 1825 | 485 | 14565 |
| | Low N mixture | 1340 | 1860 | 1715 | 2750 | 1945 | 1790 | 1470 | 2745 | 920 | 16540 |
| | Low N white clover monoculture | 0 | 3160 | 1635 | 2645 | 1955 | 1610 | 1200 | 1735 | 480 | 14420 |
| SED (within N treatment) | | 67.3 | 123.5 | 52.7 | 104.5 | 78.9 | 71.9 | 70.2 | 73.5 | 33.1 | 380.1 |
| *P* value | Perennial ryegrass presence | ---- | < 0.001 | 0.091 | < 0.001 | 0.360 | < 0.001 | < 0.001 | < 0.001 | < 0.001 | < 0.001 |
| | N x perennial ryegrass presence interaction | ---- | < 0.001 | 0.697 | < 0.001 | 0.282 | 0.841 | 0.495 | 0.194 | < 0.05 | < 0.001 |

To examine if the inclusion of different perennial ryegrass cultivars in mixtures with different white clover cultivars affected pasture production, ANOVA was conducted on the DM yield of the mixtures and the white clover monocultures, considering the latter to represent a zero perennial ryegrass (No perennial ryegrass) treatment. In this way, the analyses were conducted as a factorial of 5 perennial ryegrass "cultivars" × 4 white clover cultivars. No interaction was detected between perennial ryegrass and white clover cultivar, or between N, perennial ryegrass and white clover cultivars, on DM yield, at any harvest or in the annual total of the season (Figure 4.10).

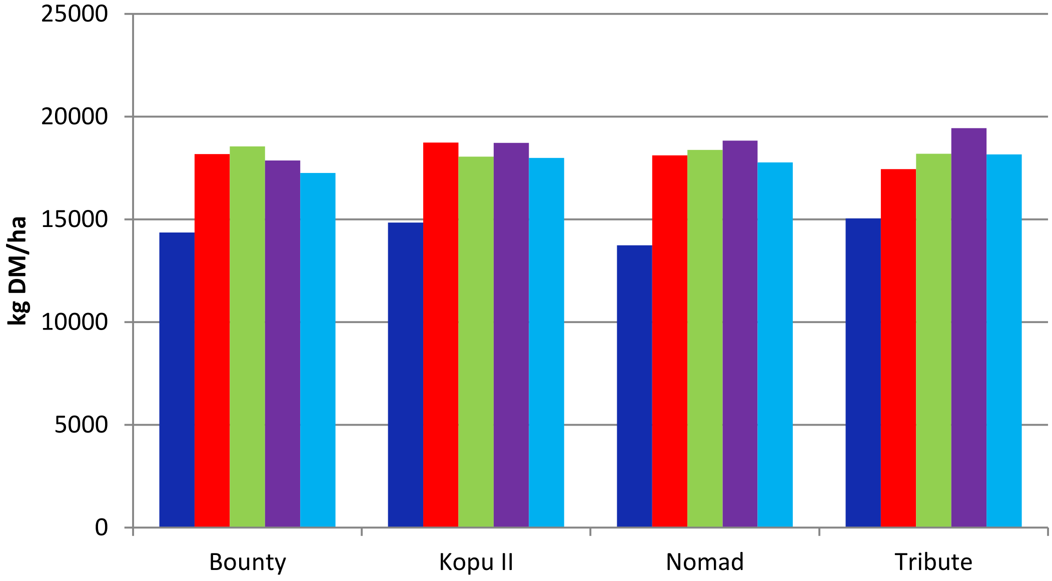


**Figure 4.10 Annual total DM yield (kg DM/ha) of pastures sown with or without perennial ryegrass. No perennial ryegrass (dark blue bar), Abermagic AR1 (red bar), Arrow AR1 (green bar), Bealey NEA2 (purple bar), Prospect AR37 (light blue bar). SED – 679.9 kg DM/ha.**

## 4.4.8 Perennial ryegrass and white clover population density

### Perennial ryegrass tiller density (tillers/m$^2$)

#### *4.4.8..1 Tiller density in perennial ryegrass monocultures*

There was no significant effect of N treatment on tiller density at the beginning of winter 2014 (June) or during spring 2014 (November), but during summer 2014 – 15 and autumn 2015 (January and May respectively) the tiller density of pastures was greater under High N than under Low N treatment (Table 4.13).

**Table 4.13 Tiller density (tillers/m²) in perennial ryegrass monocultures receiving high (325 kg N/ha) or low (100 kg N/ha) rates of N fertiliser annually.**

| | | Jun-14 | | Nov-14 | | Jan-15 | | May-15 | |
|---|---|---|---|---|---|---|---|---|---|
| N treatment | High N | 7023 | | 8723 | | 8440 | | 6917 | |
| | Low N | 7521 | | 7285 | | 5646 | | 5058 | |
| SED | | 393.6 | | 1320.7 | | 677.3 | | 516.3 | |
| Perennial ryegrass | Abermagic AR1 | 9275 | a | 9904 | a | 8083 | a | 7162 | a |
| | Arrow AR1 | 6933 | b | 7154 | bc | 7975 | a | 6838 | a |
| | Bealey NEA2 | 5038 | c | 6221 | c | 5400 | b | 4221 | b |
| | Prospect AR37 | 7842 | b | 8738 | ab | 6712 | ab | 5729 | a |
| SED | | 639.3 | | 874.1 | | 987.0 | | 714.4 | |
| *P* value | N | 0.295 | | 0.356 | | < 0.05 | | < 0.05 | |
| | Perennial ryegrass | < 0.001 | | < 0.01 | | < 0.05 | | < 0.01 | |
| | N x Perennial ryegrass | 0.616 | | 0.053 | | 0.503 | | 0.085 | |

Perennial ryegrass cultivars differed in tiller density at all four sampling times. In June, Abermagic AR1 was the densest cultivar and Bealey NEA2 the least dense, while Prospect AR37 and Arrow AR1 were intermediate. In November, Abermagic AR1 still had the highest tiller density, but was not significantly different than Prospect AR37, which was followed by Arrow AR1, with Bealey NEA2 being again the least dense cultivar. In January and May 2015 the three diploids did not differ in their tiller density while the tetraploid, Bealey NEA2, had fewer tillers/m² (Table 4.13).

#### *4.4.8..2 Tiller density in pastures sown with or without the presence of white clover*

At the beginning of the season, in June 2014, mixed pastures had 10 % fewer tillers/m² than the perennial ryegrass monocultures ($P = 0.005$; Table 4.14).

**Table 4.14 Tiller density (tillers/m²) in pastures sown with (mixture) or without the presence of white clover (perennial ryegrass monoculture) and receiving high (325 kg N/ha) or low (100 kg N/ha) rates of N fertiliser annually.**

| | | Jun-14 | Nov-14 | Jan-15 | May-15 |
|---|---|---|---|---|---|
| White clover presence | Mixture | 6542 | 7586 | 6722 | 4029 |
| | Perennial ryegrass monoculture | 7272 | 8004 | 7043 | 5987 |
| SED | | 253.1 | 338.6 | 330.5 | 240.7 |
| N x White clover presence | High N mixture | 6669 | 8597 | 8208 | 5119 |
| | High N perennial ryegrass monoculture | 7023 | 8723 | 8440 | 6917 |
| | Low N mixture | 6416 | 6575 | 5236 | 2940 |
| | Low N perennial ryegrass monoculture | 7521 | 7285 | 5646 | 5058 |
| SED (within N treatment) | | 358.0 | 478.9 | 467.4 | 340.4 |
| *P* value | White clover presence | < 0.01 | 0.220 | 0.334 | < 0.001 |
| | N x White clover presence interaction | 0.141 | 0.390 | 0.788 | 0.506 |

By November 2014, tiller numbers had increased in mixtures and grass monocultures although no effect of clover presence was detected ($P$ = 0.220), and mixtures and monocultures had similar mean tiller density. Meanwhile in January 2015, there was no effect of white clover inclusion on tiller density ($P$ = 0.334) or interactions between treatments on tiller density. In May, mean tiller density of mixed pastures was 32.7 % lower than mean tiller density of ryegrass monocultures ($P$ < 0.001).

To examine if the inclusion of different white clover cultivars in mixtures with different perennial ryegrass cultivars affected tiller density, ANOVA was conducted on the tiller density of the mixtures and the perennial ryegrass monocultures, considering the latter to represent a zero white clover (No white clover) treatment. In this way, the analyses were conducted as a factorial of 4 perennial ryegrass cultivars × 5 white clover "cultivars". A significant interaction between perennial ryegrass and white clover cultivars ($P$ = 0.043) was present in November, but not at other times. In November, pastures sown with Arrow AR1, Bealey NEA2 or Prospect AR37 had similar tiller density when grown in monoculture or when grown in association with any of the four clover cultivars. However for Abermagic AR1, tiller density was greater when grown in association with Bounty and Nomad than with Kopu II and Tribute, while the monoculture was intermediate (Figure 4.11).

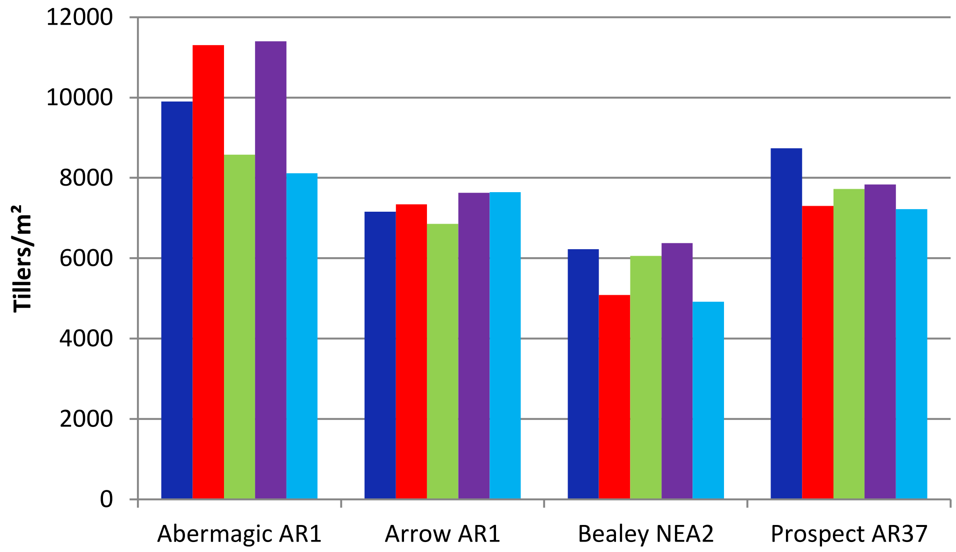


**Figure 4.11 Perennial ryegrass tiller density (tillers/m$^2$) in pastures sown with or without white clover – November 2014. No white clover (dark blue bar), Bounty (red bar), Kopu II (green bar), Nomad (purple bar), Tribute (light blue bar). SED – 856.6 kg DM/ha.**

#### *4.4.8..3 Tiller density of mixtures*

There was no significant effect of N treatment on tiller density of mixtures at the beginning of winter 2014 (June) (Table 4.15).

**Table 4.15 Tiller density (tillers/m$^2$) of mixtures receiving high (325 kg N/ha) or low (100 kg N/ha) rates of nitrogen fertiliser annually.**

| | | Jun-14 | | Nov-14 | | Jan-15 | | May-15 | |
|---|---|---|---|---|---|---|---|---|---|
| N treatment | High N | 6669 | | 8597 | | 8208 | | 5119 | |
| | Low N | 6416 | | 6575 | | 5236 | | 2940 | |
| SED | | 236.2 | | 746.7 | | 348.1 | | 565.4 | |
| Perennial ryegrass cultivar | Abermagic AR1 | 7845 | a | 9848 | a | 8118 | a | 4792 | a |
| | Arrow AR1 | 6708 | b | 7366 | b | 6872 | b | 4105 | b |
| | Bealey NEA2 | 4814 | c | 5609 | c | 5400 | c | 3516 | b |
| | Prospect AR37 | 6802 | b | 7522 | b | 6500 | b | 3704 | b |
| White clover cultivar | Bounty | 6319 | | 7757 | ab | 6852 | | 3867 | |
| | Kopu II | 6798 | | 7301 | b | 6449 | | 3934 | |
| | Nomad | 6696 | | 8310 | a | 6719 | | 4527 | |
| | Tribute | 6356 | | 6976 | b | 6870 | | 3789 | |
| SED | Perennial ryegrass (White clover) | 324.0 | | 423.1 | | 410.5 | | 296.5 | |
| *P* value | N | 0.363 | | 0.073 | | < 0.01 | | < 0.05 | |
| | Perennial ryegrass | < 0.001 | | < 0.001 | | < 0.001 | | < 0.001 | |
| | White clover | 0.353 | | < 0.05 | | 0.719 | | 0.058 | |
| | N x Perennial ryegrass interaction | 0.638 | | 0.297 | | 0.086 | | 0.877 | |
| | N x White clover interaction | 0.610 | | 0.128 | | 0.205 | | 0.280 | |
| | Perennial ryegrass x White clover interaction | 0.336 | | < 0.05 | | 0.171 | | 0.379 | |
| | N x Perennial ryegrass x White clover interaction | 0.880 | | 0.134 | | 0.497 | | 0.855 | |

A few months later during spring 2014 (November), the tiller density of the mixtures under the High N treatment had increased considerably (28.9 % more tillers than in June) while under the Low N treatment they had increased only 2.5 %, showing a trend towards higher tiller density when more N was available (*P* = 0.073). This trend was confirmed during summer 2014 – 15 and autumn 2015 (January and May respectively) when the tiller density of pastures was greater under High N than under Low N treatment (56.8 % greater in January and 74.1 % greater in May 2015).

Meanwhile, the perennial ryegrass cultivar effect was present at all four sampling times; during June, November and January Abermagic AR1 was the densest cultivar and Bealey NEA2 the least dense, while Prospect AR37 and Arrow AR1 were intermediate and not different between them. In May, Abermagic AR1 remained the cultivar with the highest density, but during this month there was no difference between Arrow AR1, Bealey NEA2 and Prospect AR37.

White clover cultivar affected grass tiller density only during November (*P* = 0.014), when there was also an interaction between perennial ryegrass and white clover cultivars (*P* value = 0.023). When Arrow AR1, Bealey NEA2 and Prospect AR37 were included in the mixtures, the inclusion of different white clover cultivars did not affect the tiller density of the pasture. On the other hand, when Abermagic AR1 was the grass cultivar included, mixtures including Nomad and Bounty were denser than mixtures including Kopu II and Tribute (Figure 4.11).

No interaction between N treatment and perennial ryegrass and white clover cultivars on tiller density was detected during the year.

### White clover growing point density (growing points/m$^2$)

#### *4.4.8..1 Growing point density in white clover monocultures*

Growing point density was similar when clover monocultures were grown under the High or under the Low N treatment at every sampling (Table 4.16).

**Table 4.16 Growing point density (growing points/m$^2$) in white clover monocultures receiving high (325 kg N/ha) or low (100 kg N/ha) rates of N fertiliser annually.**

| | | Jun-14 | | Nov-14 | | Jan-15 | | May-15 | |
|---|---|---|---|---|---|---|---|---|---|
| N treatment | High N | 3829 | | 2690 | | 2683 | | 3446 | |
| | Low N | 3617 | | 2215 | | 2719 | | 3298 | |
| SED | | 103.7 | | 281.8 | | 251.3 | | 389.6 | |
| White clover | Bounty | 4358 | a | 2725 | ab | 3062 | a | 3846 | a |
| | Kopu II | 2883 | b | 1633 | c | 1867 | c | 2758 | b |
| | Nomad | 4246 | a | 3042 | a | 3304 | a | 3579 | a |
| | Tribute | 3404 | b | 2408 | b | 2571 | b | 3304 | ab |
| SED | | 291.7 | | 246.0 | | 158.7 | | 308.1 | |
| *P* value | N | 0.133 | | 0.190 | | 0.897 | | 0.729 | |
| | White clover | < 0.001 | | < 0.001 | | < 0.001 | | < 0.05 | |
| | N x White clover | 0.611 | | 0.730 | | 0.316 | | 0.801 | |

By contrast, the differences between clover cultivars in growing point density were always significant, with Bounty and Nomad consistently amongst the cultivars with the most growing points/m$^2$, and Kopu II and Tribute amongst the cultivars with the fewest growing points/m$^2$.

#### *4.4.8..2 Growing point density in pastures sown with or without the presence of perennial ryegrass*

During the entire season, the number of growing points/m$^2$ was higher in white clover monocultures than in mixtures (Table 4.17).

**Table 4.17 Growing point density (growing points/m$^2$) in pastures sown with (mixture) or without the presence of perennial ryegrass (white clover monoculture) and receiving high (325 kg N/ha) or low (100 kg N/ha) rates of N fertiliser annually.**

| | | Jun-14 | Nov-14 | Jan-15 | May-15 |
|---|---|---|---|---|---|
| Perennial ryegrass presence | Mixture | 702 | 533 | 1185 | 649 |
| | White clover monoculture | 3723 | 2452 | 2701 | 3372 |
| SED | | 83.4 | 81.3 | 123.3 | 96.6 |
| N x Perennial ryegrass presence | High N mixture | 621 | 336 | 698 | 438 |
| | High N white clover monoculture | 3829 | 2690 | 2683 | 3446 |
| | Low N mixture | 783 | 730 | 1672 | 860 |
| | Low N white clover monoculture | 3617 | 2215 | 2719 | 3298 |
| SED (within N treatment) | | 118 | 115 | 174.4 | 136.6 |
| *P* value | Perennial ryegrass presence | < 0.001 | < 0.001 | < 0.001 | < 0.001 |
| | N x perennial ryegrass presence interaction | 0.053 | < 0.001 | < 0.001 | 0.001 |

In this table the number of growing points/m$^2$ and the SED are from the analysis without square root transformation, but *P* values are from the analysis with square root transformation.

While in June 2014 the effect of the inclusion of grass in the pasture was similar under both N treatments, from November onwards, the reduction in the number of growing points in the pasture due to the inclusion of perennial ryegrass was greater under the High than under Low N treatment.

To examine if the inclusion of different perennial ryegrass cultivars in mixtures with different white clover cultivars affected growing point density, ANOVA was conducted on the growing point density of the mixtures and the white clover monocultures, considering the latter to represent a zero perennial ryegrass (No perennial ryegrass) treatment. In this way, the analyses were conducted as a factorial of 5 perennial ryegrass “cultivars” × 4 white clover cultivars. Based on the power of the trial, no interaction was detected between perennial ryegrass and white clover cultivar or between N, perennial ryegrass and white clover cultivars on growing point density at any of the samplings.

#### *4.4.8..3 Growing point density of mixtures*

Mixtures had greater growing point density under Low than under High N treatment at every sampling (Table 4.18).

**Table 4.18 Growing point density (growing points/m²) of mixtures receiving high (325 kg N/ha) or low (100 kg N/ha) rates of N fertiliser annually.**

| | | Jun-14 | | Nov-14 | | Jan-15 | May-15 |
|---|---|---|---|---|---|---|---|
| N treatment | High N | 621 | | 336 | | 698 | 438 |
| | Low N | 783 | | 730 | | 1672 | 860 |
| SED | | 70.4 | | 136.6 | | 228.9 | 88.0 |
| Perennial ryegrass cultivar | Abermagic AR1 | 753 | | 527 | b | 1173 | 678 |
| | Arrow AR1 | 650 | | 523 | b | 1379 | 666 |
| | Bealey NEA2 | 809 | | 742 | a | 1203 | 603 |
| | Prospect AR37 | 596 | | 342 | c | 985 | 650 |
| White clover cultivar | Bounty | 842 | a | 545 | | 1298 | 659 |
| | Kopu II | 631 | bc | 402 | | 1028 | 739 |
| | Nomad | 803 | ab | 652 | | 1209 | 552 |
| | Tribute | 532 | c | 534 | | 1205 | 647 |
| SED | Perennial ryegrass (White clover) | 94.4 | | 96.9 | | 158.5 | 110.9 |
| *P* value | N | < 0.05 | | 0.050 | | < 0.01 | < 0.05 |
| | Perennial ryegrass | 0.082 | | < 0.001 | | 0.134 | 0.865 |
| | White clover | < 0.01 | | 0.131 | | 0.243 | 0.701 |
| | N x Perennial ryegrass interaction | 0.640 | | 0.261 | | 0.153 | 0.823 |
| | N x White clover interaction | 0.259 | | 0.605 | | 0.813 | 0.493 |
| | Perennial ryegrass x White clover interaction | 0.690 | | 0.825 | | 0.468 | 0.950 |
| | N x Perennial ryegrass x White clover interaction | 0.242 | | 0.143 | | 0.713 | 0.722 |

In this table the number of growing points/m² and the SED are from the analysis without square root transformation, but *P* values and letters are from the analysis with square root transformation

Meanwhile, the effect of perennial ryegrass cultivar on growing point density was present only in November 2014 ($P < 0.001$), when mixtures including Bealey NEA2 had the greatest growing point density and mixtures including Prospect AR37 were the least dense, while pastures including Abermagic AR1 and Arrow AR1 were intermediate.

The effect of the white clover cultivar on growing point density was only present during the first sampling of the season ($P = 0.003$), when mixtures including Bounty and Nomad had more growing points/m² than mixtures with Tribute, while mixtures including Kopu II were intermediate but not significantly different from Nomad and Tribute.

There was no interaction between N treatment and perennial ryegrass or white clover cultivars, or between perennial ryegrass and white clover cultivars, or between N treatment, perennial ryegrass and white clover cultivars on clover growing point density at any sampling time.

### 4.4.9 Light interception and canopy height

#### Light interception

##### *4.4.9..1 Light interception in perennial ryegrass monocultures*

At every sampling perennial ryegrass monocultures receiving high N application rate intercepted more photosynthetically active radiation (PAR) than monocultures grown under low N application (Table 4.19).

**Table 4.19 Percentage of PAR intercepted by perennial ryegrass monocultures receiving high (325 kg N/ha) or low (100 kg N/ha) rates of N fertiliser annually.**

| | | Dec-14 | Jan-15 | Apr-15 |
|---|---|---|---|---|
| N treatment | High N | 95.3 | 84.3 | 97.7 |
| | Low N | 87.4 | 67.0 | 91.5 |
| SED | | 1.43 | 1.02 | 1.49 |
| Perennial ryegrass | Abermagic AR1 | 90.6 | 73.3 | 93.4 |
| | Arrow AR1 | 91.1 | 74.8 | 93.3 |
| | Bealey NEA2 | 92.7 | 80.3 | 96.2 |
| | Prospect AR37 | 91.1 | 74.3 | 95.4 |
| SED | | 1.31 | 2.39 | 1.43 |
| *P* value | N | < 0.01 | < 0.001 | < 0.01 |
| | Perennial ryegrass | 0.293 | 0.051 | 0.065 |
| | N x Perennial ryegrass | 0.145 | 0.251 | 0.176 |

In this table the PAR interceptance (%) and the SED are from the analysis without angular transformation, but *P* values are from the analysis with angular transformation.

Perennial ryegrass cultivar effect was very close to significance in January, where Bealey NEA2 intercepted more light than all other cultivars.

##### *4.4.9..2 Light interception in pastures sown with or without the presence of white clover*

Mixtures intercepted more PAR than perennial ryegrass monocultures at every sampling time, and the increment in light intercepted due to the presence of clover was greater under Low than under high N treatment (Table 4.20).

**Table 4.20 Percentage of PAR intercepted by pastures sown with (mixture) or without the presence of white clover (perennial ryegrass monoculture) and receiving high (325 kg N/ha) or low (100 kg N/ha) rates of N fertiliser annually.**

| | | Dec-14 | Jan-15 | Apr-15 |
|---|---|---|---|---|
| White clover presence | Mixture | 96.0 | 88.2 | 99.1 |
| | Perennial ryegrass monoculture | 91.4 | 75.6 | 94.6 |
| SED | | 0.47 | 1.08 | 0.39 |
| N x White clover presence | High N mixture | 97.2 | 89.3 | 99.2 |
| | High N perennial ryegrass monoculture | 95.3 | 84.3 | 97.7 |
| | Low N mixture | 94.8 | 87.2 | 99.0 |
| | Low N perennial ryegrass monoculture | 87.4 | 67.0 | 91.5 |
| SED (within N treatment) | | 0.66 | 1.53 | 0.55 |
| *P* value | White clover presence | < 0.001 | < 0.001 | < 0.001 |
| | N x White clover presence interaction | < 0.001 | < 0.001 | < 0.001 |

In this table the PAR interceptance (%) and the SED are from the analysis without angular transformation, but *P* values are from the analysis with angular transformation.

To examine if the inclusion of different white clover cultivars in mixtures with different perennial ryegrass cultivars affected the light intercepted by the canopy, ANOVA was conducted on the PAR interceptance of the mixtures and the perennial ryegrass monocultures, considering the latter to represent a zero white clover (No white clover) treatment. In this way, the analyses were conducted as a factorial of 4 perennial ryegrass cultivars × 5 white clover "cultivars". Based on the power of the trial, no interaction was detected between perennial ryegrass and white clover cultivar or between N, perennial ryegrass and white clover cultivars on PAR interceptance at any of the samplings.

#### *4.4.9..3 Light interception in white clover monocultures*

During December and April, pastures sown under high or low N application rate intercepted similar percentage of PAR, but during January 2015, white clover monocultures intercepted more light when grown under High N treatment (Table 4.21).

**Table 4.21 Percentage of PAR intercepted by white clover monocultures receiving high (325 kg N/ha) or low (100 kg N/ha) rates of N fertiliser annually.**

| | | Dec-14 | | Jan-15 | | Apr-15 |
|---|---|---|---|---|---|---|
| N treatment | High N | 98.3 | | 95.3 | | 98.9 |
| | Low N | 98.7 | | 92.9 | | 98.3 |
| SED | | 0.52 | | 0.38 | | 0.32 |
| White clover | Bounty | 99.1 | a | 96.2 | a | 98.5 |
| | Kopu II | 97.6 | b | 93.8 | b | 98.6 |
| | Nomad | 98.6 | ab | 92.5 | b | 98.1 |
| | Tribute | 98.5 | ab | 94.0 | b | 99.1 |
| SED | | 0.52 | | 0.99 | | 0.45 |
| *P* value | N | 0.550 | | < 0.01 | | 0.087 |
| | White clover | < 0.05 | | < 0.01 | | 0.130 |
| | N x White clover | 0.974 | | 0.667 | | 0.650 |

In this table the PAR interceptance (%) and the SED are from the analysis without angular transformation, but *P* values and letters are from the analysis with angular transformation.

In December 2014, monocultures sown with Bounty intercepted more light than pastures sown with Kopu II, while monocultures sown with Nomad and Tribute were intermediate. Meanwhile during January, Bounty pastures intercepted more light than the rest of the cultivars. No white clover cultivar effect was present during April 2015.

#### *4.4.9..4 Light interception in pastures sown with or without the presence of perennial ryegrass*

White clover monocultures intercepted more light than mixed pastures during December and January (Table 4.22); in December the decrease in the light intercepted by the canopy when grown in mixtures respect to clover monoculture was greater under Low than under High N treatment, while in January this decrease was not influenced by the N application rate. In April, mixtures intercepted more light than white clover monocultures.

**Table 4.22 Percentage of PAR intercepted by pastures sown with (mixture) or without the presence of perennial ryegrass (white clover monoculture) and receiving high (325 kg N/ha) or low (100 kg N/ha) rates of N fertiliser annually.**

| | | Dec-14 | Jan-15 | Apr-15 |
|---|---|---|---|---|
| Perennial ryegrass presence | Mixture | 96.0 | 88.2 | 99.1 |
| | White clover monoculture | 98.5 | 94.1 | 98.6 |
| SED | | 0.41 | 1.03 | 0.13 |
| N x Perennial ryegrass presence | High N mixture | 97.2 | 89.3 | 99.2 |
| | High N white clover monoculture | 98.3 | 95.3 | 98.9 |
| | Low N mixture | 94.8 | 87.2 | 99.0 |
| | Low N white clover monoculture | 98.7 | 92.9 | 98.3 |
| SED (within N treatment) | | 0.58 | 1.46 | 0.18 |
| *P* value | Perennial ryegrass presence | < 0.001 | < 0.001 | < 0.001 |
| | N x perennial ryegrass presence interaction | < 0.001 | 0.485 | 0.170 |

In this table the PAR interceptance (%) and the SED are from the analysis without angular transformation, but *P* values are from the analysis with angular transformation.

To examine if the inclusion of different perennial ryegrass cultivars in mixtures with different white clover cultivars affected the light intercepted by the canopy, ANOVA was conducted on the PAR interceptance of the mixtures and the white clover monocultures, considering the latter to represent a zero perennial ryegrass (No perennial ryegrass) treatment. In this way, the analyses were conducted as a factorial of 5 perennial ryegrass “cultivars” × 4 white clover cultivars. Based on the power of the trial, no interaction was detected between perennial ryegrass and white clover cultivar or between N, perennial ryegrass and white clover cultivars on PAR interceptance at any of the samplings.

#### *4.4.9..5 Light interception in mixtures*

During December 2014, mixtures sown with Abermagic AR1 and Bealey NEA2 intercepted similar light under the High and Low N treatment, while mixtures sown with Arrow AR1 and Prospect AR37 intercepted less light under the Low N treatment. As a result, when more N was available all the mixtures intercepted similar PAR, but when less Low N was available Abermagic AR1 and Bealey NEA2 intercepted more light than mixtures including Prospect AR37, while mixtures including Arrow AR1 were intermediate (interaction N x perennial ryegrass, *P* = 0.008; Table 4.23).

**Table 4.23 Percentage of PAR intercepted by mixtures receiving high (325 kg N/ha) or low (100 kg N/ha) rates of N fertiliser annually.**

| | | Dec-14 | Jan-15 | Apr-15 |
|---|---|---|---|---|
| N treatment | High N | 97.2 | 89.3 | 99.2 |
| | Low N | 94.8 | 87.2 | 99.0 |
| SED | | 0.43 | 0.88 | 0.06 |
| Perennial ryegrass cultivar | Abermagic AR1 | 96.1 | 88.1 | 99.0 |
| | Arrow AR1 | 96.3 | 88.7 | 99.1 |
| | Bealey NEA2 | 96.2 | 89.3 | 99.1 |
| | Prospect AR37 | 95.6 | 86.8 | 99.2 |
| White clover cultivar | Bounty | 96.3 | 89.6 | 99.2 |
| | Kopu II | 96.6 | 89.0 | 99.1 |
| | Nomad | 95.8 | 88.0 | 99.1 |
| | Tribute | 95.5 | 86.3 | 99.0 |
| SED | Perennial ryegrass (White clover) | 0.56 | 1.43 | 0.13 |
| *P* value | N | < 0.05 | 0.188 | < 0.05 |
| | Perennial ryegrass | 0.664 | 0.433 | 0.238 |
| | White clover | 0.158 | 0.125 | 0.858 |
| | N x Perennial ryegrass interaction | < 0.01 | 0.079 | 0.075 |
| | N x White clover interaction | 0.742 | 0.891 | 0.843 |
| | Perennial ryegrass x White clover interaction | 0.448 | 0.936 | 0.831 |
| | N x Perennial ryegrass x White clover interaction | 0.315 | 0.261 | 0.303 |

In this table the PAR interceptance (%) and the SED are from the analysis without angular transformation, but *P* values and letters are from the analysis with angular transformation. In the April sampling, because the light intercepted by the mixtures was close to 100 % with very little variation, the full analysis is not appropriate.

During January the light intercepted by mixtures sown under High or Low N treatment or with different grass cultivars was similar; meanwhile, during April 2015, although the effect of N treatment was significant, the light intercepted was close to 100 % with very little variation, and for this reason the full analysis is not appropriate. The only effect of the grass cultivar was through the interaction with N in December as described above. There was no white clover cultivar effect on the PAR intercepted by mixtures at any sampling. Similarly, the inclusion of different clover cultivars in pastures sown with different grass cultivars did not affect the amount of light intercepted by the canopy.

## Canopy height

### *4.4.9..1 Perennial ryegrass height in monocultures*

Perennial ryegrass plants grown in monoculture under High N treatment formed taller canopy than the canopy formed under the Low N treatment during December and January, but not in April when canopy height did not differ between N treatments (Table 4.24).

**Table 4.24 Undisturbed perennial ryegrass height (cm) in grass monocultures receiving high (325 kg N/ha) or low (100 kg N/ha) rates of N fertiliser annually.**

| | | Dec-14 | Jan-15 | | Apr-15 | |
|---|---|---|---|---|---|---|
| N treatment | High N | 33.3 | 19.9 | | 25.2 | |
| | Low N | 26.9 | 17.6 | | 23.3 | |
| SED | | 0.68 | 0.35 | | 0.83 | |
| Perennial ryegrass | Abermagic AR1 | 29.5 | 19.0 | b | 23.3 | b |
| | Arrow AR1 | 29.5 | 17.7 | b | 24.0 | b |
| | Bealey NEA2 | 31.1 | 21.0 | a | 25.9 | a |
| | Prospect AR37 | 30.3 | 17.5 | b | 23.8 | b |
| SED | | 0.97 | 0.79 | | 0.67 | |
| *P* value | N | < 0.01 | < 0.01 | | 0.111 | |
| | Perennial ryegrass | 0.301 | < 0.01 | | < 0.01 | |
| | N x Perennial ryegrass | 0.057 | 0.490 | | 0.625 | |

In January and April, Bealey NEA2 plants formed a taller canopy than plants from the other cultivars. When the N effect was present, this effect was similar across grass cultivars.

#### *4.4.9..2 Perennial ryegrass height (cm) in pastures sown with or without the presence of white clover and receiving high (325 kg N/ha) or low (100 kg N/ha) rates of N fertiliser annually.*

During December and April, perennial ryegrass plants in mixed pastures were taller than plants in monoculture when grown under the Low N treatment, but under the High N treatment, the inclusion of clover did not increase the grass height. In January, however, ryegrass plants in mixed pastures were taller than in monocultures under both N treatments (Table 4.25).

**Table 4.25 Undisturbed perennial ryegrass height (cm) in pastures sown with (mixtures) or without the presence of white clover (perennial ryegrass monocultures) and receiving high (325 kg N/ha) or low (100 kg N/ha) rates of N fertiliser annually.**

| | | Dec-14 | Jan-15 | Apr-15 |
|---|---|---|---|---|
| White clover presence | Mixture | 30.9 | 19.7 | 25.8 |
| | Perennial ryegrass monoculture | 30.1 | 18.8 | 24.2 |
| SED | | 0.41 | 0.29 | 0.27 |
| N x White clover presence | High N mixture | 32.8 | 20.9 | 25.9 |
| | High N perennial ryegrass monoculture | 33.3 | 19.9 | 25.2 |
| | Low N mixture | 29.1 | 18.6 | 25.7 |
| | Low N perennial ryegrass monoculture | 26.9 | 17.6 | 23.3 |
| SED (within N treatment) | | 0.57 | 0.41 | 0.38 |
| *P* value | White clover presence | 0.050 | < 0.01 | < 0.001 |
| | N x White clover presence interaction | < 0.01 | 0.995 | < 0.01 |

To examine if the inclusion of different white clover cultivars in mixtures with different perennial ryegrass cultivars affected the undisturbed grass height, ANOVA was conducted on the grass height in the mixtures and in the perennial ryegrass monocultures, considering the latter to represent a zero white clover (No white clover) treatment. In this way, the analyses were conducted as a factorial of 4 perennial ryegrass cultivars × 5 white clover “cultivars”. Based on the power of the trial, no interaction was detected between perennial ryegrass and white clover cultivar or between N, perennial ryegrass and white clover cultivars on the grass height at any of the samplings.

#### *4.4.9..3 Perennial ryegrass height (cm) in mixtures receiving high (325 kg N/ha) or low (100 kg N/ha) rates of N fertiliser annually.*

Perennial ryegrass plants grown in mixture under the High N treatment were taller than plants grown under the Low N treatment during December and January but they had similar height during April 2015 (Table 4.26).

**Table 4.26 Undisturbed perennial ryegrass height (cm) in mixed pastures receiving high (325 kg N/ha) or low (100 kg N/ha) rates of N fertiliser annually**

| | | Dec-14 | | Jan-15 | | Apr-15 | |
|---|---|---|---|---|---|---|---|
| N treatment | High N | 32.8 | | 20.9 | | 25.9 | |
| | Low N | 29.1 | | 18.6 | | 25.7 | |
| SED | | 0.28 | | 0.14 | | 0.32 | |
| Perennial ryegrass cultivar | Abermagic AR1 | 29.2 | b | 19.2 | b | 25.9 | a |
| | Arrow AR1 | 31.3 | a | 19.4 | b | 26.4 | a |
| | Bealey NEA2 | 31.2 | a | 21.4 | a | 26.2 | a |
| | Prospect AR37 | 31.9 | a | 19.0 | b | 24.7 | b |
| White clover cultivar | Bounty | 31.2 | | 20.0 | | 25.9 | |
| | Kopu II | 31.4 | | 20.0 | | 25.6 | |
| | Nomad | 30.7 | | 19.4 | | 26.2 | |
| | Tribute | 30.4 | | 19.6 | | 25.5 | |
| SED | Perennial ryegrass (White clover) | 0.52 | | 0.37 | | 0.34 | |
| *P* value | N | < 0.001 | | < 0.001 | | 0.563 | |
| | Perennial ryegrass | < 0.001 | | < 0.001 | | < 0.001 | |
| | White clover | 0.207 | | 0.289 | | 0.178 | |
| | N x Perennial ryegrass interaction | 0.184 | | 0.189 | | 0.420 | |
| | N x White clover interaction | 0.493 | | 0.159 | | < 0.01 | |
| | Perennial ryegrass x White clover interaction | 0.983 | | 0.653 | | 0.365 | |
| | N x Perennial ryegrass x White clover interaction | 0.911 | | 0.099 | | 0.459 | |

In canopies of mixed pastures, Abermagic AR1 plants did not reach the same height as plants of the other three cultivars during December, while during January plants from the cultivar Bealey NEA2 were higher in mixture canopies than plants from the other cultivars in their respective mixture canopies. In April 2015, plants from Abermagic AR1, Arrow AR1 and Bealey NEA2 reached similar heights in their mixture canopies, while plants form Prospect AR37 were shorter.

Undisturbed perennial ryegrass height (cm) was similar in mixtures grown with different white clover cultivars during December and January, and under High N treatment in April 2015, but in this last sampling and under Low N treatment, ryegrass plants grown in mixtures with Nomad were taller than plants grown with Tribute and Kopu II, while plants in mixtures including Bounty were intermediate.

The inclusion of different clover cultivars in mixtures of different perennial ryegrass under High or Low N treatment did not affect the undisturbed height of the grass plants.

#### *4.4.9..4 White clover height in monocultures*

White clover plants grown in monoculture under High or Low N treatment formed a canopy of similar undisturbed height (Table 4.27).

**Table 4.27 Undisturbed white clover height (cm) in clover monocultures receiving high (325 kg N/ha) or low (100 kg N/ha) rates of N fertiliser annually.**

| | | Dec-14 | | Jan-15 | | Apr-15 | |
|---|---|---|---|---|---|---|---|
| N treatment | High N | 18.8 | | 13.3 | | 18.2 | |
| | Low N | 18.7 | | 11.6 | | 16.7 | |
| SED | | 1.63 | | 0.89 | | 0.81 | |
| White clover | Bounty | 18.5 | b | 12.7 | b | 16.9 | b |
| | Kopu II | 20.4 | a | 14.0 | a | 19.0 | a |
| | Nomad | 17.6 | b | 10.8 | c | 15.1 | c |
| | Tribute | 18.3 | b | 12.3 | b | 18.7 | a |
| SED | | 0.81 | | 0.52 | | 0.80 | |
| *P* value | N | 0.955 | | 0.153 | | 0.167 | |
| | White clover | < 0.05 | | < 0.001 | | < 0.001 | |
| | N x White clover | 0.780 | | 0.220 | | 0.510 | |

On the other hand, white clover cultivar effect on canopy height was significant at every sampling. During December, Kopu II plants formed a taller canopy than plants from the other three cultivars. In January, Kopu II plants continued forming the taller canopy; however during this month mean height of canopies sown with Bounty and Tribute were also taller than canopies formed by Nomad plants. During the last sampling of the season, April 2015, canopies formed by Kopu II and Tribute plants were taller than canopies formed by Bounty plants, and these ones taller than canopies formed by Nomad plants.

#### *4.4.9..5 White clover height (cm) in pastures sown with or without the presence of perennial ryegrass and receiving high (325 kg N/ha) or low (100 kg N/ha) rates of N fertiliser annually.*

White clover plants grown in mixtures were taller than plants grown in monoculture at every sampling (Table 4.28).

**Table 4.28 Undisturbed white clover height (cm) in pastures sown with (mixture) or without the presence of perennial ryegrass (white clover monoculture) and receiving high (325 kg N/ha) or low (100 kg N/ha) rates of N fertiliser annually.**

| | | Dec-14 | Jan-15 | Apr-15 |
|---|---|---|---|---|
| Perennial ryegrass presence | Mixture | 19.9 | 14.0 | 21.2 |
| | White clover monoculture | 18.7 | 12.5 | 17.4 |
| SED | | 0.38 | 0.30 | 0.31 |
| N x Perennial ryegrass presence | High N mixture | 20.2 | 14.3 | 21.1 |
| | High N white clover monoculture | 18.8 | 13.3 | 18.2 |
| | Low N mixture | 19.5 | 13.7 | 21.2 |
| | Low N white clover monoculture | 18.7 | 11.6 | 16.7 |
| SED (within N treatment) | | 0.53 | 0.43 | 0.43 |
| *P* value | Perennial ryegrass presence | < 0.01 | < 0.001 | < 0.001 |
| | N x perennial ryegrass presence interaction | 0.409 | 0.056 | < 0.05 |

During April 2015, although the inclusion of perennial ryegrass increased the clover height at both N levels, the increment was greater under the Low N treatment than under the High N treatment.

To examine if the inclusion of different perennial ryegrass cultivars in mixtures with different white clover cultivars affected the undisturbed clover height, ANOVA was conducted on the white clover plants height in the mixtures and in the white clover monocultures, considering the latter to represent a zero perennial ryegrass (No perennial ryegrass) treatment. In this way, the analyses were conducted as a factorial of 5 perennial ryegrass "cultivars" × 4 white clover cultivars. An interaction between perennial ryegrass cultivar and white clover cultivar was only detected in April 2015 ($P$ = 0.019); when adding different perennial ryegrass cultivars to pastures sown with Bounty, Kopu II and Tribute, the increment in the height of clover plants in the mixture with ryegrass was similar for all the grass cultivars (Figure 4.12). However, when Nomad was the cultivar, clover plants grew taller with respect to the monoculture when Abermagic AR1 was the grass cultivar included in the mixtures than when Bealey NEA2 was included, while mixtures including Arrow AR1 and Prospect AR37 were intermediate.

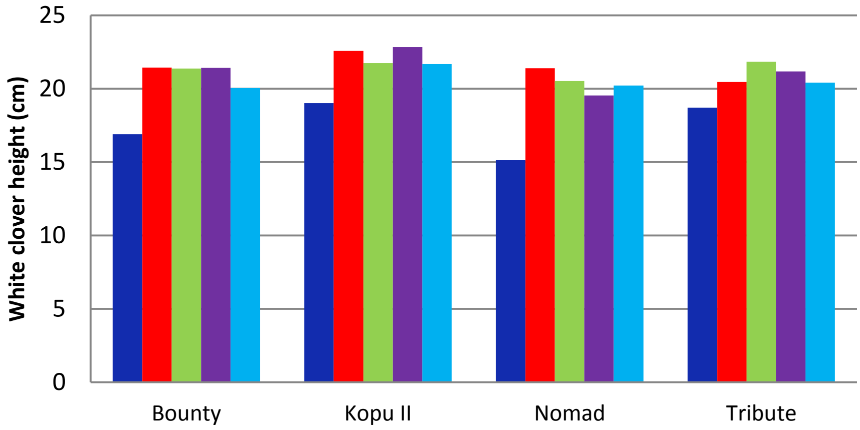


**Figure 4.12 Undisturbed white clover height (cm) in pastures sown with (mixture) or without perennial ryegrass (white clover monoculture) and receiving high (325 kg N/ha) or low (100 kg N/ha) rates of N fertiliser annually – April 2015. No perennial ryegrass (dark blue bar), Abermagic AR1 (red bar), Arrow AR1 (green bar), Bealey NEA2 (purple bar), Prospect AR37 (light blue bar). SED – 0.78 cm.**

#### *4.4.9..6 White clover height (cm) in mixtures receiving high (325 kg N/ha) or low (100 kg N/ha) rates of N fertiliser annually.*

In mixed pastures, the white clover height was similar under the High and under the Low N treatment and when grown with different perennial ryegrass cultivars (Table 4.29).

**Table 4.29 Undisturbed white clover height (cm) in mixed pastures receiving high (325 kg N/ha) or low (100 kg N/ha) rates of N fertiliser annually**

| | | Dec-14 | | Jan-15 | | Apr-15 | |
|---|---|---|---|---|---|---|---|
| N treatment | High N | 20.2 | | 14.3 | | 21.1 | |
| | Low N | 19.5 | | 13.7 | | 21.2 | |
| SED | | 0.46 | | 0.24 | | 0.30 | |
| Perennial ryegrass cultivar | Abermagic AR1 | 19.7 | | 14.2 | | 21.5 | |
| | Arrow AR1 | 20.2 | | 13.9 | | 21.4 | |
| | Bealey NEA2 | 19.7 | | 14.4 | | 21.3 | |
| | Prospect AR37 | 19.8 | | 13.4 | | 20.6 | |
| White clover cultivar | Bounty | 20.1 | b | 14.4 | b | 21.1 | b |
| | Kopu II | 22.0 | a | 15.5 | a | 22.2 | a |
| | Nomad | 18.2 | d | 12.9 | c | 20.4 | b |
| | Tribute | 19.2 | c | 13.2 | c | 21.0 | b |
| SED | Perennial ryegrass (White clover) | 0.46 | | 0.39 | | 0.36 | |
| *P* value | N | 0.216 | | 0.106 | | 0.762 | |
| | Perennial ryegrass | 0.615 | | 0.054 | | 0.066 | |
| | White clover | < 0.001 | | < 0.001 | | < 0.001 | |
| | N x Perennial ryegrass interaction | 0.308 | | 0.190 | | 0.458 | |
| | N x White clover interaction | 0.851 | | 0.526 | | 0.583 | |
| | Perennial ryegrass x White clover interaction | 0.907 | | 0.913 | | 0.118 | |
| | N x Perennial ryegrass x White clover interaction | 0.099 | | 0.057 | | 0.515 | |

However, different white clover cultivars had different undisturbed clover height at every sampling. Kopu II plants were the tallest, followed by Bounty, then Tribute and the shortest plants in the mixtures were from Nomad white clover; nevertheless, these differences were not always significant.

No interactions between perennial ryegrass and white clover cultivars on clover height were detected at any season.

### 4.4.10 White clover leaf size

Analysis of variance was conducted on the calculated centre leaflet area of leaves collected in November 2015 on the white clover monocultures grown under the Low N treatment. Although the cultivar effect was not significant ($P$ = 0.227), the leaflet area followed the expected trend: Kopu II leaflets (5.81 $cm^2$) were bigger than Tribute (5.52 $cm^2$), which were bigger than Bounty (4.93 $cm^2$), while Nomad leaflets were the smallest (4.64 $cm^2$).

## 4.5 Discussion

### 4.5.1 Do combinations of different perennial ryegrass and white clover phenotypes result in different total annual yield of a mixed pasture?

Many factors affect the DM yield and botanical composition of mixed pastures on New Zealand dairy farms. These include soil type, availability of nutrients and water in the soil, grazing management, N fertilizer application rates, along with the perennial ryegrass and white clover cultivars included in the mixture (Camlin, 1981; Collins & Rhodes, 1989; Frame & Boyd, 1986a, 1986b; Gilliland, 1996; S. L. Harris & Clark, 1996; S. L. Harris, Clark, et al., 1996; S. L. Harris, Thom, et al., 1996; W. Harris & Hoglund, 1977; Whitehead, 1970). In general, mixtures based on different perennial ryegrass and white clover cultivars tend to yield similarly despite differences in botanical composition, due to substitution or compensatory effects between the two main components of the sward (Camlin, 1981; Connolly, 1968; Elgersma et al., 1998; Hoen, 1970; Widdup & Turner, 1983; Williams et al., 2000). Therefore the working hypothesis was that there would be no difference in the DM production of the sward when modern perennial ryegrass and white clover cultivars with different phenotypes are grown in mixture. With the exception of one occasion (harvest conducted in November 2014), no interactions between perennial ryegrass and white clover cultivars on DM yield (kg DM/ha) of the mixtures were observed, meaning that the hypothesis was supported by the results of the trial.

The white clover content (% DM) of pastures was affected by the perennial ryegrass cultivar included in the mixture in only three of the nine harvests, and by the white clover cultivar in seven of the nine harvests. However, with the exception of one occasion (harvest conducted in May 2015), no interaction between perennial ryegrass and white clover cultivars in white clover content (% DM) was detected, meaning that in general, it was the effect of the ryegrass or the clover cultivar alone that determined the proportion of the legume in the sward, and not the combination of different phenotypes.

### 4.5.2 Ryegrass and white clover phenotype variation

The N treatment and pasture types selected to test the hypothesis created different environments for plant growth, as evidenced by the range in yield between treatments and the sward characteristics. Total annual DM production varied from 12.7 t DM/ha in the perennial ryegrass monocultures under Low N treatment to 19.9 t DM/ha in the mixtures grown under High N treatment (Table 4.11). When considering the mixtures only, the yields varied from 15.3 t DM/ha for the pastures sown with Bealey NEA2 and Bounty and receiving Low N treatment, to 20.9 t DM/ha for the pastures sown with Prospect AR37 and Tribute under High N treatment. When averaged over N treatments, mean annual yield of mixtures sown with Bealey NEA2 (18.7 t DM/ha) was 5.2 % greater than mean yield of pastures sown with Prospect AR37 (17.8 t DM/ha). The differences between highest and lowest yielding cultivars (that were not always the same) over the different harvests, were on average of 8.3 % for the summer months, 12.9 % for the autumn months, 33.4 % for the spring months, and 93.2 % for the only harvest conducted in Winter (August 2014), when Bealey NEA2 yield almost doubled the yield of Abermagic AR1.

The perennial ryegrass and white clover cultivars included in the experiment were selected because of their differences in a suite of characteristics (Tables 4.1 and 4.2): perennial ryegrass leaf width (from narrow to medium – broad/wide) and length (from short to medium – long), and tendency toward higher or lower tiller density when managed under similar conditions. The white clovers were selected because of their differences in leaf size, denoted as difference in the length (from short to long) and width (from narrow to broad) of the central leaflet, differences in the length (from short to long) and thickness (from thin to thick) of the petiole, as well as differences in the thickness of the stolon (from thin/medium to thick). To measure all these characteristics was not a purpose of this experiment, because most of them are part of the Objective Description of Variety (Intellectual Property Office of New Zealand). However, it was important to measure the expression in the field of some of these characteristics which play an important role in the competition (or combining ability) between the perennial ryegrass and white clover, and as a result the DM yield and quality of the sward. In this experiment, perennial ryegrass and white clover cultivars showed differences in their population density as well as in their canopy height when grown in monocultures, according to their expected characteristics.

### 4.5.3 N and clover effects on DM yield

**N effect**

N had a significant effect on the total annual DM yield of the mixture which, across the whole year, was 20.5 % greater in the High than in the Low N treatment (Tables 4.6 and 4.30). This effect was mainly due to differences in yield in the harvests conducted during spring, early summer and late autumn (August, October, December and May), but not during the rest of the summer or in early autumn. Variation in response to N is common and has been reported in previous research (Ball & Field, 1982; Ball et al., 1978; Clark & Harris, 1996; Feyter et al., 1985; S. L. Harris & Clark, 1996; S. L. Harris, Clark, et al., 1996; Hennessy et al., 2012; C. W. Holmes, 1982; Laidlaw, 1980; Moir et al., 2003; Shepherd & Lucci, 2011; L. C. Smith, Morton, Catto, & Trainor, 2000; Sun, Luo, Longhurst, & Luo, 2008; Whitehead, 1995); soil temperature, N supply by the soil and pasture composition are amongst the factors that affect this response.

In this experiment, the lack of response to N during summer and early autumn, resulted from the increased clover component yield of the mixture when less N was applied, which compensated for the lesser perennial ryegrass component yield under these conditions of lower N availability (Figure 4.13, Tables 4.7 and 4.8).

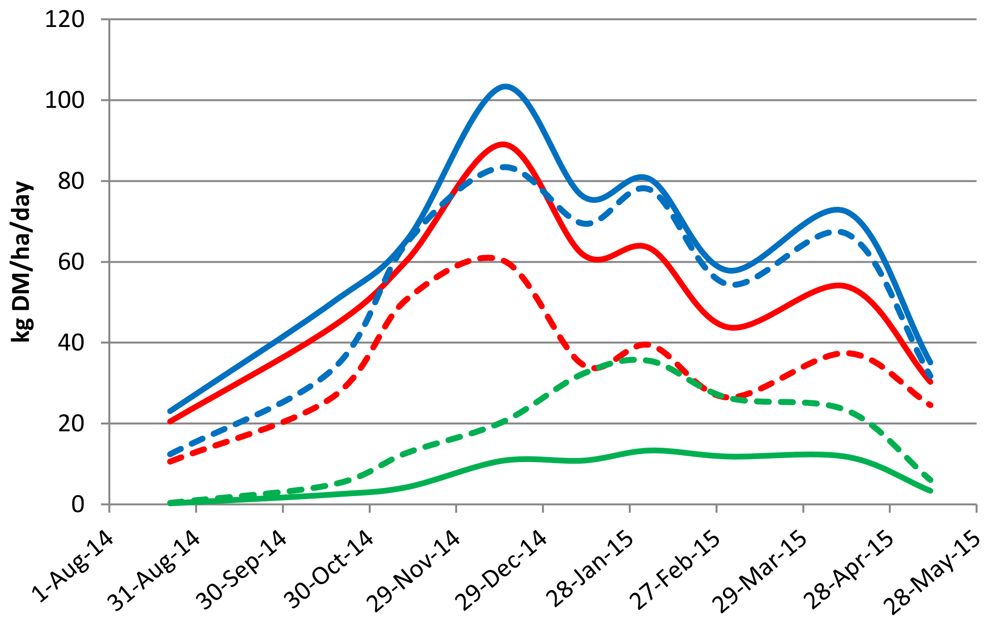


**Figure 4.13 Growth per day (kg DM/ha/day) of the mixture and the perennial ryegrass and white clover components of the mixture, under High or Low N treatments. High N mixture (solid blue line), High N perennial ryegrass component (solid red line), High N white clover component (solid green line); Low N mixture (dashed blue line), Low N perennial ryegrass component (dashed red line), Low N white clover component (dashed green line).**

In contrast to the temporal variability in the effect of N on the mixture yield, the higher N application rate significantly increased the perennial ryegrass component yield at every harvest (Tables 4.7 and 4.30; red lines in Figure 4.13), resulting in an annual grass yield 54 % greater in the High N treatment compared with the Low N treatment. Along with this increase in grass yield a decrease in white clover yield was observed from November to the end of the season when more N was applied (Tables 4.8 and 4.30; green lines in Figure 4.13). These results were not surprising since the effect of N in promoting perennial ryegrass growth (e.g. Ball & Field, 1982; Donald, 1963; Lemaire & Chapman, 1996; O'Connor, 1982; Robson & Deacon, 1978; Whitehead, 1995; Wilman & Asiegbu, 1982a; Wilman & Wright, 1983b), and the negative effect of this increased grass growth on the white clover component of the mixture (e.g. Caradus et al., 1993; Dennis & Woledge, 1987; S. L. Harris, Clark, et al., 1996; Hoglind & Frankow-Lindberg, 1998; Laidlaw & Withers, 1998; Pinxterhuis, 2000; Thompson, 1995; Wilman & Asiegbu, 1982b) has been well documented. So although the mixtures yielded similarly under both N treatments during five of the nine harvests, the composition of the sward was very different.

The effect of N on the perennial ryegrass monoculture yield was significant in seven of the nine harvests and in the total annual yield, which resulted 47 % higher under the High than under the Low N treatment (Tables 4.9 and 4.30). In contrast, the yield of the white clover monoculture was not affected by N treatment at any harvest or in the total annual of the season (Table 4.10 and 4.30), similar to previous findings (Cowling, 1961). White clover monoculture grew more than grass monoculture under the Low N treatment and vice-versa under the High N treatment, similar to findings of Reid (1983). In general white clover monoculture yielded almost as well as perennial ryegrass monoculture (14.5 versus 15.7 t DM/ha respectively, average of both N levels), a result that was not expected based on previous research (8 t/ha, Cowling, 1961; 58% of perennial ryegrass monoculture, Davidson & Robson, 1986; 6.8 t/ha total yield, Frame & Harkess, 1987; 10.5 t/ha, Suckling, 1960).

**Table 4.30 Annual DM yield (kg DM/ha) of monocultures and mixture grown under High or Low N treatment, and apparent response to N (kg DM/kg N).**

| | Low N | High N | Apparent response to N (kg DM/kg N)[1] |
|---|---|---|---|
| Perennial ryegrass monoculture | 12665 | 18650 | 21.0 |
| White clover monoculture | 14420 | 14565 | 0.5 |
| Mixture - total[2] | 16540 | 19920 | 11.9 |
| Mixture – perennial ryegrass component | 10795 | 16635 | 20.5 |
| Mixture – white clover component | 4880 | 2135 | -9.6 |

[1] Calculated based on a difference of 285 kg N/ha/year (instead of 225 kg N/ha/year) between the High and the Low N treatment due to the extra fertiliser applied to both treatments to replace the N removed.
[2] The difference between the total mixture yield and the sum of perennial ryegrass and white clover components is explained by the presence of other species and dead matter.

The apparent response to N of the perennial ryegrass monoculture was very similar to the response of the ryegrass component in the mixture, suggesting that grass and clover were not competing for this nutrient in the mixed sward. Meanwhile, there was almost no response to N in the white clover monoculture (0.5 kg DM/kg N), but there was a negative response to N on the white clover component of the mixture (-9.6 kg DM/kg N). As a result, the apparent response to N in the mixture (total) was 11.9 kg DM/kg N, close to the average response of 10 kg DM/kg N found by Ledgard et al. (2001) and lower than the response found by Glassey et al. (2013) in mixed pastures (16 kg DM/kg N). In this experiment, for every 10% of increase in the white clover content of the sward, the response to N decreased 5.3 kg DM/kg N.

## Clover effect

Increments in pasture yield due to clover inclusion have been reported previously. Amongst the factors contributing to this increase are: different seasonal growth patterns of ryegrass and white clover resulting in a more efficient use of the 'resource space' and a temporal separation of competition. Also the ability of clover to fix $N_2$ increasing the pool of this nutrient in the soil and the difference in their leaf types (plagiophile the grass and planophhile the clover) which could be considered to separate the two species into different 'light spaces' thus improving the efficiency of the use of this resource (Brougham, 1958; Clark & Harris, 1996; W. Harris, 2001; W. Harris & Thomas, 1973; Ledgard et al., 1990; Mitchell, 1956a; Reid, 1983; Turkington & Harper, 1979a).

The growth patterns of perennial ryegrass and white clover observed in the monoculture and mixture treatments agreed with the seasonal growths described in the literature (Brougham, 1959; W. Harris & Hoglund, 1977; W. Harris & Thomas, 1973; Mitchell, 1956b). White clover growth rate was greater in the warmer months of the year, regardless of the rate of N application used (Figure 4.13). The different patterns of growth contributed to the increased yield due to clover inclusion in the sward. In this sense in this experiment, the white clover played a similar role to the red clover (*Trifolium pratense* L.) in the experiment described by W. Harris and Hoglund (1977, p. 242):

> *Trifolium pratense L. filled the niche provided by the decline of L. perenne growth in summer whereas T. repens did not.*

The interception of photosynthetically active radiation was greater in mixtures than in perennial ryegrass monocultures (Table 4.20) under both N treatments, and this difference must have been reflected in increased total canopy photosynthesis (Parsons & Chapman, 2000), which would also contribute to the increased yield in mixed pastures. The increase in PAR intercepted due to the inclusion of clover in the sward was greater under the Low than under the High N treatment. There are two factors to consider here. First, the grass monoculture under the High N treatment was already intercepting a high percentage of light, 95.3 %, compared with 87.4 % in the grass monoculture under the Low N treatment, probably due to the increased leaf extension rate that is expected when more N is available (Parsons & Chapman, 2000). As a consequence, the inclusion of clover increased significantly but marginally the PAR intercepted at the high N application rate. Second, the proportion of white clover in the sward was greater under the Low N than under the High N treatment (Figure 4.6). Clover laminae are oriented horizontally which favours capture of light, allowing white clover to reach a similar percentage of light interception at a lower leaf area index (LAI) compared with perennial ryegrass (Brougham, 1958). The increased light intercepted in the mixture compared with the grass monoculture agrees with the description of different 'light spaces' by W. Harris (2001) as a mechanism of optimizing the use of pools of growth resources.

As mentioned, the contribution of clover to pasture yield was significant (Table 4.11). To explain these results as well as to detect evidence of competition between grass and clover, the yield of these components in mixture and monoculture was analysed. The perennial ryegrass component yield of the mixture was lower than the yield of perennial ryegrass in monoculture at five of the nine harvests (November, $P = 0.005$; February, $P = 0.007$; March, $P < 0.001$; April, $P < 0.001$; and May, $P = 0.001$), confirming interference or competition for resources among the two main components of the mixed sward. Nevertheless, the contribution of clover to the mixture (kg DM/ha) was similar to, or greater than, the decrease in grass yield, resulting in similar (November and May) or greater (February, March, April) yield of the mixture compared with the grass monoculture (Table 4.11). Meanwhile at three harvests (August, $P = 0.867$; October, $P = 0.321$; and January, $P = 0.120$) the perennial ryegrass component yield of the mixture did not differ from the yield of perennial ryegrass grown in monoculture. As a result, in August the mixture yield was similar to the monoculture yield due to the small contribution of the clover during this time of the year (Table 4.8) while in October and January the yield of the mixture was greater than the yield of the ryegrass monoculture due to a larger yield of clover during these months (Table 4.11). The similarity in grass yield under mixture and monoculture suggests that during these months, the clover was not exerting strong competitive pressure over the grass (August and October) or that factors other than the presence of clover were limiting the growth of grass in monoculture (January). In December, however, the yield of the grass component was similar in mixture and monoculture under the High N treatment, and the inclusion of clover did not increase total pasture yield, but was lower in monoculture than in mixture under the Low N treatment, indicating a possible N transfer from clover to grass via $N_2$ fixation and cycling. In this harvest, the contribution of clover increased the DM yield of the mixture compared with the grass monoculture (Table 4.11).

Total annual yield of mixed pasture was greater than yield of perennial ryegrass monoculture evidencing the contribution of clover to DM yield (Table 4.11). There was a small but significant facilitation effect of white clover under the High N treatment (+ 1.3 t DM/ha), and a strong facilitation effect under the Low N treatment (+ 3.9 t DM/ha) (Table 4.30).

The finding that under the Low N treatment the white clover component of the mixture was able to grow as much (or more) DM as the grass component (Figure 4.15, b) and d), solid red and green lines) in summer and early autumn agrees with Brougham (1959) working with a mixed pasture (short rotation ryegrass and white clover) under irrigation and no N application. However, it differs from W. Harris and Hoglund (1977); these authors, working with a perennial ryegrass/white clover pasture with no N application, found that the grass dominated the clover throughout the year, and attributed the variance from Brougham (1959) results to the use of irrigation in Brougham's experiment. In the present experiment, irrigation was used and this could explain the similarity to Brougham's findings.  Under the High N treatment however, perennial ryegrass was always the dominant component of the pasture; nevertheless, white clover was able to contribute to the total annual yield of the mixture due to increase growth in summer and early autumn (Figure 4.14, a) and c)).

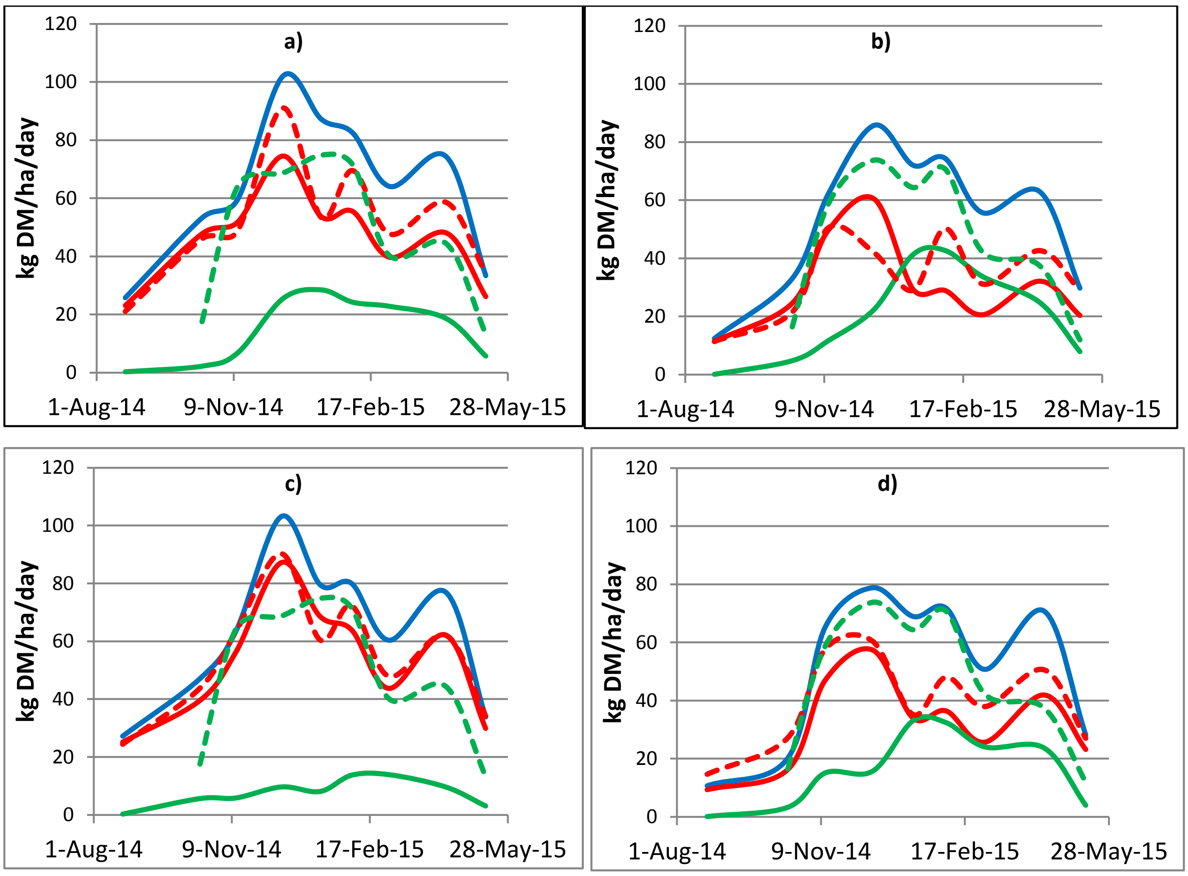


**Figure 4.14** **Growth per day (kg DM/ha/day) – a) Arrow AR1 + Bounty High N, b) Arrow AR1 + Bounty Low N, c) Bealey NEA2 + Bounty High N, d) Bealey NEA2 + Bounty Low N. Total mixture (solid blue line), perennial ryegrass component in mixture (solid red line), white clover component in mixture (solid green line), perennial ryegrass component in monoculture (dashed red line), white clover component in monoculture (dashed green line).**

### 4.5.4 Perennial ryegrass cultivar effect on DM yield and interaction with N treatment

In six of the nine harvests, the mean yield of mixed pastures sown with different perennial ryegrass cultivars differed significantly due to the grass cultivar effect. At the first harvest (August 2014), the yield of pastures sown with Abermagic AR1 was almost half of the yield of pastures sown with the other three cultivars. This is consistent with the relatively low performance value for Abermagic AR1 in winter and early spring in the Forage Value Index (Chapman et al., 2016; DairyNZ, evaluation date December 2015), based on data from outside Canterbury. Mixtures with Prospect AR37 and Bealey NEA2 were the highest yielding pastures, while Arrow AR1 was intermediate. In spring the cultivars began to reflect their expected differences in the timing of reproductive development; the first to show this effect was Arrow AR1 (heading date +7) which was the highest yielding cultivar in October, followed by Abermagic AR1 (+19) in November, then Prospect AR37 and Bealey NEA2 (+12 and +25 respectively) in December (although the cultivar effect was not significant at this time). During the only harvest in summer, when there was a significant cultivar effect on DM yield of the mixture (January 2015), as well as in the two harvests in which there was difference in autumn (April and May 2015), Prospect AR37 was the lowest yielding cultivar. This was not expected, considering that this is one of the highest ranked cultivars in the Forage Value Index (Chapman et al., 2016; DairyNZ, evaluation date December 2015) for summer and autumn yields. The management of this experiment with cutting only, may not be the best alternative for this cultivar. At the end of the season, the annual total DM yield of the mixtures sown with different grass cultivars was not statistically different, and the highest yielding mixtures, which included Bealey NEA2, produced only 5.2 % more DM than the lowest yielding mixtures.

The dominance of the grass yield in determining the total yield of the mixture is a consequence of the grass being the major component of the sward (Figure 4.15). With the exception of mixtures based on Bounty and Kopu II under the Low N treatment during January, February and March, the average white clover content was below 50 % of total DM. These findings agree with Camlin (1981) who compared perennial ryegrass/white clover mixtures fertilized with 200 – 240 kg N/ha/year and observed that mixture yield tended to reflect the yield of the grass component. Additionally, when there were significant differences in the white clover yield due to perennial ryegrass cultivars (August, October, November and May, Table 4.8), these yields were not large enough to change the trend in ranking for yield of the perennial ryegrass treatments.

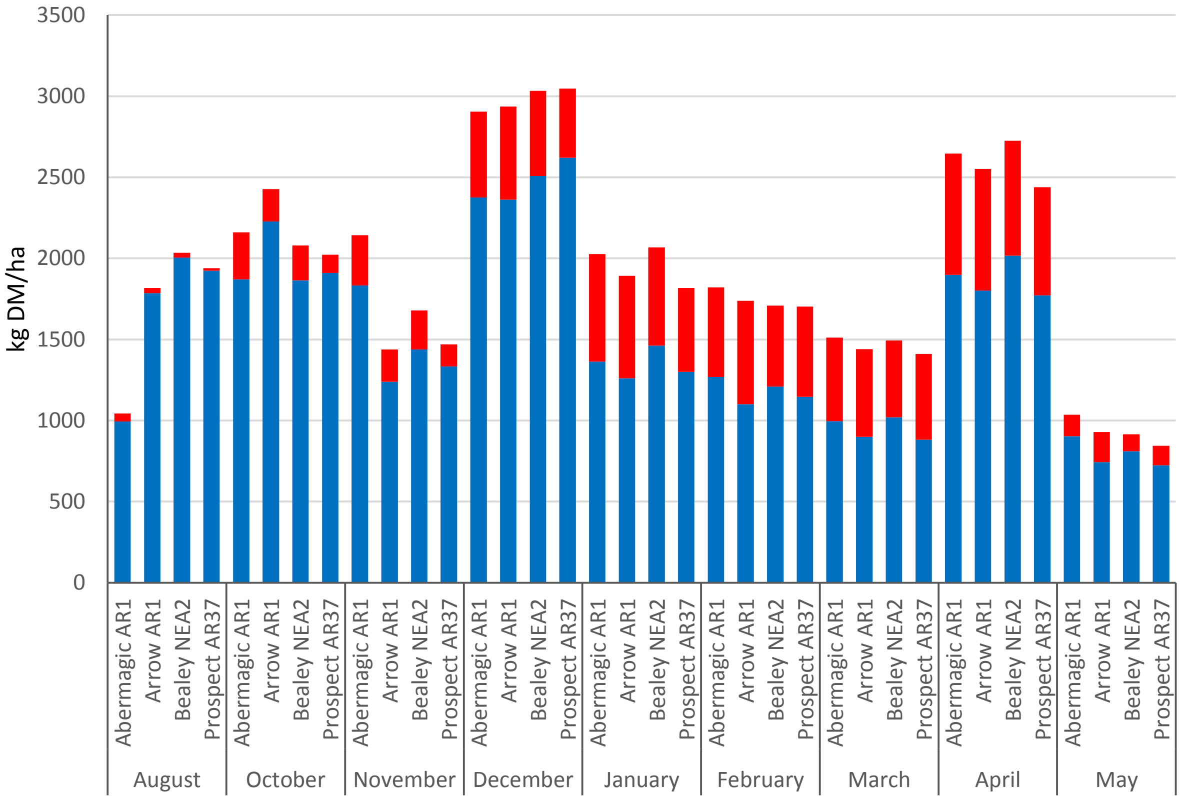


**Figure 4.15 Perennial ryegrass (blue bar) and white clover (red bar) yields (kg DM/ha) in mixed pastures based on different perennial ryegrass cultivars (average of both N treatments).**

Meanwhile, when comparing the yield of the grass monocultures, the perennial ryegrass effect was present only in two of the nine harvests (August and November), and showed a similar trend to that observed from the analysis of mixture yields. Regression analyses confirmed that in these two months, there was a significant positive association between the mean perennial ryegrass cultivar yield in monoculture and in mixture (August: $P$ = 0.006; $R^2$ = 0.989; November: $P$ = 0.002; $R^2$ = 0.997). The generally similar yield of the cultivars when grown in monoculture was an advantage from a methodological point of view, because the differences that subsequently appeared in component yields and sward composition were probably more due to the effect of the plant phenotype and seasonality of growth, which was the objective of this experiment, than to relative differences in yield potential of the grass component.

To examine if tiller density was a factor determining ryegrass yield, regression analyses were conducted with the data from November measurements, when significant effects of grass cultivar on the yield of grass monoculture, the grass component in mixture, and the total mixed sward were detected. There were not significant associations between tiller density in monoculture and ryegrass yield in monoculture ($P$ = 0.362, $R^2$ = 0.407), or between tiller density in mixture and ryegrass component yield in mixture ($P$ = 0.309, $R^2$ = 0.478), or between tiller density in monoculture and the grass yield in mixture ($P$ = 0.344, $R^2$ = 0.430). These results illustrate the limitations of the use of tiller density data under cutting management as an indicator of productivity, due to the size-density compensation response of grass to environmental and management factors (Matthew et al., 2000; Yoda, 1963).

### 4.5.5 White clover cultivar effect on DM yield and interaction with N treatment

The mean yield of mixed swards sown with different white clover cultivars differed significantly due to the clover cultivar effect on only three occasions (October, February and May, Table 4.6). While there were fewer effects of clover cultivar on total mixture yield compared with perennial ryegrass cultivar, clover cultivar significantly affected grass and clover component yields within the mixture at six of the nine harvests.

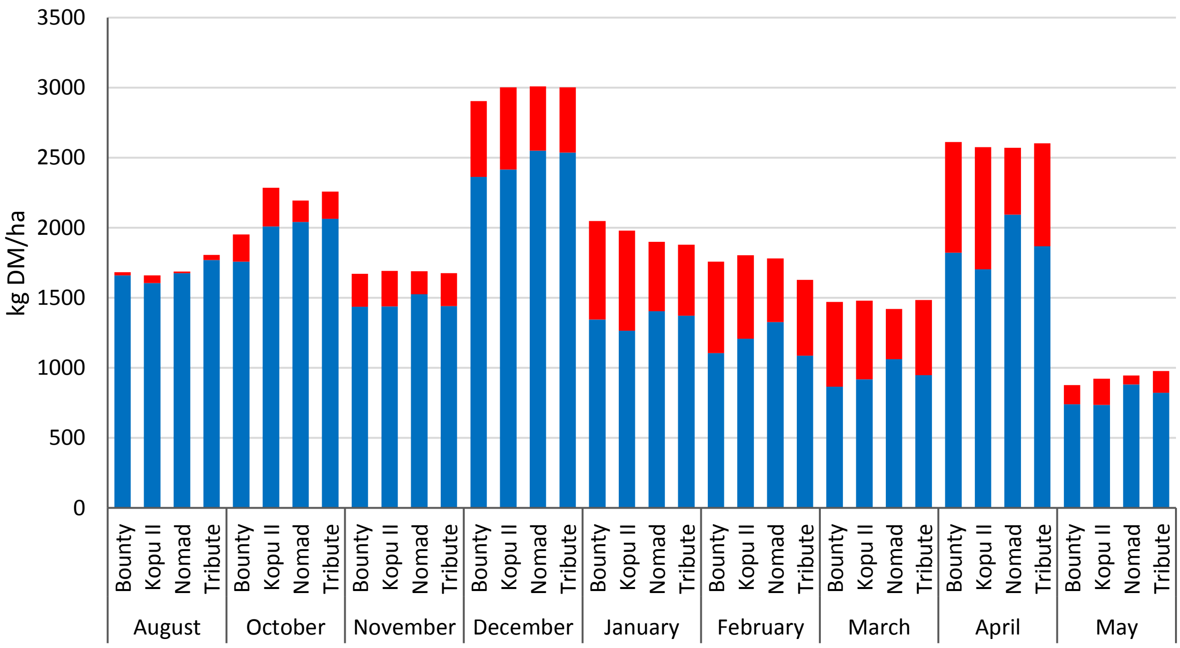


**Figure 4.16 Perennial ryegrass (blue bar) and white clover (red bar) yields (kg DM/ha) in mixed pastures based on different white clover cultivars (average of both N treatments).**

On the three occasions when there was effect of clover cultivar on total mixture yield, the clover yield was similar between cultivars (October) or not large enough to compensate for the lower grass yield (February and May), and therefore the yield of the mixture reflected the yield of the grass component. At the remaining harvests there was a compensatory effect between white clover and ryegrass and the total yield of the mixture was similar.

From February onwards, a consistent trend was observed: pastures based on Nomad white clover tended to yield more grass and less clover than pastures based on the rest of the cultivars (Table 4.7), resulting in a mean grass yield for the season approximately 10 % greater (+ 1363 kg DM/ha) in mixtures based on this cultivar than in mixtures including Kopu II and Bounty. This indicates that the smallest-leaved cultivar, Nomad, was less able to compete with the grass than the other clover cultivars. The annual clover yield of mixtures based on Nomad was 1352 kg DM/ha less than in swards based on Kopu II and Bounty, illustrating evidencing the compensatory effect mentioned above and resulting in a similar total annual yield of mixtures based on different clover cultivars.

The lower clover annual yield of Nomad is consistent with the breeding objective for this cultivar which was selected for a higher root-to-shoot dry matter ratio than other small leaved cultivars to improve its adaptation to dry environments (Widdup & Barrett, 2011). This difference in the priority for allocation of resources likely contributed to the lower clover plant height observed in mixtures with this cultivar compared with the larger leaved cultivars (Table 4.29), a trend that was also present in the white clover monoculture (Table 4.27).

When grown in monoculture, the white clover cultivars only differed in their DM production in the three harvests conducted in autumn, when Tribute was the cultivar with the highest yield, along with Kopu II in two of these harvests, while Nomad and Bounty were the lowest yielding cultivars. Although their annual yield was not significantly different ($P$ = 0.054) a similar trend to that noted in mixtures was observed: monocultures based on Nomad yielded less than monocultures based on Tribute and Kopu II. However, in contrast to what was observed with ryegrass, when regression analyses were conducted to detect associations between mean yield of the white clover component when grown in monoculture, and mean yield of the clover component when grown in mixture, there was no significant association for those harvests when there was a clover cultivar effect on white clover monoculture yield (March: $P$ = 0.343; $R^2$ = 0.431; April: $P$ = 0.545; $R^2$ = 0.207; May: $P$ = 0.234 $R^2$ = 0.587). On average, for these three harvests, while the lowest yielding cultivar in monoculture yielded almost three quarters of the highest yielding cultivar, it yielded only half in the mixture, illustrating the effect of grass competition on clover yield during this time of the year.

### 4.5.6 White clover content (% DM) in mixed swards

#### Effect of N, perennial ryegrass cultivar, white clover cultivar and their interactions on clover content of the sward

##### *4.5.6..1 N effect*

Similarly to findings from previous research (Caradus et al., 1993; A. Davies & Evans, 1990; Egan et al., 2015; Enriquez-Hidalgo et al., 2016; Frame & Boyd, 1986a, 1987a; Hennessy et al., 2012; Ledgard, 2001; Ledgard et al., 1995; Nassiri & Elgersma, 2002), the white clover content (% DM) of mixed swards was lower under High than under Low N treatment.

There are several reasons for the decrease in clover content of mixed swards when more N is applied. They relate to the promotion of the competitive advantage of the grass component of the mixture when more N is available (Blackman, 1938; Laidlaw & Withers, 1998), stemming from an increased rate of tillering and increased leaf length contributing to a greater grass leaf area index (LAI) and greater light interception by the grass component of the pasture. In addition to competition effects, increased mineral N supply can inhibit the N fixation by the legume (Cowling, 1961; Crush et al., 1982; Whitehead, 1995).

In this experiment, when more N was applied to the clover monocultures, the DM yield did not change (Table 4.10), meaning that the plants were able to cope with the low N application rate through $N_2$ fixation. Moreover, if at high N fertiliser level the clover plants had substituted part of their N needs previously met from $N_2$ fixation with mineral N uptake from the soil, which has a lower metabolic cost for each unit of N assimilated for the plant compared with biologically-fixed N (Ryle et al., 1979), then, we should have seen an increase in clover yield. But, this was not the case, so it is possible to conclude that $N_2$ fixation was dominant and consistent across treatments (including clover cultivar, since there was no cultivar x N interaction in white clover monoculture yields). Therefore, there was no negative or positive effect of an increased N supply on the clover yield. In the same way, there was no N effect on the growing point density (growing points/$m^2$) of the monocultures (Table 4.16), or in the height of the clover plants (Table 4.27), or in the light intercepted by the canopy in December and April (Table 4.21). The only increase in light intercepted by the monocultures due to a higher N supply was in January, but the level of light interception was above 90 % in both N treatments and the increase was less than 3 %.

To understand the factors that could have been involved in this decrease of the white clover content in the pasture when more N was available, an examination of the sward characteristics and the impact of the higher N application level is needed. Both population and canopy processes need to be considered in this analysis.

*Population processes*

At the beginning of the study (June 2014) the tiller density of the pastures was similar under both N levels, and greater than the 5000 tillers/$m^2$ threshold suggested by Brereton et al. (1985) as the tiller density above which clover is suppressed (Table 4.15). Already at that sampling, the white clover growing point density of the mixtures was lower in the High than in the Low N treatment (Table 4.18), probably as a consequence of the N treatments applied in autumn 2014, before the start of the measurement period. By November, the tiller density of the swards in the High N treatment had increased considerably, while the increase in the Low N treatments was modest; nevertheless, the effect of N on tiller density was still not significant. At the same time, the decrease in the growing point density in the Low N treatment was modest, while the growing point density in the High N treatment almost halved from the number present in June (N effect on growing point density in

November *P* = 0.050). These results indicate the establishment of different environments for competition under the two N treatments early in the season. This trend continued from January onwards when pastures under the High N treatment had greater tiller density and lower growing point density than pastures under the Low N treatment.

Over the four population density sampling dates, the maximum tiller density in the mixtures was recorded in November (under both N treatments), in contrast to S. L. Harris, Thom, et al. (1996) in Waikato, New Zealand, who recorded maximum tiller density in January – February. Meanwhile, the white clover growing point density in the mixtures reached a maximum in January (under both N treatments) indicating a certain shift in population dynamics that could be indicating an improved combining ability of the components of the pasture under the environment and management of this experiment. In the Waikato however, maximum clover plant density was reached in spring. A possible reason for this difference is the application of irrigation in this experiment, which could have minimised the restriction to clover growth imposed by the drier summer conditions normally experienced in the Waikato.

The seasonal pattern of population density in perennial ryegrass was similar for both monoculture and mixture swards, indicating the dominance of ryegrass in the mixed sward. Meanwhile, the trend for white clover was different. In monoculture the highest growing point density was not observed in January; instead it was later in the season (June 2014 and May 2015).

*Canopy processes*

Nevertheless, the competition between grass and clover in the sward is not limited to interactions at the base of the canopy; competition for light in the canopy is critically important. Thus trends in those attributes that improve plant access to light, also play a role in competition. The undisturbed height of both ryegrass and clover was affected when grown in mixtures compared with monocultures (Tables 4.25 and 4.28), indicating the presence of inter-specific competition for light in the former. For ryegrass this increment was always significant under the Low N treatment, when clover was growing better and competing for light more actively; under the High N treatment however, the inclusion of clover only increased grass height in January. Meanwhile for white clover, the inclusion of grass in the pasture always caused an increment in height, under both N treatments. Over the three sampling times and N treatments, the perennial ryegrass height in the canopy increased 4.5% when grown in mixture compared to monoculture, while for white clover this increment was 13.6%. This change in height due to competition implies a difference in the allocation of resources for growth, such as longer petiole length in white clover or longer leaves in ryegrass at the expense of resource allocation to other organs or plant growth processes.

Results of the undisturbed grass and clover height when grown in mixture and under both N treatments are presented in Figure 4.17.

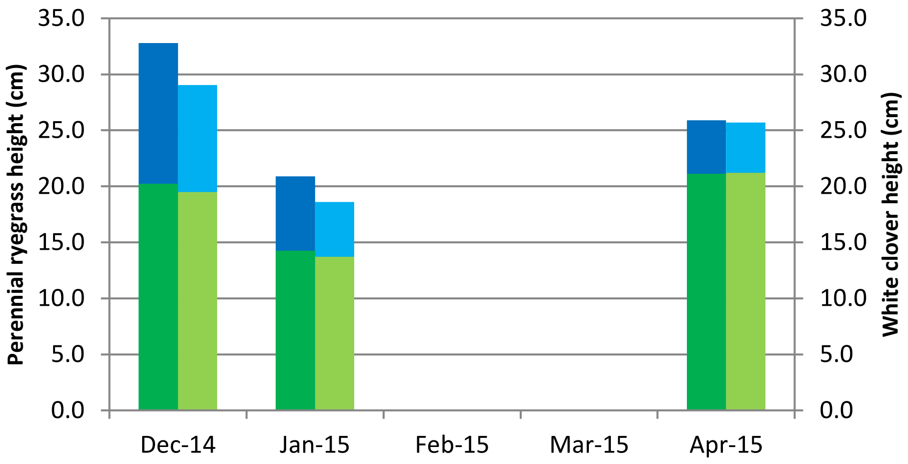


**Figure 4.17 Perennial ryegrass and white clover undisturbed height (cm) in mixed pastures under High or Low N treatment. Perennial ryegrass height High N (dark blue bar), perennial ryegrass height Low N (light blue bar), white clover height High N (dark green bar), white clover height Low N (light green bar).**

In December, when more N was applied, the increase in the grass height was significant, while the height of the clover plants was similar under both N treatments and considerably less than the grass (Tables 4.26 and 4.29); this may have limited the ability of the clover to capture a similar quantity and quality of light as the grass, and consequently limited its photosynthesis (Laidlaw & Withers, 1998). Wilman and Asiegbu (1982a) also found greater increase in grass height than in clover height when N was applied to a mixed sward. Similar results were obtained in summer (January 2015) when the application of a higher N fertiliser rate created again favourable conditions for grass growth and dominance. When the height of the plants was measured again in autumn (April 2015), neither the grass nor the clover plants increased significantly their height when more N was available, and this applied both in mixture and in monocultures.

Thus, the effect of a higher N input to the system, tipped the competition balance towards the grass, which was able to position its leaves higher in the canopy relative to the clover than was the case under lower N in fertiliser. In turn, this likely increased the proportion of the light energy reaching the canopy that was captured by the grass which was then able to transform this resource into a faster rate of DM accumulation.

#### *4.5.6..2 Perennial ryegrass and white clover cultivar effects and interactions with N*

*Perennial ryegrass cultivar*

Legume cultivar appeared to have a greater effect than grass cultivar on white clover percentage in the mixtures. The grass cultivar effect was significant only at three of the nine harvests; two of these harvests were in spring and one in autumn. The first occasion was in August, when the clover percentage was very low in all treatments. Mixtures including Abermagic AR1 had greater clover content than the rest of the cultivars, and although the yield of these swards was the lowest at that harvest (almost half of the yield of the other three cultivars), the difference in percentage was large enough to create a significant (although modest in absolute terms) difference in clover yield. In October 2014, the initial advantage of Abermagic AR1 continued, although mixtures with Bealey NEA2 had increased their clover content considerably, reaching a similar level to the Abermagic AR1 swards. It was not until May 2015 that the grass cultivar affected the clover content again, when mixtures including Arrow AR1 had the greater clover percentage and yield. The relationship between white clover percentage and white clover yield was positive and significant at two of these harvests (October: $P = 0.018$; $R^2 = 0.965$; May: $P = 0.040$; $R^2 = 0.922$) and trended toward significance at the other (August: $P = 0.063$; $R^2 = 0.878$).

To examine if greater ryegrass yield in monoculture was associated with lower clover content in mixture, regression analyses were conducted for the only harvest at which both grass yield in monoculture and clover percentage in mixture varied significantly due to grass cultivar (August). A significant negative association was observed ($P = 0.043$, $R^2 = 0.916$), driven mostly by the big difference in grass yield between Abermagic AR1 and the rest of the grass cultivars. Similar results were obtained when the same analysis was conducted between grass yield in mixture and white clover content in the mixture ($P = 0.019$, $R^2 = 0.963$). However, in October and May, when grass yield and clover content in mixture both varied significantly due to ryegrass cultivar, no significant associations were found ($P = 0.669$ and 0.483 respectively). The limited number of harvests in which both ryegrass component yield (in monoculture and mixture) and clover content differed due to ryegrass cultivar restricted the scope for exploring these associations further.

It has been suggested that a low tiller density favours clover growth in mixed swards (Frame & Boyd, 1986a) due to reduced competition for light from the grass component. In this experiment however, pastures based on Abermagic AR1 were the densest throughout the season, but they had the greatest clover content in two of the three harvests when the ryegrass cultivar effect was significant, suggesting that higher tiller density does not necessarily lead to lower sward clover content. Relationships between tiller density of ryegrass in mixtures and the white clover percentage of mixed swards in May (when there was an effect of grass cultivar on clover content), or between tiller density and clover yield in November and May (when a grass cultivar effect on clover yield was present) were not significant (*P* = 0.854, 0.544 and 0.621 respectively). Similarly, when regression analyses between tiller density in perennial ryegrass monocultures and clover content in mixtures were conducted for the same sampling times, no significant associations were detected. In November when the effect of grass cultivar on clover growing point density was significant (Table 4.18) and swards based on Bealey NEA2 had more growing points/m$^2$, the relationships between tiller density in monoculture or tiller density in mixture with white clover growing point density in mixture were not significant (*P* = 0.359 and 0.854 respectively). These results suggest that under the conditions and management of this experiment, differences in tiller population densities among the different perennial ryegrass cultivars had no effect on clover content, clover yield or growing point density in mixed swards, similar to the findings of Elgersma and Schlepers (1997a).

Moreover, there was no evidence that the light intercepted by the canopy of grass monocultures was affected by differences in tiller density among the perennial ryegrass cultivars (Table 4.19), although swards based on the tetraploid cultivar Bealey NEA2 were the least dense in the four samplings conducted. Interestingly, canopies formed by plants of the tetraploid when grown in monoculture were the tallest at two of the three measurements conducted. This tall canopy together with the large leaf morphology of this cultivar (Griffiths et al., 2016) explain partly the similarity in the light intercepted by the canopy of this cultivar and the denser diploids. When grown in mixtures, the grass cultivar effect on light interception was not significant either, with one exception in December when swards based on Prospect AR37 under the Low N treatment intercepted less light than the swards of the other three cultivars. This was probably due to the lower clover content of these mixtures under this treatment at this time. Similarly in mixtures, swards based on Bealey NEA2 were the least dense and formed the tallest grass canopy in January, and were amongst the tallest in December and April. Therefore in this experiment, a lower tiller density was not associated with lower light interception by the canopy in monoculture or mixture.

There have been previous indications that seasonal growth patterns of the ryegrass cultivars associated with reproductive development may influence clover growth (Camlin, 1981; Gooding et al., 1996). Reduced competitive ability of the mid heading date cultivars at the time when clover growth starts to accelerate in spring as temperatures increase appears to facilitate better growth of the legume (Camlin, 1981). Although no mid-heading cultivars were included in this experiment, the range in heading date (from +7 to +25), presents an opportunity to examine this issue. The lesser growth rate of the earliest heading ryegrass (Arrow AR1) during autumn (Figure 4.18) may explain why this cultivar had the highest clover percentage (and clover yield) in the last harvest of the season (May). Lower ryegrass growth rate was also observed in pastures based on Prospect AR37 during the same time, although this cultivar did not reach the same clover content and yield in the last harvest (Figure 4.7 and Table 4.8).

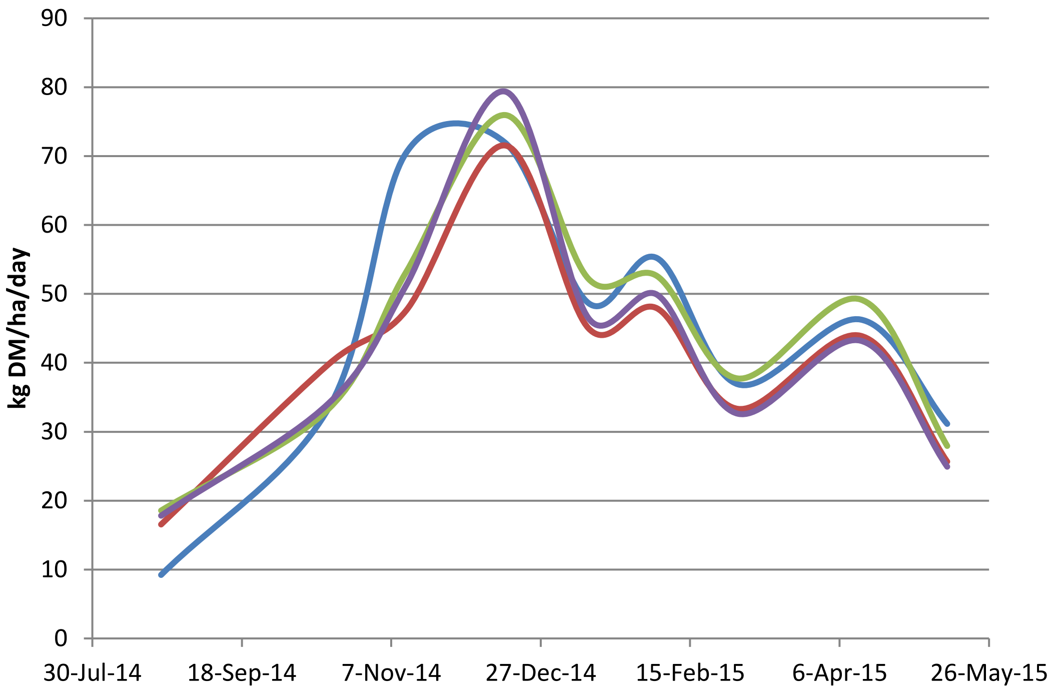


**Figure 4.18 Growth per day (kg DM/ha/day) of the perennial ryegrass component of mixed pastures based on different cultivars (average of High and Low N treatments). Abermagic AR1 (blue line), Arrow AR1 (red line), Bealey NEA2 (green line), Prospect AR37 (purple line).**

The plasticity of the clover to adapt to the different environments created by the variable characteristics of the grass component of the sward allowed the presence of similar amounts of clover in the swards based on perennial ryegrass cultivars differing in their phenotypes.

*White clover cultivar*

Meanwhile, the clover cultivar effect on clover percentage was significant at seven of the nine harvests, and was accompanied sometimes by an interaction with N; the trend in general was that Kopu II was amongst the cultivars with the highest content at every harvest when the difference was significant, although Bounty and/or Tribute were at times also in the same group, while Nomad was amongst the cultivars with the lowest clover content (Figures 4.8 and 4.19). These results could be the consequence of a change of harvest index due to breeding for increased stature (Rhodes & Harris, 1979). Clover yield (kg DM/ha) followed the same trend as clover percentage because the total DM produced by the mixtures was relatively similar amongst all clover cultivars. Regression analyses between clover content (% DM) and clover yield (kg DM/ha) in those harvests when significant differences due to clover cultivar in both variables were observed revealed significant positive associations that explained more than 95 % of the variation. Similar to Widdup and Turner

(1983), the reduction in the clover yield when grown in mixture relative to the clover yield in monoculture was greater for the small leaved clover (Nomad, 79 %) than for the larger leaved cultivar (Kopu II, 71 %) probably due to differences in their respective ability to compete with grass for light.

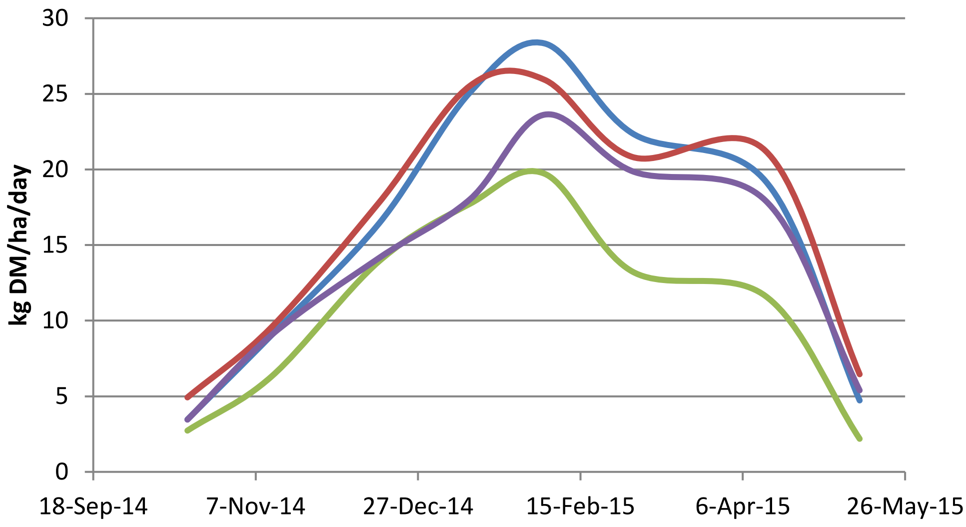


**Figure 4.19 Growth per day (kg DM/ha/day) of the white clover component of mixed pastures based on different cultivars (average of High and Low N treatments). Bounty (blue line), Kopu II (red line), Nomad (green line), Tribute (purple line).**

When the effect of the clover cultivar on the sward structure of the mixtures was analysed, a difference in the tiller density of the pastures due to clover cultivar was detected in only one of the four samplings (November 2014) where swards including Kopu II and Tribute had lower tiller density than swards including Nomad. In the context of competition for light in the mixture and considering that Kopu II was the tallest clover cultivar both in mixture and monoculture at every harvest (the opposite happened with Nomad that was the shortest cultivar), it could be expected that the ryegrass plants became taller and probably less dense due to different resource allocation within the plant in order to compete with the clover. However, there was no difference in the perennial ryegrass undisturbed height due clover cultivar when grown in mixture at any harvest meaning that there was no one clover cultivar that induced a greater increase in grass height than others. The explanation for the lower ryegrass tiller density in swards including Kopu II and Tribute is therefore unclear.

Meanwhile the number of clover growing points differed between clover cultivars when grown in mixtures only in the first sampling of the season (June 2014), when swards with Kopu II and Tribute had the lowest growing point density. Surprisingly, during the other three samplings, when grown in mixtures, the growing point density of the pasture was similar across clover cultivars, while when grown in monoculture, the cultivars differed at every sampling, with Kopu II and Tribute being the cultivars with less growing points/m$^2$. Apparently competition diluted stolon morphological differences, perhaps because leaf growth became the over-riding priority in order for plant to capture enough light.

Peak white clover growth was reached later in mixtures than in monoculture (solid and dashed green lines in Figure 4.14), due to the effect of competition with grass and this was similar for the four white clover cultivars. This trend in white clover was also mentioned by Smetham (1973) and is similar that observed by W. Harris and Hoglund (1977) with red clover (*Trifolium pratense* L.). This displacement in peak growth did not happen with ryegrass, illustrating its dominance of the mixture.

Only a few interactions between N and perennial ryegrass cultivars or N and clover cultivars were observed. Under the High N treatment the white clover content and yield tended to be similar amongst cultivars but under the Low N treatment the increment in clover content differed. Although a trend was noted towards Prospect AR37 preventing the same level of increase in clover content compared to other ryegrass cultivars when less N was available (in clover content and clover yield in December, and in clover content in January), the presence of these interactions was exceptional and for this reason no clear conclusions could be drawn. Similar comments apply to the interactions between clover cultivar and N; when less N was applied, there was a trend towards Kopu II increasing its clover content in the mixture more than other cultivars. However this interaction was present only for the clover percentage of the pasture, and not for the clover yield. Therefore no clear conclusion arises from these interactions.

### 4.5.7 Reasons for the lack of interaction between perennial ryegrass and white clover cultivars on DM yield and botanical composition

Although the phenotype of the grass and clover cultivars selected was different, as shown by the variation in tiller and growing point density and canopy height when grown in monocultures, these differences did not result in dissimilar total annual DM yield of the mixture for the factorial combinations of grass and clover cultivars ($P$ = 0.459). Different conditions for pasture growth were also created by the use of low and high N application rates, which affect competition between grass and clover in the mixture, but in general no interactions involving N, perennial ryegrass cultivar and white clover cultivar were detected. One of the reasons for the absence of interactions is the role of perennial ryegrass as the dominant component of the sward throughout the season, despite the more aggressive competition from some clover cultivars in summer and autumn, shown by the greater clover yield in mixture (Table 4.8) associated with a lower grass yield in mixture with those cultivars (Table 4.7). Substitution of grass by clover herbage occurred (Figure 4.20); the ability of the legume to growth at similar rates to ryegrass, especially under low N application rates, and the observation that the clover yield potential was very close to the ryegrass yield potential when grown in monocultures, resulted in similar total annual yield of the mixtures.

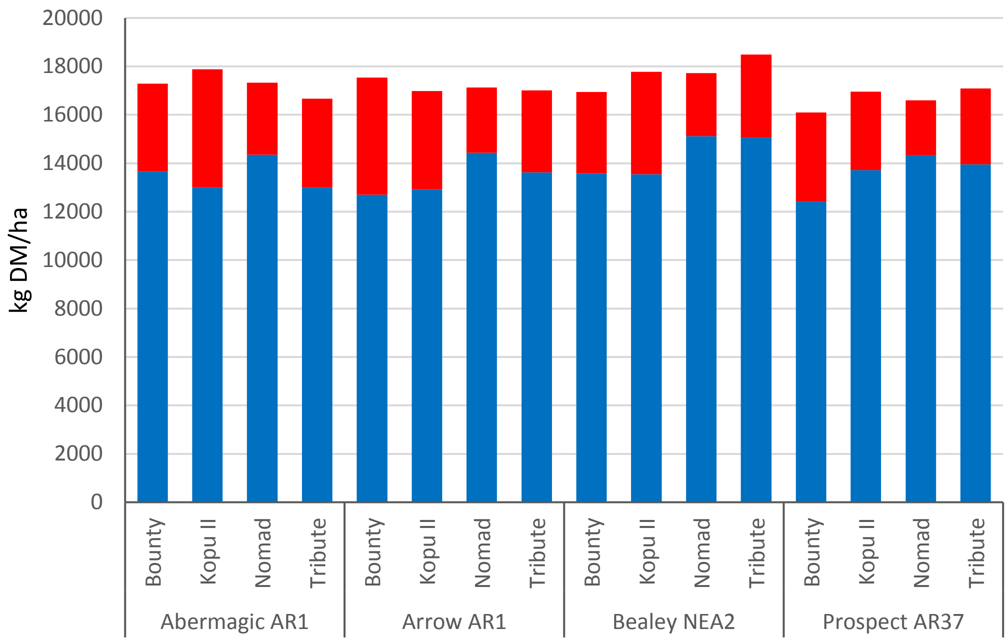


**Figure 4.20 Annual perennial ryegrass (blue bar) and white clover (red bar) yields (kg DM/ha) in mixed pastures sown with combinations of different cultivars.**

The results of this experiment agree with previous research showing no effect of the interaction between perennial ryegrass and white clover cultivar on total yield of the mixture (Camlin, 1981; Connolly, 1968; Elgersma et al., 1998; Ledgard et al., 1990; Widdup & Turner, 1983) and highlight the compensatory effect of both components of the sward on total yield under the management of this study, with contrasting N application regimes and irrigation.

Only one significant interaction was detected between perennial ryegrass and white clover cultivars in clover content during the year, indicating that in general, it was the effect of the ryegrass or the clover cultivar alone that determined the proportion of the legume in the sward, and not the combination of different phenotypes. The effect of grass cultivar was more important in spring and autumn indicating that during these times of the year this component of the grass becomes more competitive, while the effect of clover cultivar was significant in seven of the harvests throughout the season.

### 4.5.8 Limitations of this study

The present experiment covered only one year (1st June 2014 to 31st May 2015): the first complete growing season after sowing of the pasture in the previous spring (November). This means that most of the clover plants were in the second phase of development (*tap-rooted phase*) that usually lasts between 1 and 2 years (Brock et al., 2000; Brock & Hay, 2001), and in which the legume tends to produce the greatest amount of DM per unit area (Widdup & Barrett, 2011). Therefore, the high clover yields observed in this experiment may not be present in established pastures, where the clover plants are in the *clonal phase*. To extend the measurement period for 1 or more years (Brock et al., 2000) would allow conclusions regarding the contribution of clover to pasture yield in mixture and monoculture on a situation fully representative of a developed pasture to be drawn.

Another limitation of this study is the management under cutting only. In New Zealand conditions, pastures are managed mostly under grazing, and are exposed to selection for some sward components over others by the grazing animals. Preference for clover has been shown in previous research (Chapman et al., 2007; Rutter, 2006) and has implications for the outcome of competition between the components of the pasture (Gilliland, 1996). A more selective grazing behaviour towards clover may carry an initial disadvantage for the legume, which loses a greater proportion of the photosynthetic area compared with grass when defoliated. In these conditions, different sward structure at the base of the canopy could result in clover plants more exposed or more protected affecting the persistence and yield of this component of the pasture. Thus, the cutting regime may have created different conditions for competition compared with the normal grazing management.

The lack of grazing created also a more uniform environment with respect to N distribution in the pasture, compared with the patchiness created as the result of the return of N through urine. The uneven distribution of N in a grazed pasture plays an important role in the stability of the clover component by creating different areas in the pasture 'out of phase' with respect to grass or legume dominance (Chapman et al., 1996; Schwinning & Parsons, 1996a, 1996c). As a consequence, the ecosystem created by the management of this experiment, although convenient to study the effect of competition between different perennial ryegrass and clover phenotypes in pasture yield and composition, may not be the best approximation to the pasture system under grazing.

The short duration of this study also limited the ability to detect the development of the exploitation interaction that occurs between the grass and clover components of the pasture due to the increase in soil N pool by $N_2$ fixation and N cycling (Chapman et al., 1996; Schwinning & Parsons, 1996a, 1996c). Observations over a longer period of time would be also needed to overcome this limitation.

### 4.5.9 Conclusions

Under the conditions and management of this experiment the total annual yield of the mixtures was similar for the different combinations of perennial ryegrass and white clover cultivars due to substitution between the components of the sward.

The composition of these pastures varied through the duration of the experiment; the role of perennial ryegrass in determining clover content was greater in spring and autumn. During spring it is not clear what characteristics of the grass favoured clover growth. It could be a combination of lower DM yield, lesser light interception immediately after harvest (not measured in this experiment), or a later heading date as is the case of cultivars Abermagic AR1 and Bealey NEA2. In autumn however, the increase in clover content may have been linked to an earlier heading date of the grass cultivars, as is the case with Arrow AR1. These results concord with results of the previous experiment, where the effect of perennial ryegrass cultivar on clover content was significant in spring in the two years and in summer of the second year. Moreover, Abermagic AR1 and Bealey NEA2 were amongst the cultivars with greater clover content in spring; when the clover content was analysed in cultivars with contrasting heading date, in summer both years and autumn of the first year, mid heading date cultivars had greater clover content, similar to what was observed with Arrow AR1 in of this experiment.

The role of white clover cultivar in determining clover content was relevant during most of the season. The smallest leaved cultivar Nomad contributed less to the harvested herbage than the larger leaved cultivars, and the differences in clover contribution were more important under the Low N treatment, when the grass component exerted less competition.

The inclusion of perennial ryegrass monoculture as one of the treatments permitted the estimation of the white clover contribution to herbage yield under the different N treatments and the comparison of the canopy characteristics of the different cultivars without the interference of white clover. Such is the case of the observation that the tetraploid Bealey NEA2, despite being the cultivar with the lowest tiller density, was intercepting similar PAR to the diploid cultivars when measured shortly before harvest.

Similarly the inclusion of white clover monoculture plots confirmed the absence of response to N of these swards, and permitted the comparison of population density characteristics under inter- and intra-specific competition. As an example, the growing point density in clover monocultures differed significantly between cultivars in all the samplings, while when grown in mixed swards they differed in only one sampling. The presence of clover monocultures plots permitted also the observation that under irrigation, swards of the legume in their taproot phase are able to yield similarly to grass monoculture.

This design of this experiment permitted to extend the analysis of the interaction between perennial ryegrass and white clover to the level of cultivar in both species. Similarly to the previous experiment, in general, there was no interaction between perennial ryegrass cultivar and white clover presence on total DM yield. Moreover in general, there was no interaction between perennial ryegrass cultivar and white clover cultivar on total DM yield. As in the previous experiment, the effect of perennial ryegrass cultivar on DM yield was more important in spring and autumn and less important in summer. During part of summer and early autumn, there was no effect of N on total DM yield of mixtures, due to the increased contribution of clover when less N fertiliser was available. Similar results were observed in the previous experiment in summer of the first year and in autumn both years.

The results of this experiment are consistent with Laidlaw and Withers (1998) observations that a larger-leaved cultivar would be able to make a greater contribution to the upper layers of the canopy and be less affected by N fertiliser. These authors also commented that the lower stolon population density of this type of cultivars could be a disadvantage in successive regrowths. In the present experiment however, only in one of the four samplings the larger-leaved cultivars had lower growing point density than the medium and small-leaved cultivars when grown in mixtures, but this relatively similar density could have been facilitated by the management of this experiment under cutting. Different could have been the result under grazing pressure.

# Chapter 5

# Overall conclusion

The two experiments presented in this thesis involved two management systems: simulated grazing by harvest and animal grazing by dairy cows. The perennial ryegrass and white clover cultivars included represented a range of morphologies, heading dates and potential yield. Moreover, the two paddocks had different soil type and management history that may have resulted in dissimilar soil organic N content. However, despite these differences, the results of these experiments show that in general, there was no evidence of re-ranking of cultivars based on their relative dry matter yield (kg DM/ha) when sown in mixed perennial ryegrass/white clover swards compared to ryegrass monoculture under the two N fertiliser application rates and irrigation regimes imposed in these experiments. Furthermore, although in one of the experiments there was some re-ranking of perennial ryegrass cultivars for ME density (MJ/kg DM) when sown with clover compared to ryegrass monoculture, the magnitude of change was too small. Therefore performance values in the Forage Value Index (Chapman et al., 2016; DairyNZ) which are calculated using dry matter yield data from cultivar evaluation trials conducted using perennial ryegrass monocultures do not need adjustment to account for grass-clover interactions over time and their effects on total pasture dry matter yield.

The finding that in the experiment reported in Chapter 4 the total annual yield of the mixtures was similar for the different combinations of perennial ryegrass and white clover cultivars due to substitution between the components of the sward agrees with previous research and reaffirms A. V. Stewart (2006, p. 11) statement regarding the challenge that represents to lift overall pasture performance in mixtures because '*any increase in the ryegrass yield is often partially cancelled by decreased clover yields*'.

This could suggest that attempts to improve feeding value of swards through an increased clover content, might be even more productive if other factors, such as complementarity in ryegrass and clover cultivars seasonal growth are considered, resulting in a more efficient use of the 'resource space' (de Wit, 1960; W. Harris, 2001). Nevertheless, despite the multiple roles that white clover plays in the pasture-based systems in New Zealand, A. Stewart and Hayes (2011, p. 40) indicated that there were '*no reports of breeders targeting clover compatibility as a breeding objective*'.

The results of the experiments included in this thesis agree with previous research (Camlin, 1981), and suggest that that the ideotype (Donald, 1968) of ryegrass to improve white clover content should have mid-heading date. The possible explanation for the increased clover content with this type of cultivars has been referred in the literature as related to the decreased competitive ability of these cultivars at the start of the period of more active clover growth.

Meanwhile there was no evidence that a lower tiller density promoted greater clover content in any of the experiments nor that tetraploids allowed a higher cover content.

Management practices such as defoliation also play an important role in the balance of competition between perennial ryegrass and white clover in the sward. In the experiment reported in Chapter 3, the results suggest that a high grazing efficiency as evidenced by a lower post-grazing mass promotes clover content, especially during spring, when the competition from grass is greater. This observation was possible due to the design of the experiment that allowed the grazing of different cultivars at the same time and the exhibition of preference by grazing animals for some cultivars. Possible explanations for this preference were not studied in this experiment, but a greater ME density (MJ/kg DM) of tetraploids or the increased water soluble carbohydrates of the high-sugar grass (Cosgrove, Mapp, Taylor, Harvey, & Knowler, 2014) may have contributed to these results. Similarly, Gilliland et al. (2002) found that tetraploids had better intake characteristics than diploids, and high water soluble carbohydrates concentration, only surpassed by a high-sugar diploid ryegrass.

Other practical implications of the results of these experiments refer to the effect of N on DM yield. The variable response to N on the DM yield amongst season and clover treatments (plus or minus clover) highlights the important role of clover in the sustainability of pasture-based production systems through the reduced need for N fertiliser. However, the contribution of clover to the sward has limitations. Its yield and presence is highly variable, it oscillates over time, it is often patchily-distributed within the sward, and is vulnerable to pests (clover root weevil, nematodes) and diseases (Chapman et al., 1996; Schwinning & Parsons, 1996a; Wakelin, Eslami, Dake, Dignam, & O'Callaghan, 2016). Therefore, it is important to consider these limitations when evaluating the contribution of the legume to total herbage yield and quality. The results of this thesis remind also the negative impact of N fertiliser application on the contribution of clover to sward composition.

Finally the contribution of white clover to dry matter yield of the mixture under the two N treatments imposed in these trials reaffirms the need to continue regarding this legume as '*New Zealand's Competitive Edge*' (Woodfield, 1996).

# Appendix A

**Table A. 1 Monthly total rainfall (mm), mean air temperature (°C), EVT potential (mm) (NIWA, Broadfield station), irrigation (mm) (A. Clement, personal communication, July 24, 2014), rainfall plus irrigation for the period April 2012 to May 2014 and historical data (NIWA, Broadfield station) for the period 1981 to 2010.**

| Month | Total Rainfall (mm) | Mean Air Temperature (°C) | Total Penman Potential Evapo-Transpiration (mm) | Irrigation (mm) | Total Rainfall + Irrigation (mm) | Rainfall (mm) historical data 1981 - 2010 | Mean Air Temperature (°C) historical data 1981 - 2010 |
|---|---|---|---|---|---|---|---|
| Jun-12 | 74.8 | 5.5 | 14.5 | 0.0 | 74.8 | 57.2 | 6.7 |
| Jul-12 | 44.0 | 6.5 | 16.6 | 0.0 | 44.0 | 57.8 | 6.1 |
| Aug-12 | 107.0 | 9.0 | 29.0 | 0.0 | 107.0 | 61.0 | 7.6 |
| Sep-12 | 29.0 | 10.0 | 64.4 | 0.0 | 29.0 | 39.7 | 9.6 |
| Oct-12 | 70.0 | 11.2 | 101.2 | 13.0 | 83.0 | 50.6 | 11.5 |
| Nov-12 | 52.8 | 11.5 | 110.0 | 41.0 | 93.8 | 48.6 | 13.3 |
| Dec-12 | 32.0 | 16.6 | 156.3 | 48.0 | 80.0 | 52.7 | 15.4 |
| Jan-13 | 29.8 | 17.6 | 178.6 | 71.0 | 100.8 | 41.7 | 16.9 |
| Feb-13 | 21.2 | 16.2 | 117.3 | 46.0 | 67.2 | 40.6 | 16.6 |
| Mar-13 | 41.8 | 16.1 | 87.1 | 68.0 | 109.8 | 46.5 | 14.9 |
| Apr-13 | 58.8 | 12.3 | 38.4 | 0.0 | 58.8 | 44.5 | 12.2 |
| May-13 | 109.2 | 9.8 | 26.8 | 0.0 | 109.2 | 58.0 | 9.3 |
| Jun-13 | 208.7 | 7.0 | 13.1 | 0.0 | 208.7 | 57.2 | 6.7 |
| Jul-13 | 30.0 | 7.8 | 22.9 | 0.0 | 30.0 | 57.8 | 6.1 |
| Aug-13 | 41.0 | 9.6 | 31.9 | 0.0 | 41.0 | 61.0 | 7.6 |
| Sep-13 | 30.6 | 9.5 | 49.3 | 0.0 | 30.6 | 39.7 | 9.6 |
| Oct-13 | 71.0 | 12.4 | 104.7 | 0.0 | 71.0 | 50.6 | 11.5 |
| Nov-13 | 44.6 | 14.3 | 118.5 | 10.0 | 54.6 | 48.6 | 13.3 |
| Dec-13 | 64.2 | 16.4 | 144.4 | 45.4 | 109.6 | 52.7 | 15.4 |
| Jan-14 | 12.2 | 15.8 | 157.0 | 94.3 | 106.5 | 41.7 | 16.9 |
| Feb-14 | 53.8 | 17.0 | 113.7 | 43.8 | 97.6 | 40.6 | 16.6 |
| Mar-14 | 121.2 | 14.0 | 77.5 | 0.0 | 121.2 | 46.5 | 14.9 |
| Apr-14 | 161.2 | 12.7 | 32.2 | 0.0 | 161.2 | 44.5 | 12.2 |
| May-14 | 44.6 | 10.3 | 32.8 | 0.0 | 44.6 | 58.0 | 9.3 |
| Season 2012 - 2013 | 670.4 | 11.9 | 940.2 | 287.0 | 957.4 | 598.9 | 11.7 |
| Season 2013 - 2014 | 883.1 | 12.2 | 898.0 | 193.5 | 1076.6 | 598.9 | 11.7 |

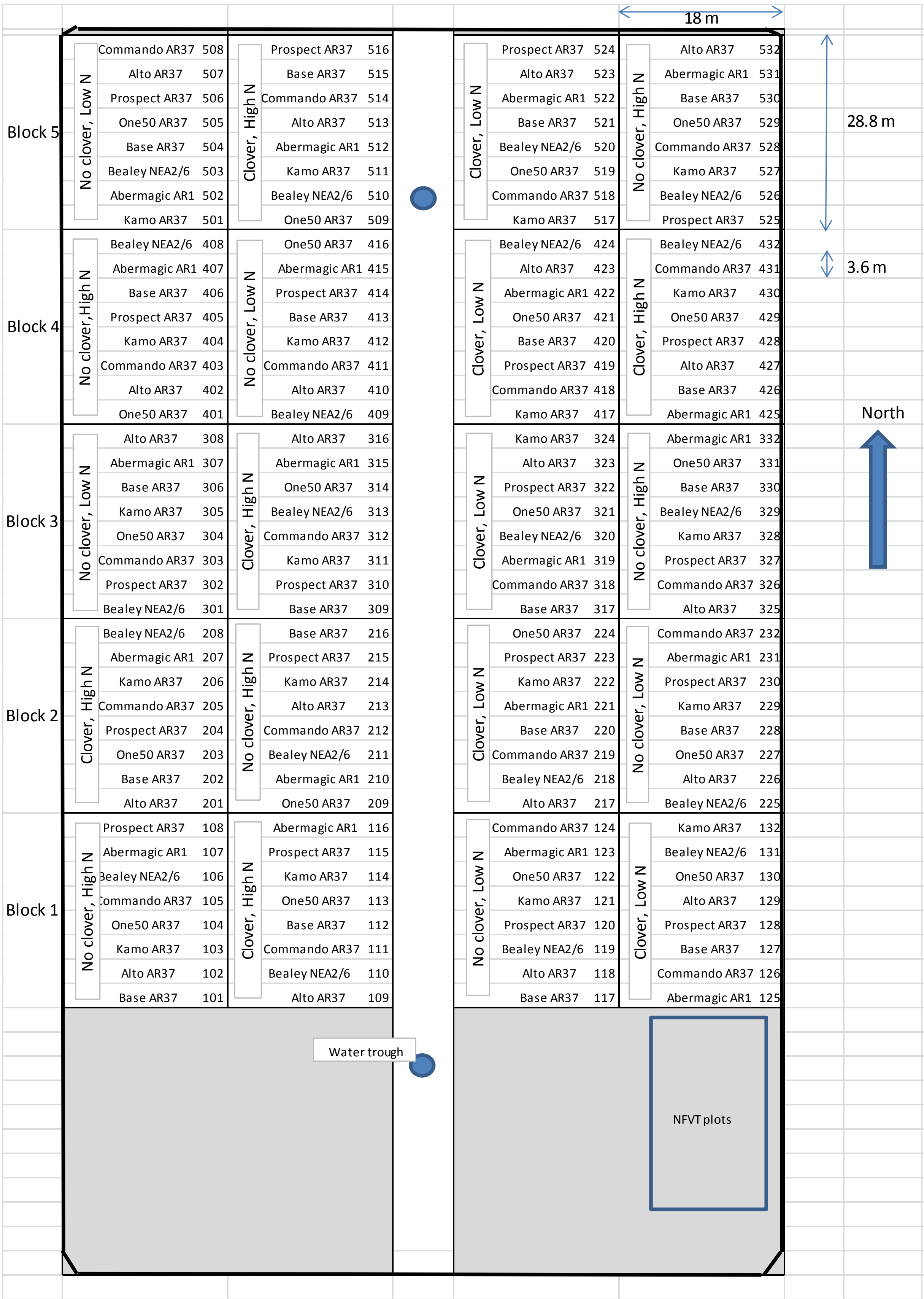


**Figure A.1 Species Interaction x Management Trial layout**

**Table A.2 Monthly total rainfall (mm), mean air temperature (°C), EVT potential (mm) (NIWA, Broadfield station), irrigation (mm) (A. Clement, personal communication, 2015), rainfall plus irrigation for the period June 2014 to May 2015 and historical data (NIWA, Broadfield station) for the period 1981 to 2010.**

| Month | Total Rainfall (mm) | Mean Air Temperature (°C) | Total Penman Potential Evapo-Transpiration (mm) | Irrigation (mm) | Total Rainfall + Irrigation (mm) | Rainfall (mm) historical data 1981 - 2010 | Mean Air Temperature (°C) historical data 1981 - 2010 |
|---|---|---|---|---|---|---|---|
| Jun-14 | 45.2 | 8.4 | 16.2 | 0.0 | 45.2 | 57.2 | 6.7 |
| Jul-14 | 49.0 | 6.7 | 24.7 | 0.0 | 49.0 | 57.8 | 6.1 |
| Aug-14 | 15.2 | 7.6 | 38.3 | 0.0 | 15.2 | 61.0 | 7.6 |
| Sep-14 | 24.6 | 9.6 | 62.4 | 0.0 | 24.6 | 39.7 | 9.6 |
| Oct-14 | 19.6 | 11.9 | 106.3 | 24.6 | 44.2 | 50.6 | 11.5 |
| Nov-14 | 53.4 | 13.7 | 144.4 | 82.0 | 135.4 | 48.6 | 13.3 |
| Dec-14 | 18.4 | 15.5 | 144.2 | 71.2 | 89.6 | 52.7 | 15.4 |
| Jan-15 | 7.2 | 18.2 | 158.4 | 113.3 | 120.5 | 41.7 | 16.9 |
| Feb-15 | 19.0 | 16.9 | 123.2 | 75.1 | 94.1 | 40.6 | 16.6 |
| Mar-15 | 40.4 | 15.7 | 87.1 | 33.8 | 74.2 | 46.5 | 14.9 |
| Apr-15 | 77.6 | 13.6 | 54.5 | 0.0 | 77.6 | 44.5 | 12.2 |
| May-15 | 6.4 | 10.1 | 31.0 | 0.0 | 6.4 | 58.0 | 9.3 |
| Season 2014-2015 | 376.0 | 12.3 | 990.7 | 400.0 | 776.0 | 598.9 | 11.7 |

| | Low N | | | | High N | | | |
|---|---|---|---|---|---|---|---|---|
| | 421 Bealey NEA2 + Kopu II | 422 Bealey NEA2 + Nomad | 423 Bealey NEA2 + Tribute | 424 Abermagic AR1+Bounty | 445 Prospect AR37 + Tribute | 446 Arrow AR1 + Kopu II | 447 Prospect AR37 + Kopu II | 448 Abermagic AR1 + Bounty |
| | 417 Prospect AR37 | 418 Arrow AR1 + Tribute | 419 Prospect AR37 + Nomad | 420 Prospect AR37+Bounty | 441 Prospect AR37 + Bounty | 442 Abermagic AR1+Nomad | 443 Bealey NEA2 + Kopu II | 444 Bounty |
| Block 4 | 413 Abermagic AR1+Nomad | 414 Abermagic AR1+Tribute | 415 Kopu II | 416 Abermagic AR1+Kopu II | 437 Bealey NEA2 | 438 Arrow AR1 + Nomad | 439 Abermagic AR1+Kopu II | 440 Abermagic AR1+Tribute |
| | 409 Bealey NEA2 | 410 Arrow AR1 + Kopu II | 411 Bounty | 412 Prospect AR37 + Kopu II | 433 Arrow AR1 + Tribute | 434 Arrow AR1 + Bounty | 435 Prospect AR37 + Nomad | 436 Abermagic AR1 |
| | 405 Prospect AR37+Tribute | 406 Arrow AR1 | 407 Tribute | 408 Arrow AR1 + Bounty | 429 Bealey NEA2 + Bounty | 430 Bealey NEA2 + Nomad | 431 Tribute | 432 Prospect AR37 |
| | 401 Arrow AR1 + Nomad | 402 Abermagic AR1 | 403 Nomad | 404 Bealey NEA2 + Bounty | 425 Bealey NEA2 + Tribute | 426 Arrow AR1 | 427 Nomad | 428 Kopu II |

| | Low N | | | | High N | | | |
|---|---|---|---|---|---|---|---|---|
| | 321 Arrow AR1 | 322 Bealey NEA2 + Bounty | 323 Prospect AR37+Tribute | 324 Arrow AR1 + Kopu II | 345 Abermagic AR1+Tribute | 346 Bealey NEA2 + Kopu II | 347 Tribute | 348 Prospect AR37 + Kopu II |
| | 317 Arrow AR1 + Nomad | 318 Prospect AR37+Bounty | 319 Arrow AR1 + Bounty | 320 Prospect AR37+Kopu II | 341 Kopu II | 342 Abermagic AR1+Kopu II | 343 Bealey NEA2 + Nomad | 344 Abermagic AR1+Nomad |
| Block 3 | 313 Kopu II | 314 Bealey NEA2 + Nomad | 315 Abermagic AR1+Tribute | 316 Abermagic AR1 | 337 Prospect AR37 | 338 Prospect AR37+Bounty | 339 Bealey NEA2 | 340 Prospect AR37+Tribute |
| | 309 Bealey NEA2 + Kopu II | 310 Abermagic AR1+Bounty | 311 Nomad | 312 Abermagic AR1+Kopu II | 333 Nomad | 334 Arrow AR1 + Nomad | 335 Bealey NEA2 + Tribute | 336 Arrow AR1 + Bounty |
| | 305 Tribute | 306 Bounty | 307 Arrow AR1 + Tribute | 308 Prospect AR37 | 329 Bealey NEA2 + Bounty | 330 Bounty | 331 Arrow AR1 | 332 Arrow AR1 + Tribute |
| | 301 Bealey NEA2 + Tribute | 302 Prospect AR37+ Nomad | 303 Abermagic AR1+Nomad | 304 Bealey NEA2 | 325 Prospect AR37+Nomad | 326 Arrow AR1 + Kopu II | 327 Abermagic AR1 | 328 Abermagic AR1+Bounty |

| | Low N | | | | High N | | | |
|---|---|---|---|---|---|---|---|---|
| | 221 Bealey NEA2 + Nomad | 222 Abermagic AR1+ Bounty | 223 Arrow AR1 + Tribute | 224 Prospect AR37 + Kopu II | 245 Abermagic AR1 | 246 Bealey NEA2 | 247 Nomad | 248 Arrow AR1 + Nomad |
| | 217 Abermagic AR1 | 218 Arrow AR1 + Nomad | 219 Abermagic AR1+ Nomad | 220 Tribute | 241 Bounty | 242 Abermagic AR1+Nomad | 243 Tribute | 244 Prospect AR37+Nomad |
| Block 2 | 213 Prospect AR37 + Bounty | 214 Bealey NEA2 + Kopu II | 215 Abermagic AR1 + Kopu II | 216 Abermagic AR1 + Tribute | 237 Abermagic AR1+Tribute | 238 Arrow AR1 | 239 Prospect AR37 + Bounty | 240 Arrow AR1 + Bounty |
| | 209 Bealey NEA2 | 210 Bealey NEA2 + Tribute | 211 Arrow AR1 + Bounty | 212 Bealey NEA2 + Bounty | 233 Bealey NEA2 + Kopu II | 234 Bealey NEA2 + Bounty | 235 Kopu II | 236 Bealey NEA2 + Nomad |
| | 205 Arrow AR1 + Kopu II | 206 Prospect AR37 + Tribute | 207 Prospect AR37 + Nomad | 208 Arrow AR1 | 229 Arrow AR1 + Koopu II | 230 Prospect AR37 | 231 Prospect AR37 + Tribute | 232 Abermagic AR1+Bounty |
| | 201 Bounty | 202 Kopu II | 203 Nomad | 204 Prospect AR37 | 225 Abermagic AR1+ Kopu II | 226 Arrow AR1 + Tribute | 227 Bealey NEA2 + Tribute | 228 Prospect AR37 + Kopu II |

| | High N | | | | Low N | | | |
|---|---|---|---|---|---|---|---|---|
| | 121 Arrow AR1 + Bounty | 122 Abermagic AR1 + Tribute | 123 Prospect AR37 | 124 Prospect AR37 + Nomad | 145 Bealey NEA2 + Kopu II | 146 Abermagic AR1 + Bounty | 147 Bounty | 148 Prospect AR37 + Kopu II |
| | 117 Abermagic AR1 + Kopu II | 118 Bealey NEA2 + Tribute | 119 Bealey NEA2 | 120 Prospect AR37 + Kopu II | 141 Abermagic AR1+Nomad | 142 Arrow AR1 + Nomad | 143 Prospect AR37 + Tribute | 144 Bealey NEA2 + Nomad |
| Block 1 | 113 Abermagic AR1 | 114 Arrow AR1 + Nomad | 115 Tribute | 116 Abermagic AR1 + Bounty | 137 Kopu II | 138 Abermagic AR1 | 139 Tribute | 140 Prospect AR37 + Nomad |
| | 109 Prospect AR37 + Tribute | 110 Bounty | 111 Kopu II | 112 Arrow AR1 + Tribute | 133 Arrow AR1 | 134 Bealey NEA2 + Tribute | 135 Prospect AR37 + Bounty | 136 Arrow AR1 + Bounty |
| | 105 Bealey NEA2 + Kopu II | 106 Abermagic AR1+Nomad | 107 Arrow AR1 | 108 Nomad | 129 Abermagic AR1+Kopu II | 130 Bealey NEA2 + Bounty | 131 Nomad | 132 Arrow AR1 + Kopu II |
| | 101 Arrow AR1+Kopu II | 102 Prospect AR37 + Bounty | 103 Bealey NEA2 + Nomad | 104 Bealey NEA2 + Bounty | 125 Abermagic AR1+Tribute | 126 Prospect AR37 | 127 Arrow AR1 + Tribute | 128 Bealey NEA2 |

**Figure A.2 Experiment layout**

**Table A. 3 Botanical composition (% of DM) of pastures during 2012 – 13.**

| | | Spring 2012 | | | | | | | | Summer 2012 - 2013 | | | | | | | | Autumn 2013 | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | PRG% | | WC% | | Other% | | Dead% | | PRG% | | WC% | | Other% | | Dead% | | PRG% | | WC% | | Other% | | Dead% | |
| Nitrogen treatment | High | 83.0 | | 2.7 | | 8.3 | | 6.0 | | 83.2 | | 8.0 | | 2.5 | | 6.3 | | 89.2 | | 2.6 | | 2.4 | | 5.8 | |
| | Low | 81.0 | | 6.2 | | 5.7 | | 7.1 | | 57.6 | | 30.2 | | 4.0 | | 8.2 | | 75.6 | | 12.1 | | 2.7 | | 9.6 | |
| Clover treatment | + clover | 79.5 | | 8.9 | | 5.7 | | 6.0 | | 60.4 | | 32.6 | | 1.6 | | 5.5 | | 77.9 | | 14.1 | | 0.9 | | 7.1 | |
| | - clover | 84.5 | | 0.1 | | 8.3 | | 7.2 | | 80.4 | | 5.6 | | 4.9 | | 9.0 | | 87.0 | | 0.6 | | 4.1 | | 8.3 | |
| SED | | 1.3 | | 1.2 | | 0.9 | | 0.7 | | 3.5 | | 3.8 | | 1.4 | | 0.9 | | 2.1 | | 1.3 | | 1.0 | | 0.8 | |
| Perennial ryegrass cultivar | Abermagic AR1 | 75.4 | d | 6.0 | a | 14.7 | a | 3.8 | e | 71.7 | ab | 19.6 | a | 2.9 | | 5.8 | bcd | 81.7 | | 9.2 | a | 2.9 | | 6.1 | cd |
| | Alto AR37 | 81.1 | bcd | 2.4 | b | 7.4 | bcd | 9.0 | ab | 70.5 | ab | 17.6 | ab | 4.3 | | 7.6 | abc | 84.7 | | 5.0 | c | 2.5 | | 7.7 | bc |
| | Base AR37 | 85.5 | ab | 5.8 | a | 4.1 | cde | 4.5 | de | 75.0 | a | 18.4 | a | 1.6 | | 4.9 | d | 84.4 | | 7.2 | bc | 2.9 | | 5.5 | cd |
| | Bealey NEA2 | 78.4 | cd | 6.8 | a | 10.3 | ab | 4.6 | de | 70.6 | ab | 20.1 | a | 3.6 | | 5.7 | cd | 84.7 | | 7.4 | bc | 3.0 | | 4.9 | d |
| | Commando AR37 | 86.2 | a | 2.9 | b | 4.2 | cde | 6.7 | bcd | 66.5 | bc | 20.1 | a | 4.3 | | 9.0 | a | 82.2 | | 8.2 | abc | 2.0 | | 7.6 | bcd |
| | Kamo AR37 | 84.0 | abc | 4.3 | ab | 4.4 | de | 7.2 | bc | 64.2 | c | 23.3 | a | 3.2 | | 9.2 | a | 78.9 | | 9.3 | ab | 2.8 | | 9.1 | ab |
| | One50 AR37 | 82.3 | abc | 4.3 | ab | 7.7 | bc | 5.7 | cd | 71.1 | ab | 18.1 | a | 3.4 | | 7.4 | abc | 80.3 | | 6.3 | bc | 2.4 | | 11.1 | a |
| | Prospect AR37 | 82.7 | abc | 3.1 | b | 3.1 | e | 11.0 | a | 73.5 | a | 15.6 | b | 2.6 | | 8.3 | ab | 82.6 | | 6.1 | c | 1.7 | | 9.6 | ab |
| SED | | 2.6 | | 1.4 | | 2.1 | | 1.2 | | 3.2 | | 2.6 | | 1.6 | | 1.2 | | 2.5 | | 1.8 | | 1.0 | | 1.6 | |
| N x Clover treatment | High N + clover | 81.6 | | 5.4 | b | 6.9 | | 6.1 | | 77.8 | | 14.6 | b | 2.3 | b | 5.3 | | 87.2 | | 5.2 | b | 1.8 | ab | 5.8 | |
| | High N - clover | 84.3 | | 0.1 | c | 9.7 | | 5.9 | | 88.6 | | 1.3 | c | 2.7 | ab | 7.3 | | 91.2 | | 0.0 | d | 3.0 | a | 5.8 | |
| | Low N + clover | 77.4 | | 12.3 | a | 4.5 | | 5.8 | | 42.9 | | 50.5 | a | 0.9 | b | 5.7 | | 68.5 | | 23.0 | a | 0.1 | b | 8.4 | |
| | Low N - clover | 84.6 | | 0.0 | c | 6.9 | | 8.5 | | 72.3 | | 9.9 | b | 7.1 | a | 10.7 | | 82.7 | | 1.1 | c | 5.3 | a | 10.9 | |
| | | 1.8 | | 1.7 | | 1.3 | | 1.0 | | 5.0 | | 5.4 | | 1.9 | | 1.3 | | 3.0 | | 1.9 | | 1.4 | | 1.1 | |
| *P* value | N effect | 0.183 | | < 0.05 | | < 0.01 | | 0.084 | | < 0.001 | | < 0.001 | | 0.635 | | < 0.05 | | < 0.001 | | < 0.001 | | 0.468 | | < 0.001 | |
| | Clover effect | < 0.01 | | < 0.001 | | < 0.05 | | 0.108 | | < 0.001 | | < 0.001 | | < 0.05 | | < 0.01 | | < 0.001 | | < 0.001 | | < 0.01 | | 0.248 | |
| | Cultivar effect | < 0.01 | | < 0.01 | | < 0.001 | | < 0.001 | | < 0.05 | | < 0.05 | | 0.443 | | < 0.01 | | 0.087 | | < 0.05 | | 0.746 | | < 0.001 | |
| | N x Clover interaction | 0.117 | | < 0.05 | | 0.730 | | 0.064 | | 0.063 | | < 0.05 | | < 0.05 | | 0.113 | | 0.096 | | < 0.001 | | < 0.05 | | 0.209 | |
| | N x Cultivar interaction | 0.749 | | 0.522 | | 0.637 | | 0.391 | | 0.364 | | 0.423 | | 0.715 | | 0.456 | | 0.108 | | 0.452 | | 0.581 | | 0.137 | |
| | Clover x Cultivar interaction | 0.525 | | < 0.05 | | 0.892 | | 0.925 | | 0.764 | | 0.136 | | 0.271 | | 0.072 | | 0.484 | | 0.093 | | 0.807 | | 0.389 | |

SED = standard error of the difference between means. Different letters within a column indicate statistical differences. In this table % and SED are from the analysis without angular transformation, but *P* value and letters are from the analysis with angular transformation.

**Table A. 4 Botanical composition (% of DM) of pastures during 2013 - 14.**

| | | Spring 2013 | | | | | | | | Summer 2013 - 2014 | | | | | | | | Autumn 2014 | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | PRG% | | WC% | | Other% | | Dead% | | PRG% | | WC% | | Other% | | Dead% | | PRG% | | WC% | | Other% | | Dead% | |
| Nitrogen treatment | High | 92.0 | | 2.9 | | 2.7 | | 2.5 | | 84.8 | | 5.9 | | 2.6 | | 6.7 | | 90.1 | | 3.0 | | 0.7 | | 6.3 | |
| | Low | 88.0 | | 5.8 | | 3.1 | | 3.2 | | 70.7 | | 18.3 | | 7.1 | | 3.8 | | 84.6 | | 9.3 | | 1.8 | | 4.3 | |
| Clover treatment | + clover | 85.6 | | 8.4 | | 3.2 | | 2.8 | | 69.2 | | 24.1 | | 2.8 | | 3.9 | | 82.3 | | 11.8 | | 0.5 | | 5.3 | |
| | - clover | 94.3 | | 0.3 | | 2.5 | | 2.9 | | 86.3 | | 0.2 | | 6.9 | | 6.6 | | 92.4 | | 0.5 | | 1.9 | | 5.3 | |
| SED | | 1.5 | | 1.2 | | 0.9 | | 0.2 | | 2.1 | | 1.5 | | 1.0 | | 0.7 | | 1.1 | | 0.6 | | 0.3 | | 1.1 | |
| Perennial ryegrass cultivar | Abermagic AR1 | 90.4 | ab | 4.7 | a | 3.2 | | 1.6 | d | 75.0 | bc | 12.1 | abc | 8.0 | | 4.9 | bcde | 90.0 | a | 5.6 | | 0.9 | | 3.6 | b |
| | Alto AR37 | 89.3 | abc | 4.3 | a | 3.2 | | 3.3 | ab | 78.2 | abc | 13.0 | a | 4.5 | | 4.3 | cde | 85.9 | c | 7.0 | | 1.3 | | 5.8 | a |
| | Base AR37 | 90.5 | ab | 4.1 | a | 3.3 | | 2.2 | cd | 81.7 | a | 9.4 | bc | 5.2 | | 3.8 | e | 90.3 | a | 5.2 | | 1.1 | | 3.3 | b |
| | Bealey NEA2 | 87.3 | c | 6.3 | a | 3.6 | | 2.7 | bc | 78.6 | abc | 13.4 | ab | 3.8 | | 4.1 | de | 89.2 | ab | 5.6 | | 1.8 | | 3.4 | b |
| | Commando AR37 | 91.2 | ab | 4.2 | a | 2.5 | | 2.1 | cd | 72.9 | c | 15.6 | a | 5.5 | | 6.0 | abc | 85.5 | c | 7.3 | | 1.2 | | 6.1 | a |
| | Kamo AR37 | 90.8 | a | 5.4 | a | 1.7 | | 2.0 | cd | 73.8 | bc | 15.2 | a | 3.9 | | 7.0 | a | 84.5 | c | 6.5 | | 1.5 | | 7.4 | a |
| | One50 AR37 | 88.5 | bc | 3.9 | a | 3.3 | | 4.3 | a | 79.5 | ab | 10.2 | abc | 4.9 | | 5.3 | abcd | 86.8 | bc | 5.5 | | 1.2 | | 6.5 | a |
| | Prospect AR37 | 91.8 | a | 1.7 | b | 2.0 | | 4.4 | a | 82.3 | a | 8.1 | c | 2.9 | | 6.7 | ab | 86.6 | bc | 6.3 | | 0.7 | | 6.4 | a |
| SED | | 1.4 | | 1.1 | | 1.0 | | 0.5 | | 3.3 | | 2.3 | | 2.1 | | 1.0 | | 1.6 | | 1.2 | | 0.6 | | 1.0 | |
| N x Clover treatment | High N + clover | 87.8 | | 5.7 | | 3.7 | | 2.8 | | 79.7 | b | 11.7 | b | 2.9 | b | 5.7 | | 86.1 | | 6.0 | b | 0.7 | b | 7.2 | |
| | High N - clover | 96.2 | | 0.0 | | 1.6 | | 2.2 | | 90.0 | a | 0.1 | c | 2.2 | b | 7.7 | | 94.0 | | 0.1 | d | 0.6 | b | 5.3 | |
| | Low N + clover | 83.5 | | 11.0 | | 2.6 | | 2.8 | | 58.8 | c | 36.4 | a | 2.6 | b | 2.2 | | 78.6 | | 17.6 | a | 0.3 | b | 3.5 | |
| | Low N - clover | 92.5 | | 0.5 | | 3.5 | | 3.5 | | 82.6 | b | 0.3 | c | 11.6 | a | 5.5 | | 90.7 | | 0.9 | c | 3.2 | a | 5.2 | |
| | | 2.1 | | 1.6 | | 1.3 | | 0.3 | | 2.9 | | 2.1 | | 1.4 | | 1.0 | | 1.5 | | 0.9 | | 0.4 | | 1.5 | |
| *P* value | N effect | < 0.05 | | < 0.01 | | 0.616 | | < 0.05 | | < 0.001 | | < 0.001 | | < 0.01 | | < 0.05 | | < 0.001 | | < 0.001 | | < 0.05 | | 0.078 | |
| | Clover effect | < 0.001 | | < 0.001 | | 0.760 | | 0.832 | | < 0.001 | | < 0.001 | | < 0.01 | | < 0.001 | | < 0.001 | | < 0.001 | | < 0.001 | | 0.923 | |
| | Cultivar effect | < 0.05 | | < 0.01 | | 0.631 | | < 0.001 | | < 0.05 | | < 0.01 | | 0.438 | | < 0.01 | | < 0.001 | | 0.608 | | 0.514 | | < 0.001 | |
| | N x Clover interaction | 0.629 | | 0.228 | | 0.103 | | 0.059 | | < 0.05 | | < 0.001 | | < 0.01 | | 0.091 | | 0.389 | | < 0.001 | | < 0.001 | | 0.060 | |
| | N x Cultivar interaction | 0.585 | | 0.128 | | 0.698 | | 0.435 | | 0.786 | | 0.624 | | 0.626 | | 0.191 | | 0.727 | | 0.244 | | 0.212 | | 0.177 | |
| | Clover x Cultivar interaction | < 0.01 | | < 0.01 | | 0.286 | | < 0.05 | | 0.421 | | < 0.05 | | 0.819 | | 0.269 | | 0.793 | | 0.154 | | 0.325 | | 0.830 | |

SED = standard error of the difference between means. Different letters within a column indicate statistical differences. In this table % and SED are from the analysis without angular transformation, but *P* value and letters are from the analysis with angular transformation.

# References


Ammerman, J. (2003). Determination of phosphorus in 0.5 M sodium bicarbonate soil extracts by flow injection analysis. Lachat Instruments, USA.

Anslow, R. C. (1965). Grass growth in midsummer. *Grass and Forage Science, 20*(1), 19-26. doi:10.1111/j.1365-2494.1965.tb00391.x

Assuero, S. G., & Tognetti, J. A. (2010). Tillering regulation by endogenous and environmental factors and its agricultural management. *The Americas Journal of Plant Science and Biotechnology, 4*, 35-48.

Bahmani, I. (1999). *Tiller dynamics and leaf growth processes of the perennial ryegrass cultivars 'Ellett'and 'Grasslands Ruanui' as influenced by environmental factors.* (Doctoral thesis), Massey University, Palmerston North, New Zealand, and l'Institut National Polytechnique de Lorraine, Nancy, France.

Bahmani, I., Thom, E. R., Matthew, C., Hooper, R. J., & Lemaire, G. (2003). Tiller dynamics of perennial ryegrass cultivars derived from different New Zealand ecotypes: effects of cultivar, season, nitrogen fertiliser, and irrigation. *Australian Journal of Agricultural Research, 54*(8), 803-817. doi:10.1071/ar02135

Ball, P. R. (1969). Legume and fertilizer nitrogen in New Zealand pastoral farming. *Proceedings of the New Zealand Grassland Association, 31*, 117-126.

Ball, P. R., & Field, T. R. O. (1982). Responses to nitrogen as affected by pasture characteristics, season and grazing management. In P. B. Lynch (Ed.), *Nitrogen fertilisers in New Zealand agriculture* (pp. 45-64). Wellington, New Zealand: NZIAS.

Ball, P. R., & Field, T. R. O. (1985). Productivity and economics of legume-based pastures and grass swards receiving fertiliser nitrogen in New Zealand. In R. F. Barnes, P. R. Ball, R. W. Brougham, G. C. Marten, & D. J. Minson (Eds.), *Forage legumes for energy-efficient animal production* (pp. 47-55). Springfield, VA: United States Department of Agriculture, Australian Commonwealth Scientific and Industrial Research Organization, New Zealand Department of Scientific and Industrial Research. .

Ball, P. R., Molloy, L. F., & Ross, D. J. (1978). Influence of fertiliser nitrogen on herbage dry matter and nitrogen yields, and botanical composition of a grazed grass-clover pasture. *New Zealand Journal of Agricultural Research, 21*(1), 47-55.

Ballare, C. L., & Casal, J. J. (2000). Light signals perceived by crop and weed plants. *Field Crops Research, 67*(2), 149-160. doi:10.1016/s0378-4290(00)00090-3

Beecher, M., Hennessy, D., Boland, T. M., McEvoy, M., O'Donovan, M., & Lewis, E. (2015). The variation in morphology of perennial ryegrass cultivars throughout the grazing season and effects on organic matter digestibility. *Grass and Forage Science, 70*(1), 19-29. doi:10.1111/gfs.12081

Begon, M., Harper, J. L., & Townsend, C. R. (1986). *Ecology: Individuals, populations and communities*. Oxford, United Kingdom: Blackwell Scientific Publications.

Black, A. D., Laidlaw, A. S., Moot, D. J., & O'Kiely, P. (2009). Comparative growth and management of white and red clovers. *Irish Journal of Agricultural and Food Research, 48*(2), 149-166.

Blackman, G. E. (1938). The interaction of light intensity and nitrogen supply in the growth and metabolism of grasses and clover (*Trifolium repens*). I. The effects of light intensity and nitrogen supply on the clover content of a sward. *Annals of Botany, 2*, 257-280.

Blakemore, L. C., Searle, P. L., & Daly, B. K. (1987). *Methods for chemical analysis of soils. NZ Soil Bureau Scientific Report 80.* ([Rev. ed.].. ed.). Lower Hutt, New Zealand: NZ Soil Bureau, Dept. of Scientific and Industrial Research.

Bluett, S. J., & Macdonald, K. A. (2002). Managing dairy calves using continuous stocking and sward surface height - a review. *Proceedings of the New Zealand Society of Animal Production, 62*, 124-127.

Boller, B. C., & Nosberger, J. (1985). Photosynthesis of white clover leaves as influenced by canopy position, leaf age, and temperature. *Annals of Botany, 56*(1), 19-27.

Brereton, A. J., Carton, O. T., & Conway, A. (1985). The effect of grass tiller density on the performance of white clover. *Proceedings of the XV International Grassland Congress*, 756-757.

Brock, J. L., Albrecht, K. A., Tilbrook, J. C., & Hay, M. J. M. (2000). Morphology of white clover during development from seed to clonal populations in grazed pastures. *Journal of Agricultural Science, 135*, 103-111. doi:10.1017/s0021859699008060

Brock, J. L., & Hay, M. J. M. (2001). White clover performance in sown pastures: a biological/ecological perspective. *Proceedings of the New Zealand Grassland Association, 63*, 73-83.

Brougham, R. W. (1958). Interception of light by the foliage of pure and mixed stands of pasture plants. *Australian Journal of Agricultural Research, 9*(1), 39-52. doi:doi:http://dx.doi.org/10.1071/AR9580039

Brougham, R. W. (1959). The effects of season and weather on the growth rate of a ryegrass clover pasture. *New Zealand Journal of Agricultural Research, 2*(2), 283-296.

Burchill, W., James, E. K., Li, D., Lanigan, G. J., Williams, M., Iannetta, P. P. M., & Humphreys, J. (2014). Comparisons of biological nitrogen fixation in association with white clover (*Trifolium repens* L.) under four fertiliser nitrogen inputs as measured using two N-15 techniques. *Plant and Soil, 385*(1-2), 287-302. doi:10.1007/s11104-014-2199-1

Burns, G. A., Gilliland, T. J., Grogan, D., Watson, S., & O'Kiely, P. (2013). Assessment of herbage yield and quality traits of perennial ryegrasses from a national variety evaluation scheme. *The Journal of Agricultural Science, 151*(03), 331-346. doi:doi:10.1017/S0021859612000251

Camlin, M. S. (1981). Competitive effects between ten cultivars of perennial ryegrass and three cultivars of white clover grown in association. *Grass and Forage Science, 36*(3), 169-178. doi:10.1111/j.1365-2494.1981.tb01553.x

Caradus, J. R. (1990). The structure and function of white clover root systems. *Advances in Agronomy, 43*, 1-46. doi:http://dx.doi.org/10.1016/S0065-2113(08)60475-7

Caradus, J. R., & Chapman, D. F. (1991). Variability of stolon characteristics and response to shading in two cultivars of white clover (*Trifolium repens* L). *New Zealand Journal of Agricultural Research, 34*(3), 239-247.

Caradus, J. R., Harris, S. L., & Johnson, R. J. (1996). Increased clover content for increased milk production. *Proceedings of the 48th Ruakura Farmers' Conference, Hamilton, New Zealand*, 42-49.

Caradus, J. R., Hay, R. J. M., & Woodfield, D. R. (1996). The positioning of white clover cultivars in New Zealand. *Agronomy Society of New Zealand Special Publication, 11*, 45-49.

Caradus, J. R., & Mackay, A. C. (1991). Performance of white clover cultivars and breeding lines in a mixed species sward. 2. Plant characters contributing to differences in clover proportion in swards. *New Zealand Journal of Agricultural Research, 34*(2), 155-160.

Caradus, J. R., Pinxterhuis, J. B. I., Hay, R. J. M., Lyons, T., & Hoglund, J. H. (1993). Response of white clover cultivars to fertilizer nitrogen. *New Zealand Journal of Agricultural Research, 36*(3), 285-295.

Caradus, J. R., & Williams, W. M. (1989). Breeding for legume persistence in New Zealand. *Proceedings of a trilateral workshop, Honolulu, Hawaii, 18-22 July 1988.*, 523-539.

Caradus, J. R., Woodfield, D. R., & Stewart, A. V. (1996). Overview and vision for white clover. *Agronomy Society of New Zealand Special Publication, 11*, 1-6.

Casal, J. J., Deregibus, V. A., & Sanchez, R. A. (1985). Variations in tiller dynamics and morphology in *Lolium multiflorum* Lam. vegetative and reproductive plants as affected by differences in red far-red irradiation. *Annals of Botany, 56*(4), 553-559.

Casal, J. J., Sanchez, R. A., & Deregibus, V. A. (1987). Tillering responses of *Lolium multiflorum* plants to changes of red far-red ratio typical of sparse canopies. *Journal of Experimental Botany, 38*(194), 1432-1439. doi:10.1093/jxb/38.9.1432

Chapman, D. F., Bryant, J. R., McMillan, W. H., & Khaembah, E. N. (2012). Economic values for evaluating pasture plant traits. *Proceedings of the New Zealand Grassland Association, 74*, 209-216.

Chapman, D. F., Bryant, J. R., Olayemi, M. E., Edwards, G. R., Thorrold, B. S., McMillan, W. H., . . . Norriss, M. (2016). An economically based evaluation index for perennial and short-term ryegrasses in New Zealand dairy farm systems. *Grass and Forage Science*, 1-21. doi:10.1111/gfs.12213

Chapman, D. F., Edwards, G. R., Stewart, A. V., McEvoy, M., O'Donovan, M., & Waghorn, G. C. (2015). Valuing forages for genetic selection: what traits should we focus on? *Animal Production Science, 55*(7), 869-882. doi:10.1071/an14838

Chapman, D. F., Edwards, G. R., Stewart, A. V., McEvoy, M., O’Donovan, M., & Waghorn, G. C. (2014). Valuing forages for genetic selection: what traits should we focus on? *Proceedings of the 5th Australasian Dairy Science Symposium*, 189-205.

Chapman, D. F., & Lemaire, G. (1993). Morphogenetic and structural determinants of plant regrowth after defoliation. *Proceedings of the XVII International Grassland Congress*, 95-104.

Chapman, D. F., Muir, P. D., & Faville, M. J. (2015). Persistence of dry matter yield among New Zealand perennial ryegrass (*Lolium perenne* L.) cultivars: insights from a long-term data set. *Journal of New Zealand Grasslands, 77*, 177-184.

Chapman, D. F., Parsons, A. J., Cosgrove, G. P., Barker, D. J., Marotti, D. M., Venning, K. J., . . . Thompson, A. N. (2007). Impacts of spatial patterns in pasture on animal grazing behavior, intake, and performance. *Crop Science, 47*(1), 399-415. doi:10.2135/cropsci2006.01.0036

Chapman, D. F., Parsons, A. J., & Schwinning, S. (1996). Management of clover in grazed pastures: expectations, limitations and opportunities. *Agronomy Society of New Zealand Special Publication, 11*, 55-64.

Chesson, J. (1983). The estimation and analysis of preference and its relatioship to foraging models. *Ecology, 64*(5), 1297-1304. doi:10.2307/1937838

Chestnutt, D. M. B., & Lowe, J. (1970). White clover/grass relationships: agronomy of white clover/grass swards: a review. *Occasional Symposium 6 of the British Grassland Society*, 191-213.

Clark, D. A. (2011). Changes in pastoral farming practices and pasture persistence - a review. *Grassland Research and Practice Series*(15), 7-14.

Clark, D. A., Caradus, J. R., Monaghan, R. M., Sharp, P., & Thorrold, B. S. (2007). Issues and options for future dairy farming in New Zealand. *New Zealand Journal of Agricultural Research, 50*(2), 203-221.

Clark, D. A., & Harris, S. L. (1996). White clover or nitrogen fertiliser for dairying? *Agronomy Society of New Zealand Special Publication, 11*, 107-114.

Clark, D. A., Matthew, C., & Crush, J. R. (2001). More feed for New Zealand dairy systems. *Proceedings of the New Zealand Grassland Association, 63*, 283-288.

Clement, A. R., Dalley, D. E., Chapman, D. F., Edwards, G. R., & Bryant, R. H. (2016). Effect of grazing system on nitrogen partitioning in lactating dairy cows grazing irrigated pastures in Canterbury, New Zealand. *Proceedings of the New Zealand Society of Animal Production, 76*, 94-99.

Collins, R. P., Fothergill, M., MacDuff, J., & Rhodes, I. (1997). The basis of perennial ryegrass/white clover interactions in establishing swards. *British Grassland Society Fifth Research Conference*, 77-78.

Collins, R. P., Fothergill, M., Macduff, J. H., & Puzio, S. (2003). Morphological compatibility of white clover and perennial ryegrass cultivars grown under two nitrate levels in flowing solution culture. *Annals of Botany, 92*(2), 247-258. doi:10.1093/aob/mcg128

Collins, R. P., Fothergill, M., & Rhodes, I. (1996). Interactions between seedlings of perennial ryegrass and white clover cultivars in establishing swards. *Grass and Forage Science, 51*(2), 163-169. doi:10.1111/j.1365-2494.1996.tb02050.x

Collins, R. P., & Rhodes, I. (1989). Yield of white clover populations in mixture with contrasting perennial ryegrasses. *Grass and Forage Science, 44*(1), 111-115. doi:10.1111/j.1365-2494.1989.tb01918.x

Connolly, V. (1968). A comparison of six white clover and three perennial ryegrass varieties under a system of grazing management. *Irish Journal of Agricultural Research, 7*(2), 227-242.

Cornforth, I. S. (1980). Soils and fertilisers: soil analysis: interpretation. Ministry of Agriculture and Fisheries, AgLink AST 8.
Corson, D. C., Waghorn, G. C., Ulyatt, M. J., & Lee, J. (1999). NIRS: forage analysis and livestock feeding. *Proceedings of the New Zealand Grassland Association, 61*, 127-132.
Cosgrove, G. P., Mapp, N. R., Taylor, P. S., Harvey, B. M., & Knowler, K. J. (2014). The chemical composition of high-sugar and control ryegrasses in grazed pastures at different latitudes throughout New Zealand. *Proceedings of the New Zealand Grassland Association, 76*, 169-175.
Cougnon, M., Baert, J., Waes, C. v., & Reheul, D. (2012). Effect of grass species and ploidy on clover content in grass-clover mixtures. *Grassland Science in Europe, 17*, 100-102.
Cowling, D. W. (1961). The effect of nitrogenous fertilizer on an established white clover sward. *Journal of the British Grassland Society, 16*(1), 65-68.
Cowling, D. W., & Lockyer, D. R. (1965). A comparison of the reaction of different grass species to fertilizer nitrogen and to growth in association with white clover. *Grass and Forage Science, 20*(3), 197-204.
Cresswell, A., Hamilton, N. R. S., Thomas, H., Charnock, R. B., Cookson, A. R., & Thomas, B. J. (1999). Evidence for root contraction in white clover (*Trifolium repens* L.). *Annals of Botany, 84*(3), 359-369. doi:10.1006/anbo.1999.0928
Crush, J. R., Cosgrove, G. P., & Brougham, R. W. (1982). The effect of nitrogen-fertilizer on clover nitrogen-fixation in an intensively grazed Manawatu pasture. *New Zealand Journal of Experimental Agriculture, 10*(4), 395-399.
DairyNZ. Forage Value Index. Retrieved from http://www.dairynz.co.nz/feed/pasture-renewal/select-pasture-species/about-fvi/
DairyNZ, Dairy Companies Association of New Zealand, & Federated Farmers. (2009). Strategy for New Zealand Dairy Farming 2009/2020. Retrieved from http://www.dairynz.co.nz/file/fileid/28814
DairyNZ, Federated Farmers, Dairy Companies Association of New Zealand, & Dairy Women's Network. (2013). Making dairy farming work for everyone - Strategy for Sustainable Dairy Farming 2013-2020 Retrieved from http://www.dairynz.co.nz/
DairyNZ, & New Zealand Plant Breeding and Research Association. (2012). 2011 Forage review: The future for forage improvement to support New Zealand's dairy industry and the wider pastoral farming sector. Retrieved from http://www.dairynz.co.nz/page/pageid/2145866515?resourceId=723
Davidson, I. A., & Robson, M. J. (1986). Effect of temperature and nitrogen supply on the growth of perennial ryegrass and white clover. 2. A comparison of monocultures and mixed swards. *Annals of Botany, 57*(5), 709-719.
Davidson, I. A., Robson, M. J., & Dennis, W. D. (1982). The effect of nitrogenous fertilizer on the composition, canopy structure and growth of a mixed grass clover sward. *Grass and Forage Science, 37*(2), 178-179.
Davies, A. (1971). Changes in growth rate and morphology of perennial ryegrass swards at high and low nitrogen levels. *Journal of Agricultural Science, 77*(1), 123-134.
Davies, A. (1978). Structure of the grass sward. *Proceedings of the International Meeting on Animal Production from Temperate Grassland, Dublin, Ireland, June 1977.*, 36-44.
Davies, A. (1989). The structure of the grass/clover sward and its implication in sward management. *Proceedings of the XVI International Grassland Congress*, 1065-1066.
Davies, A. (1992). White clover. *Biologist, 39*(4), 129-133.
Davies, A., & Evans, M. E. (1990). Effects of spring defoliation and fertilizer nitrogen on the growth of white clover in ryegrass clover swards. *Grass and Forage Science, 45*(4), 345-356. doi:10.1111/j.1365-2494.1990.tb01959.x
Davies, A., & Thomas, H. (1983). Rates of leaf and tiller production in young spaced perennial ryegrass plants in relation to soil-temperature and solar-radiation. *Annals of Botany, 51*(5), 591-597.

Davies, D. A., & Fothergill, M. (1990). Productivity and persistence of white clover grown with three perennial ryegrass varieties and continuously stocked with sheep. *Proceedings of the 13th General Meeting of the European Grassland Federation*, 157-162.

Davies, D. A., Fothergill, M., & Morgan, C. T. (1993). Assessment of contrasting perennial ryegrasses, with and without white clover, under continuous sheep stocking in the uplands. 5. Herbage production, quality and intake in years 4-6. *Grass and Forage Science, 48*(3), 213-222. doi:10.1111/j.1365-2494.1993.tb01854.x

de Wit, C. T. (1960). On competition. *Verslagen van landbouwkundige onderzoekingen*(66), 1-82.

Dennis, W. D., & Woledge, J. (1982). Photosynthesis by white clover leaves in mixed clover/ryegrass swards. *Annals of Botany, 49*(5), 627-635.

Dennis, W. D., & Woledge, J. (1983). The effect of shade during leaf expansion on photosynthesis by white clover leaves. *Annals of Botany, 51*(1), 111-118.

Dennis, W. D., & Woledge, J. (1985). The effect of nitrogenous fertilizer on the photosynthesis and growth of white clover / perennial ryegrass swards. *Annals of Botany, 55*(2), 171-178.

Dennis, W. D., & Woledge, J. (1987). The effect of nitrogen in spring on shoot number and leaf-area of white clover in mixtures. *Grass and Forage Science, 42*(3), 265-269. doi:10.1111/j.1365-2494.1987.tb02115.x

Deregibus, V. A., Sanchez, R. A., & Casal, J. J. (1983). Effects of light quality on tiller production in *Lolium* spp. *Plant Physiology, 72*(3), 900-902. doi:10.1104/pp.72.3.900

Dillon, P., Roche, J. R., Shalloo, L., & Horan, B. (2005). Optimising financial return from grazing in temperate pastures. *Proceedings of a satellite workshop at the XX International Grassland Congress, Cork, Ireland, July 2005*, 131-147.

Donaghy, D. J. (1998). *Improving the production and persistence of temperate pasture species in subtropical dairy regions of Australia.* (Doctoral thesis), University of New England, Armidale, Australia.

Donald, C. M. (1963). Competition among crop and pasture plants. *Advances in Agronomy, 15*, 1-118. doi:http://dx.doi.org/10.1016/S0065-2113(08)60397-1

Donald, C. M. (1968). The breeding of crop ideotypes. *Euphytica, 17*, 385-403. doi:10.1007/bf00056241

Easton, H. S., Amyes, J. M., Cameron, N. E., Green, R. B., Kerr, G. A., Norriss, M. G., & Stewart, A. V. (2002). Pasture plant breeding in New Zealand: where to from here? *Proceedings of the New Zealand Grassland Association, 64*, 173-179.

Easton, H. S., Baird, D., Baxter, G., Cameron, N. E., Hainsworth, R., Johnston, C., . . . Nichol, W. (1997). Annual and hybrid ryegrass cultivars in New Zealand. *Proceedings of the New Zealand Grassland Associarion, 59*, 239-244.

Easton, H. S., Baird, D. B., Cameron, N. E., Kerr, G. A., Norriss, M., & Stewart, A. V. (2001). Perennial ryegrass cultivars: herbage yield in multi-site plot trials. *Proceedings of the New Zealand Grassland Association, 63*, 183-188.

Edmeades, D. C., & O'Connor, M. B. (2003). Sodium requirements for temperate pastures in New Zealand: a review. *New Zealand Journal of Agricultural Research, 46*(1), 37-47.

Edmeades, D. C., & Perrott, K. W. (2004). The calcium requirements of pastures in New Zealand: A review. *New Zealand Journal of Agricultural Research, 47*(1), 11-21. doi:10.1080/00288233.2004.9513566

Edwards, G. R., & Chapman, D. F. (2011). Plant responses to defoliation and relationships with pasture persistence. *Grassland Research and Practice Series*(15), 39-46.

Edwards, G. R., Parsons, A. J., Rasmussen, S., & Bryant, R. H. (2007). High sugar ryegrasses for livestock systems in New Zealand. *Proceedings of the New Zealand Grassland Association, 69*, 161-171.

Egan, M. J. (2015). *Strategies to increase white clover in intensive dairy production systems.* (Unpublished Doctoral thesis), University College Dublin, Dublin, Ireland.

Egan, M. J., Lynch, M. B., & Hennessy, D. (2015). Herbage and milk production from a grass-only sward and grass-white clover swards in an intensive grass-based system. *Proceedings of the 18th Symposium of the European Grassland Federation*, 93-95.

Elgersma, A., & Li, F. R. (1997). Effects of cultivar and cutting frequency on dynamics of stolon growth and leaf appearance in white clover grown in mixed swards. *Grass and Forage Science, 52*(4), 370-380. doi:10.1111/j.1365-2494.1997.tb02369.x

Elgersma, A., Nassiri, M., & Schlepers, H. (1998). Competition in perennial ryegrass white clover mixtures under cutting. 1. Dry-matter yield, species composition and nitrogen fixation. *Grass and Forage Science, 53*(4), 353-366.

Elgersma, A., & Schlepers, H. (1997a). Cattle production and botanical composition in continuously stocked grass-clover swards. *Proceedings of the XVIII International Grassland Congress, 2*, 87-88.

Elgersma, A., & Schlepers, H. (1997b). Performance of white clover perennial ryegrass mixtures under cutting. *Grass and Forage Science, 52*(2), 134-146. doi:10.1046/j.1365-2494.1997.00047.x

Enriquez-Hidalgo, D., Gilliland, T. J., & Hennessy, D. (2015). Herbage production in grazed grass-white clover plots: effect of N fertilizer application. *Proceedings of the 18th Symposium of the European Grassland Federation*, 404-406.

Enriquez-Hidalgo, D., Gilliland, T. J., & Hennessy, D. (2016). Herbage and nitrogen yields, fixation and transfer by white clover to companion grasses in grazed swards under different rates of nitrogen fertilization. *Grass and Forage Science, 71*(4), 559-574. doi:10.1111/gfs.12201

Ettema, P. J., & Ledgard, S. F. (1992). Getting the best out of white clover. *Proceedings of the 44th Ruakura Farmers' Conference, Hamilton, New Zealand*, 72-76.

Evans, D. R., Hill, J., Williams, T. A., & Rhodes, I. (1985). Effects of coexistence on the performance of white clover perennial ryegrass mixtures. *Oecologia, 66*(4), 536-539. doi:10.1007/bf00379346

Evans, P. S. (1977). Comparative root morphology of some pasture grasses and clovers. *New Zealand Journal of Agricultural Research, 20*(3), 331-335.

Faurie, O., Soussana, J. F., & Sinoquet, H. (1996). Radiation interception, partitioning and use in grass clover mixtures. *Annals of Botany, 77*(1), 35-45. doi:10.1006/anbo.1996.0005

Feyter, C., O'Connor, M. B., & Addison, B. (1985). Effects of rates and times of nitrogen application on the production and composition of dairy pastures in Waikato district, New Zealand. *New Zealand Journal of Experimental Agriculture, 13*(3), 247-252.

Frame, J., & Boyd, A. G. (1986a). Effect of cultivar and seed rate of perennial ryegrass and strategic fertilizer nitrogen on the productivity of grass white clover swards. *Grass and Forage Science, 41*(4), 359-366. doi:10.1111/j.1365-2494.1986.tb01826.x

Frame, J., & Boyd, A. G. (1986b). Response of white clover to strategic fertiliser N in spring and/or autumn applied to a grass/white clover sward. *British Grassland Society occasional symposium, 20*, 92-93.

Frame, J., & Boyd, A. G. (1987a). The effect of fertilizer nitrogen rate, white clover variety and closeness of cutting on herbage productivity from perennial ryegrass white clover swards. *Grass and Forage Science, 42*(1), 85-96. doi:10.1111/j.1365-2494.1987.tb02094.x

Frame, J., & Boyd, A. G. (1987b). The effect of strategic use of fertilizer nitrogen in spring and or autumn on the productivity of a perennial ryegrass white clover sward. *Grass and Forage Science, 42*(4), 429-438. doi:10.1111/j.1365-2494.1987.tb02133.x

Frame, J., & Harkess, R. D. (1987). The productivity of four forage legumes sown alone and with each of five companion grasses. *Grass and Forage Science, 42*(3), 213-223. doi:10.1111/j.1365-2494.1987.tb02109.x

Frame, J., & Laidlaw, A. S. (1998). Managing white clover in mixed swards: principles and practice. *Pastos, 28*(1), 5-33.

Frame, J., & Newbould, P. (1986). Agronomy of white clover. *Advances in Agronomy, 40*, 1-88. doi:10.1016/s0065-2113(08)60280-1

Gilliland, T. J. (1996). Assessment of perennial ryegrass variety compatibility with white clover under grazing. *Plant Varieties and Seeds, 9*(2), 65-75.

Gilliland, T. J., Barrett, P. D., Mann, R. L., Agnew, R. E., & Fearon, A. M. (2002). Canopy morphology and nutritional quality traits as potential grazing value indicators for *Lolium perenne* varieties. *Journal of Agricultural Science, 139*, 257-273. doi:10.1017/s0021859602002575

Glassey, C. B., Roach, C. G., Lee, J. M., & Clark, D. A. (2013). The impact of farming without nitrogen fertiliser for ten years on pasture yield and composition, milksolids production and profitability; a research farmlet comparison. *Proceedings of the New Zealand Grassland Association, 75*, 71-78.

Gooding, R. F., Frame, J., & Thomas, C. (1996). Effects of sward type and rest periods from sheep grazing on white clover presence in perennial ryegrass white clover associations. *Grass and Forage Science, 51*(2), 180-189. doi:10.1111/j.1365-2494.1996.tb02052.x

Gowen, N., O'Donovan, M., Casey, I., Rath, M., Delaby, L., & Stakelum, G. (2003). The effect of grass cultivars differing in heading date and ploidy on the performance and dry matter intake of spring calving dairy cows at pasture. *Animal Research, 52*(4), 321-336. doi:10.1051/animres:2003025

Grace, J. B. (1990). On the relationship between plant traits and competitive ability. In J. B. Grace & D. Tilman (Eds.), *Perspectives on plant competition.* (pp. 51-65). San Diego, CA: Academic Press

Griffiths, W. M., Matthew, C., Lee, J. M., & Chapman, D. F. (2016). Is there a tiller morphology ideotype for yield differences in perennial ryegrass (*Lolium perenne* L.)? *Grass and Forage Science*, 1-14. doi:10.1111/gfs.12268

Grime, J. P. (1973). Competitive exclusion in herbaceous vegetation. *Nature, 242*(5396), 344-347.

Grime, J. P. (1974). Vegetation classification by reference to strategies. *Nature, 250*(5461), 26-31.

Harmer, M., Stewart, A. V., & Woodfield, D. R. (2016). Genetic gain in perennial ryegrass forage yield in Australia and New Zealand. *Journal of New Zealand Grasslands, 78*, 133-138.

Harris, S. L. (1998). White clover-How much and how to get it. *Proceedings of the 50th Ruakura Farmers' Conference, Hamilton, New Zealand*, 73-79.

Harris, S. L., Auldist, M. J., Clark, D. A., & Jansen, E. B. L. (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*(3), 389-400. doi:10.1017/s0022029998002969

Harris, S. L., & Clark, D. A. (1996). Effect of high rates of nitrogen fertiliser on white clover growth, morphology, and nitrogen fixation activity in grazed dairy pasture in northern New Zealand. *New Zealand Journal of Agricultural Research, 39*(1), 149-158.

Harris, S. L., Clark, D. A., Auldist, M. J., Waugh, C. D., & Laboyrie, P. G. (1997). Optimum white clover content for dairy pastures. *Proceedings of the New Zealand Grassland Association, 59*, 29-33.

Harris, S. L., Clark, D. A., Waugh, C. D., & Clarkson, F. H. (1996). Nitrogen fertiliser effects on white clover in dairy pastures. *Agronomy Society of New Zealand Special Publication, 11*, 119-124.

Harris, S. L., Thom, E. R., & Clark, D. A. (1996). Effect of high rates of nitrogen fertiliser on perennial ryegrass growth and morphology in grazed daily pasture in northern New Zealand. *New Zealand Journal of Agricultural Research, 39*(1), 159-169.

Harris, W. (1977). An approach to evaluate a large number of mixtures under grazing. *Proceedings of the XIII International Grassland Congress*, 401-411.

Harris, W. (1987). Population dynamics and competition. In M. J. Baker & W. M. Williams (Eds.), *White clover.* (pp. 203-297). Wallingford, United Kingdom: C.A.B. International.

Harris, W. (1990). Pasture as an ecosystem. In R. H. M. Langer (Ed.), *Pastures - their ecology and management* (pp. 75-131). Auckland, New Zealand: Oxford University Press.

Harris, W. (2001). Formulation of pasture seed mixtures with reference to competition and succession in pastures. In P. G. Tow & A. Lazenby (Eds.), *Competition and Succession in Pastures* (pp. 149-174). Wallingford, United Kingdom: CAB International.

Harris, W., & Hoglund, J. H. (1977). Influence of seasonal growth periodicity and N-fixation on competitive combining abilities of grasses and legumes. *Proceedings of the XIII International Grassland Congress*, 138-149.

Harris, W., & Thomas, V. J. (1973). Competition among pasture plants III. Effects of frequency and height of cutting on competition between white clover and two ryegrass cultivars. *New Zealand Journal of Agricultural Research, 16*(1), 49-58.

Hart, A. L. (1987). Physiology. In M. J. Baker & W. M. Williams (Eds.), *White clover.* (pp. 125-151). Wallingford, United Kingdom: C.A.B. International.

Hay, R. J. M., & Lancashire, J. A. (1996). Cultivar development and links to industry. *Agronomy Society of New Zealand Special Publication, 11*, 15-18.
Haynes, R. J. (1980). Competitive aspects of the grass-legume association. *Advances in Agronomy, 33*, 227-261.
Hennessy, D., Enriquez-Hidalgo, D., O'Donovan, M., & Gilliland, T. (2012). Effect of N fertiliser application rate on herbage production and sward clover content in grazed grass clover plots. *Proceedings of the 24th General Meeting of the European Grassland Federation*, 124-126.
Heraut-Bron, V., Robin, C., Varlet-Grancher, C., Afif, D., & Guckert, A. (1999). Light quality (red : far-red ratio): does it affect photosynthetic activity, net $CO_2$ assimilation, and morphology of young white clover leaves? *Canadian Journal of Botany, 77*(10), 1425-1431.
Héraut-Bron, V., Robin, C., Varlet-Grancher, C., & Guckert, A. (2001). Phytochrome mediated effects on leaves of white clover: consequences for light interception by the plant under competition for light. *Annals of Botany, 88*(suppl 1), 737-743.
Hewitt, A. E. (2010). *New Zealand soil classification* (3rd ed.). Lincoln, NZ: Manaaki Whenua Press, Landcare Research.
Hill, J., & Michaelsonyeates, T. P. T. (1987). Effects of competition upon the productivity of white clover-perennial ryegrass mixtures - Analysis of and interrelations between characters. *Plant Breeding, 98*(2), 161-170. doi:10.1111/j.1439-0523.1987.tb01110.x
Hoen, K. (1970). Performance in mixed swards of three perennial ryegrass and three white clover varieties at two nitrogen levels. *Irish Journal of Agricultural Research, 9*(2), 215-223.
Hoglind, M., & Frankow-Lindberg, B. (1998). Growing point dynamics and spring growth of white clover in a mixed sward and the effects of nitrogen application. *Grass and Forage Science, 53*(4), 338-345.
Hoglund, J. H., Crush, J. R., Brock, J. L., Ball, P. R., & Carran, R. A. (1979). Nitrogen fixation in pasture. 12. General discussion. *New Zealand Journal of Experimental Agriculture, 7*(1), 45-51.
Hollington, P. A., & Wilman, D. (1985). Effects of white clover and fertilizer nitrogen on clover and grass leaf dimensions, percentage cover and numbers of leaves and tillers. *Journal of Agricultural Science, 104*, 595-607.
Holmes, C. W. (1982). The effect of fertiliser nitrogen on the production of pasture and milk in dairy farmlets: 1971-74. *Proceedings of the New Zealand Grassland Associarion, 43*, 53-57.
Holmes, M. G., & Smith, H. (1977). The function of phytochrome in the natural environment. 2. The influence of vegetation canopies on the spectral energy distribution of natural daylight. *Photochemistry and Photobiology, 25*(6), 539-545. doi:10.1111/j.1751-1097.1977.tb09125.x
Hume, D. E., Ryan, D. L., Cooper, B. M., & Popay, A. J. (2007). Agronomic performance of AR37-infected ryegrass in northern New Zealand. *Proceedings of the New Zealand Grassland Associarion, 69*, 201-205.
Hunt, W. F., & Easton, H. S. (1989). Fifty years of ryegrass research in New Zealand. *Proceedings of the New Zealand Grassland Association, 50*, 11-23.
Hunt, W. F., & Field, T. R. O. (1979). Growth characteristics of perennial ryegrass. *Proceedings of the New Zealand Grassland Association, 40*, 104-113.
Intellectual Property Office of New Zealand. Plant variety rights. Retrieved from https://www.iponz.govt.nz/about-ip/pvr/
Kerr, G. A. (2011). Introduction. *Grassland Research and Practice Series*(15), 1.
Korte, C. J., Watkin, B. R., & Harris, W. (1984). Effects of the timing and intensity of spring grazings on reproductive development, tillering, and herbage production of perennial ryegrass dominant pasture. *New Zealand Journal of Agricultural Research, 27*(2), 135-149.
L'Huillier, P. J., & Thomson, N. A. (1988). Estimation of herbage mass in ryegrass/white clover dairy pastures. *Proceedings of the New Zealand Grassland Association, 49*, 117-122.
Laidlaw, A. S. (1980). The effects of nitrogen fertilizer applied in spring on swards of ryegrass sown with four cultivars of white clover. *Grass and Forage Science, 35*(4), 295-299.
Laidlaw, A. S., & Withers, J. A. (1998). Changes in contribution of white clover to canopy structure in perennial ryegrass/white clover swards in response to N fertilizer. *Grass and Forage Science, 53*(3), 287-291.

Lambert, M. G., & Litherland, A. J. (2000). A practitioner's guide to pasture quality. *Proceedings of the New Zealand Grassland Associarion, 62*, 111-115.
Landcare Research. (2015). S-map: soil database for New Zealand. Retrieved from http://dx.doi.org/10.7931/L1WC7
Langer, R. H. M. (1963). Tillering in herbage grasses. *Herbage Abstracts, 33*(3), 141-148.
Langer, R. H. M. (1973). Growth of grasses and clovers. In R. H. M. Langer (Ed.), *Pastures and pasture plants* (pp. 42-63). Wellington, New Zealand: Reed.
Ledgard, S. F. (1991). Transfer of fixed nitrogen from white clover to associated grasses in swards grazed by dairy cows, estimated using 15N methods. *Plant and Soil, 131*(2), 215-223.
Ledgard, S. F. (2001). Nitrogen cycling in low input legume-based agriculture, with emphasis on legume/grass pastures. *Plant and Soil, 228*(1), 43-59. doi:10.1023/a:1004810620983
Ledgard, S. F., Brier, G. J., & Upsdel, M. P. (1990). Effect of clover cultivar on production and nitrogen fixation in clover-ryegrass swards under dairy cow grazing. *New Zealand Journal of Agricultural Research, 33*(2), 243-249.
Ledgard, S. F., Crush, J. R., & Penno, J. W. (1998). Environmental impacts of different nitrogen inputs on dairy farms and implications for the Resource Management Act of New Zealand. *Environmental Pollution, 102*(1, Supplement 1), 515-519. doi:http://dx.doi.org/10.1016/S0269-7491(98)80077-8
Ledgard, S. F., Penno, J. W., & Sprosen, M. S. (1999). Nitrogen inputs and losses from clover/grass pastures grazed by dairy cows, as affected by nitrogen fertilizer application. *Journal of Agricultural Science, 132*, 215-225. doi:10.1017/s002185969800625x
Ledgard, S. F., Sprosen, M. S., Penno, J. W., & Rajendram, G. S. (2001). Nitrogen fixation by white clover in pastures grazed by dairy cows: Temporal variation and effects of nitrogen fertilization. *Plant and Soil, 229*(2), 177-187. doi:10.1023/a:1004833804002
Ledgard, S. F., Sprosen, M. S., Steele, K. W., & West, C. P. (1995). Productivity of white clover cultivars under intensive grazing, as affected by high nitrogen fertiliser application. *New Zealand Journal of Agricultural Research, 38*(4), 473-482.
Ledgard, S. F., & Steele, K. W. (1992). Biological nitrogen fixation in mixed legume/grass pastures. *Plant and Soil, 141*(1-2), 137-153. doi:10.1007/BF00011314
Lee, J. M., Donaghy, D. J., Sathish, P., & Roche, J. R. (2010). Perennial ryegrass regrowth after defoliation - physiological and molecular changes. *Proceedings of the New Zealand Grassland Association, 72*, 127-134.
Lee, J. M., Matthew, C., Thom, E. R., & Chapman, D. F. (2012). Perennial ryegrass breeding in New Zealand: a dairy industry perspective. *Crop & Pasture Science, 63*(2), 107-127. doi:10.1071/cp11282
Lee, J. M., Thom, E. R., Wynn, K., Waugh, D., Rossi, L., & Chapman, D. F. (2016). High perennial ryegrass seeding rates reduce plant size and survival during the first year after sowing: does this have implications for pasture sward persistence? *Grass and Forage Science*, early view online. doi:10.1111/gfs.12243
Lemaire, G., & Chapman, D. F. (1996). Tissue flows in grazed plant communities. In J. Hodgson & A. W. Illius (Eds.), *The ecology and management of grazing systems* (pp. 3-37). Wallingford, United Kingdom: CAB International.
Leuchtmann, A., Bacon, C. W., Schardl, C. L., White, J. F., Jr., & Tadych, M. (2014). Nomenclatural realignment of *Neotyphodium* species with genus *Epichloe*. *Mycologia, 106*(2), 202-215. doi:10.3852/106.2.202
Litherland, A. J., Webby, R., Fraser, T. J., Matthew, C., McCleod, K., Walcroft, J., . . . Moss, R. (2008). Indirect measurement of pasture mass and pasture growth rate on sheep and beef pastures. *Proceedings of the New Zealand Grassland Association, 70*, 137-144.
Livestock Improvement Corporation Limited, & DairyNZ Limited. (2015). New Zealand Dairy Statistics 2014 - 15. Retrieved from http://www.dairynz.co.nz/publications/dairy-industry/new-zealand-dairy-statistics-2014-15/
Luxmoore, R. J., & Millington, R. J. (1971). Growth of perennial ryegrass ( *Lolium perenne* L.) in relation to water, nitrogen, and light intensity. I. Effects on leaf growth and dry weight. *Plant and Soil, 34*(2), 269-281. doi:10.1007/bf01372784

MacLeod, C. J., & Moller, H. (2006). Intensification and diversification of New Zealand agriculture since 1960: An evaluation of current indicators of land use change. *Agriculture, Ecosystems & Environment, 115*(1–4), 201-218. doi:http://dx.doi.org/10.1016/j.agee.2006.01.003
Manly, B. F. J., Miller, P., & Cook, L. M. (1972). Analysis of a selective predation experiment. *American Naturalist*, 719-736.
Martin, T. W. (1960). The role of white clover in grassland. *Herbage Abstracts, 30*(3), 159-164.
Mather, R. D. J., Melhuish, D. T., & Herlihy, M. (1996). Trends in the global marketing of white clover cultivars. *Agronomy Society of New Zealand Special Publication, 11*, 7-14.
Matthew, C., Assuero, S. G., Black, C. K., & Hamilton, N. R. S. (2000). Tiller dynamics of grazed swards. In G. Lemaire, J. Hodgson, A. d. Moraes, C. Nabinger, & P. C. d. F. Carvalho (Eds.), *Grassland ecophysiology and grazing ecology* (pp. 127-150). Wallingford, United Kingdom: CABI Publishing.
McEvoy, M., O'Donovan, M., & Shalloo, L. (2011). Development and application of an economic ranking index for perennial ryegrass cultivars. *Journal of Dairy Science, 94*(3), 1627-1639. doi:http://dx.doi.org/10.3168/jds.2010-3322
McKenzie, F. R., Jacobs, J. L., & Kearney, G. (2003). Long-term effects of multiple applications of nitrogen fertiliser on grazed dryland perennial ryegrass/white clover dairy pastures in south-west Victoria. 3. Botanical composition, nutritive characteristics, mineral content, and nutrient selection. *Australian Journal of Agricultural Research, 54*(5), 477-485. doi:10.1071/ar02189
Ministry for Primary Industries. (2015). Situation and outlook for primary industries 2015 (SOPI). Retrieved from http://www.mpi.govt.nz/
Mitchell, K. J. (1953a). Influence of light and temperature on the growth of ryegrass ( Lolium spp.) II. The control of lateral bud development. *Physiologia Plantarum, 6*(3), 425-443. doi:10.1111/j.1399-3054.1953.tb08401.x
Mitchell, K. J. (1953b). Influence of light and temperature on the growth of ryegrass ( Lolium spp.). I. Pattern of vegetative development. *Physiologia Plantarum, 6*(1), 21-46. doi:10.1111/j.1399-3054.1953.tb08930.x
Mitchell, K. J. (1956a). Growth of pasture species under controlled environment. I. Growth at various levels of constant temperature. *New Zealand Journal of Science and Technology, Section A, 38*(2), 203-215.
Mitchell, K. J. (1956b). The influence of light and temperature on the growth of pasture species. *Proceedings of the VII International Grassland Congress*, 58-69.
Mitchell, K. J., & Calder, D. M. (1958). The light regime within pastures. *New Zealand Journal of Agricultural Research, 1*(1), 61-68.
Moir, J. L., Cameron, K. C., Di, H. J., Roberts, A. H. C., & Kuperus, W. (2003). The effects of urea and ammonium sulphate nitrate (ASN) on the production and quality of irrigated dairy pastures in Canterbury, New Zealand. In L. D. Currie (Ed.), *Tools for nutrient and pollutant management: Applications to agriculture and environmental quality.* (pp. 139-145): Fertiliser and Lime Research Centre, Massey University, Palmerston North, New Zealand.
Moore, K. J., & Moser, L. E. (1995). Quantifying developmental morphology of perennial grasses. *Crop Science, 35*(1), 37-43.
Moore, K. J., Moser, L. E., Vogel, K. P., Waller, S. S., Johnson, B. E., & Pedersen, J. F. (1991). Describing and quantifying growth-stages of perennial forage grasses. *Agronomy Journal, 83*(6), 1073-1077.
Morgan, W. G. (1976). A technique for the production of polyploids in grasses. *Euphytica, 25*(2), 443-446. doi:10.1007/bf00041577
Nassiri, M., & Elgersma, A. (2002). Effects of nitrogen on leaves, dry matter allocation and regrowth dynamics in *Trifolium repens* L. and *Lolium perenne* L. in pure and mixed swards. *Plant and Soil, 246*(1), 107-121. doi:10.1023/a:1021528732218
National Institute of Water and Atmospheric Research. (2015). CliFlo: The National Climate Database. Retrieved from http://cliflo.niwa.co.nz/

Neuteboom, J. H., Lantinga, E. A., & Wind, K. (1988). Tillering characteristics of diploid and tetraploid perennial ryegrass. *Proceedings of the 12th General Meeting of the European Grassland Federation*, 498-503.
New Zealand Plant Breeding and Research Association Inc. Forage trials. Retrieved from http://www.nzpbra.org/forage-trials/
New Zealand Plant Breeding and Research Association Inc. (2016). National Forage Variety Trials ®. Retrieved May 28, 2016
Nicol, A. M., & Edwards, G. R. (2011). Why is clover better than ryegrass? *Proceedings of the New Zealand Society of Animal Production, 71*, 71-78.
O'Connor, M. B. (1982). Nitrogen fertilisers for the production of out-of-season grass. In P. B. Lynch (Ed.), *Nitrogen fertilisers in New Zealand Agriculture* (pp. 65-76). Wellington, New Zealand: New Zealand Institute of Agricultural Science.
O'Connor, M. B., & Cumberland, G. L. B. (1973). Nitrogen responses on pasture. *Proceedings of the 25th Ruakura Farmers' Conference*, 137-144.
O'Donovan, M., & Delaby, L. (2005). A comparison of perennial ryegrass cultivars differing in heading date and grass ploidy with spring calving dairy cows grazed at two different stocking rates. *Animal Research, 54*(5), 337-350. doi:10.1051/animres:2005027
Parsons, A. J., & Chapman, D. F. (2000). The principles of pasture growth and utilization. In A. Hopkins (Ed.), *Grass, its production and utilization.* (pp. 31-89). London, United Kingdom: Blackwell Science.
Parsons, A. J., Harvey, A., & Woledge, J. (1991). Plant animal interactions in a continuously grazed mixture. I. Differences in the physiology of leaf expansion and the fate of leaves of grass and clover. *Journal of Applied Ecology, 28*(2), 619-634. doi:10.2307/2404572
Pearse, P. J., & Wilman, D. (1984). Effects of applied nitrogen on grass leaf initiation, development and death in field swards. *Journal of Agricultural Science, 103*(2), 405-413.
PGG Wrightson Seeds. (2015). Pasture Options 2015. Retrieved 10 August 2015
Pinxterhuis, J. B. (2000). *White clover dynamics in New Zealand pastures.* (Doctoral thesis), Wageningen University, Wageningen, The Netherlands.
Popay, A. J., & Hume, D. E. (2011). Endophytes improve ryegrass persistence by controlling insects. *Grassland Research and Practice Series, 15*, 149-156.
Popay, A. J., Wilson, D. J., Bell, N. L., Barton, D., Ferguson, C. M., Townsend, R. J., . . . Taylor, P. (2015). *Invertebrate sampling of Forage Value Index Trials 2013-2015. Report for Dairy NZ Ltd*.
Pyke, N. B., Rolston, M. P., & Woodfield, D. R. (2004). National and export trends in herbage seed production. *Proceedings of the New Zealand Grassland Association, 66*, 95-102.
Rahman, A., James, T. K., Grbavac, N., & Mellsop, J. (1995). Evaluation of two methods for enumerating the soil weed seedbank. *Proceedings of the 48th New Zealand Plant Protection Conference*, 175-180.
Ramsbottom, G., Horan, B., Berry, D. P., & Roche, J. R. (2015). Factors associated with the financial performance of spring-calving, pasture-based dairy farms. *Journal of Dairy Science, 98*(5), 3526-3540. doi:10.3168/jds.2014-8516
Rawnsley, R. P., Langworthy, A. D., Pembleton, K. G., Turner, L. R., Corkrey, R., & Donaghy, D. J. (2014). Quantifying the interactions between grazing interval, grazing intensity, and nitrogen on the yield and growth rate of dryland and irrigated perennial ryegrass. *Crop & Pasture Science, 65*(8), 735-746. doi:10.1071/cp13453
Rayment, G. E., & Lyons, D. J. (2011). *Soil chemical methods: Australasia* (Vol. 3): CSIRO publishing. 6A1 Organic C – W & B.
Reid, D. (1961). Factors influencing the role of clover in grass-clover leys fertilized with nitrogen at different rates. II. The effects of the variety of white clover on the yields of total herbage and clover. *Journal of Agricultural Science, 56*, 155-160.
Reid, D. (1983). The combined use of fertilizer nitrogen and white clover as nitrogen-sources for herbage growth. *Journal of Agricultural Science, 100*, 613-623.
Rhodes, I. (1970). Competition between herbage grasses. *Herbage Abstracts, 40*(2), 115-121.
Rhodes, I., & Harris, W. (1979). The nature and basis of differences in sward composition and yield in ryegrass-white clover mixtures. *British Grassland Society occasional symposium, 10*, 55-60.

Rhodes, I., & Mee, S. S. (1978). Canopy structure, yielding ability and competitive ability. Ryegrass - white clover. *Welsh Plant Breeding Station: Report for 1977.*, 142-144.
Rhodes, I., & Ngah, A. W. (1983). Yielding ability and competitive ability of forage legumes under contrasting defoliation regimes. In D. G. Jones & D. R. Davies (Eds.), *Temperate legumes: physiology, genetics and nodulation* (pp. 77-88). London, United Kingdom: Pitman Books Ltd.
Rhodes, I., & Stern, W. R. (1978). Competition for light. In J. R. Wilson (Ed.), *Plant relations in pastures.* (pp. 175-189). Melbourne, Australia: Commonwealth Scientific and Industrial Research Organisation.
Roberts, A. H. C., & Morton, J. D. (2009). *Fertiliser use on New Zealand dairy farms : the principles and practice of soil fertility and fertiliser use on New Zealand dairy farms*. Auckland, New Zealand: New Zealand Fertiliser Manufacturers' Research Association.
Robson, M. J., & Deacon, M. J. (1978). Nitrogen deficiency in small closed communities of S24 ryegrass. II. Changes in the weight and chemical composition of single leaves during their growth and death. *Annals of Botany, 42*(5), 1199-1213.
Robson, M. J., & Parsons, A. J. (1978). Nitrogen deficiency in small closed communities of S24 ryegrass. I. Photosynthesis, respiration, dry matter production and partition. *Annals of Botany, 42*(181), 1185-1197.
Russell, G., Jarvis, P. G., & Monteith, J. L. (1989). Absorption of radiation by canopies and stand growth. In G. Russell, B. Marshall, & P. G. Jarvis (Eds.), *Plant canopies: their growth, form and function* (Vol. 31, pp. 21-39). Cambridge, England; New York, NY: Cambridge University Press.
Rutter, S. M. (2006). Diet preference for grass and legumes in free-ranging domestic sheep and cattle: Current theory and future application. *Applied Animal Behaviour Science, 97*(1), 17-35. doi:10.1016/j.applanim.2005.11.016
Ryle, G. J. A., Powell, C. E., & Gordon, A. J. (1979). The respiratory costs of nitrogen fixation in soyabean, cowpea, and white clover I. Nitrogen fixation and the respiration of the nodulated root. *Journal of Experimental Botany, 30*(1), 135-144.
Sackville Hamilton, N. R. (2001). Measurement of competition and competition effects in pastures. In P. G. Tow & A. Lazenby (Eds.), *Competition and Succession in Pastures* (pp. 15-42). Wallingford, United Kingdom: CAB International.
Sackville Hamilton, N. R., Matthew, C., & Lemaire, G. (1995). In defence of the -3/2 boundary rule: a re-evaluation of self-thinning concepts and status. *Annals of Botany, 76*(6), 569-577.
Salama, H., Loesche, M., Herrmann, A., Gierus, M., Loges, R., Feuerstein, U., . . . Taube, F. (2012). Limited genotype- and ploidy-related variation in the nutritive value of perennial ryegrass (*Lolium perenne* L.). *Acta Agriculturae Scandinavica Section B-Soil and Plant Science, 62*(1), 23-34. doi:10.1080/09064710.2011.563750
Sanderson, K., & Webster, M. (2009). Economic analysis of the value of pasture to the New Zealand economy. *Report to the Pasture Renewal Charitable Trust. Wellington, New Zealand: Business and Economic Research Limited, BERL*.
Sanderson, M. A., & Elwinger, G. F. (1999). Grass species and cultivar effects on establishment of grass-white clover mixtures. *Agronomy Journal, 91*(6), 889-897.
Sartie, A. M., Matthew, C., Easton, H. S., & Faville, M. J. (2011). Phenotypic and QTL analyses of herbage production-related traits in perennial ryegrass (*Lolium perenne* L.). *Euphytica, 182*(3), 295-315. doi:10.1007/s10681-011-0400-7
Savage, J., & Lewis, C. (2005). Applying science as a tool for dairy farmers. *Proceedings of the New Zealand Grassland Association, 67*, 61-66.
Schwinning, S., & Parsons, A. J. (1996a). Analysis of the coexistence mechanisms for grasses and legumes in grazing systems. *Journal of Ecology, 84*(6), 799-813. doi:10.2307/2960553
Schwinning, S., & Parsons, A. J. (1996b). Interactions between grasses and legumes: understanding variability in species composition. *Proceedings of the joint conference of the British Grassland Society and the Sustainable Farming Systems initiative*, 153-163.
Schwinning, S., & Parsons, A. J. (1996c). A spatially explicit population model of stoloniferous N-fixing legumes in mixed pasture with grass. *Journal of Ecology, 84*(6), 815-826. doi:10.2307/2960554

Shepherd, M., & Lucci, G. (2011). Fertiliser advice–What progress can we make? In L. D. Currie & C. L. Christensen (Eds.), *Adding to the knowledge base for the nutrient manager. Occasional Report No. 24.* Palmerston North, New Zealand: Fertilizer and Lime Research Centre, Massey University.
Simon, J. C., & Lemaire, G. (1987). Tillering and leaf area index in grasses in the vegetative phase. *Grass and Forage Science, 42*(4), 373-380. doi:10.1111/j.1365-2494.1987.tb02127.x
Simpson, J. R., & Stobbs, T. H. (1981). Nitrogen supply and animal production from pastures. In F. H. W. Morley (Ed.), *Grazing animals* (pp. 261-287). Amsterdam, The Netherlands: Elsevier Scientific Publishing Company.
Simpson, W. R., Schmid, J., Singh, J., Faville, M. J., & Johnson, R. D. (2012). A morphological change in the fungal symbiont *Neotyphodium lolii* induces dwarfing in its host plant *Lolium perenne*. *Fungal Biology, 116*(2), 234-240. doi:10.1016/j.funbio.2011.11.006
Smetham, M. L. (1973). Pasture legume species and strains. In R. H. M. Langer (Ed.), *Pastures and pasture plants* (pp. 85-127). Wellington, New Zealand: A. H. & A. W. Reed.
Smit, H. J., Tamminga, S., & Elgersma, A. (2006). Dairy cattle grazing preference among six cultivars of perennial ryegrass. *Agronomy Journal, 98*(5), 1213-1220. doi:10.2134/agronj2005.0264
Smith, H. (2000). Phytochromes and light signal perception by plants - an emerging synthesis. *Nature, 407*(6804), 585-591. doi:10.1038/35036500
Smith, L. C., Morton, J. D., Catto, W. D., & Trainor, K. D. (2000). Nitrogen responses on pastures in the southern South Island of New Zealand. *Proceedings of the New Zealand Grassland Association, 62*, 19-24.
Soil survey staff. (1998). *Keys to soil taxonomy*. Washington, DC: Natural Resources Conservation Service (NRCS), US Department of Agriculture (USDA)
Solangaarachchi, S., & Harper, J. (1987). The effect of canopy filtered light on the growth of white clover *Trifolium repens*. *Oecologia, 72*(3), 372-376.
Solomon, J. K. Q., Macoon, B., Lang, D. J., Vann, R. C., & Ward, S. (2014). Cattle grazing preference among tetraploid and diploid annual ryegrass cultivars. *Crop Science, 54*(1), 430-438. doi:10.2135/cropsci2013.07.0458
South Island Dairying Development Centre. (2015). Lincoln University Dairy Farm Focus Day July 2015. Retrieved from http://www.siddc.org.nz/assets/LUDF-Focus-Days/LUDF-Focus-Day-Handout-FINAL-7-July-2015-amended.pdf
Statistics New Zealand. National Accounts (Industry Benchmarks): Year ended March 2013. Retrieved from http://www.stats.govt.nz/
Stern, W. R., & Donald, C. M. (1962). Light relationships in grass-clover swards. *Australian Journal of Agricultural Research, 13*(4), 599-614. doi:10.1071/ar9620599
Stevens, D. R., & Knowles, I. (2011). Identifying the need for pasture renewal and valuing the contribution of renewal on a dairy farm–Telford Dairy, a case study. *Grassland Research and Practice Series, 15*, 211-216.
Stewart, A., & Hayes, R. (2011). Ryegrass breeding - balancing trait priorities. *Irish Journal of Agricultural and Food Research, 50*(1), 31-46.
Stewart, A. V. (2006). Genetic origins of perennial ryegrass (*Lolium perenne*) for New Zealand pastures *Proceedings of the 13th Australasian Plant Breeding Conference*, 11-20.
Suckling, F. E. T. (1960). Productivity of pasture species on hill country. *New Zealand Journal of Agricultural Research, 3*(3), 579-591. doi:10.1080/00288233.1960.10426640
Sun, X., Luo, N., Longhurst, B., & Luo, J. (2008). Fertiliser nitrogen and factors affecting pasture responses. *The Open Agriculture Journal, 2*, 35-42. doi:10.2174/1874331500802010035
Swift, G., Vipond, J. E., McClelland, T. H., Cleland, A. T., Milne, J. A., & Hunter, E. A. (1993). A comparison of diploid and tetraploid perennial ryegrass and tetraploid ryegrass white clover swards under continuous sheep stocking at controlled sward heights .1. Sward characteristics. *Grass and Forage Science, 48*(3), 279-289. doi:10.1111/j.1365-2494.1993.tb01861.x
Thom, E. R., Popay, A. J., Hume, D. E., & Fletcher, L. R. (2013). Evaluating the performance of endophytes in farm systems to improve farmer outcomes–a review. *Crop and Pasture Science, 63*(10), 927-943.

Thomas, H. (1984). Effects of drought on growth and competitive ability of perennial ryegrass and white clover. *Journal of Applied Ecology, 21*(2), 591-602. doi:10.2307/2403431

Thomas, R. J. (1992). The role of the legume in the nitrogen-cycle of productive and sustainable pastures. *Grass and Forage Science, 47*(2), 133-142. doi:10.1111/j.1365-2494.1992.tb02256.x

Thompson, L. (1995). Sites of photoperception in white clover. *Grass and Forage Science, 50*(3), 259-262. doi:10.1111/j.1365-2494.1995.tb02321.x

Thompson, L., & Harper, J. L. (1988). The effect of grasses on the quality of transmitted radiation and its influence on the growth of white clover *Trifolium repens*. *Oecologia, 75*(3), 343-347. doi:10.1007/bf00376935

Thornley, J. H. M., Bergelson, J., & Parsons, A. J. (1995). Complex dynamics in a carbon-nitrogen model of a grass-legume pasture. *Annals of Botany, 75*(1), 79-94.

Tilman, D. (1990). Mechanisms of plant competition for nutrients: the elements of a predictive theory of competition. In J. B. Grace & D. Tilman (Eds.), *Perspectives on plant competition.* (pp. 117-141). San Diego, CA: Academic Press.

Tow, P. G., & Lazenby, A. (2001). Competition and succession in pastures - Some concepts and questions. In P. G. Tow & A. Lazenby (Eds.), *Competition and Succesion in Pastures* (pp. 1-13). Wallingford, United Kingdom: CAB International.

Tozer, K. N., Chapman, D. F., Bell, N. L., Crush, J. R., King, W. M., Rennie, G. M., . . . Cameron, C. A. (2014). Botanical survey of perennial ryegrass-based dairy pastures in three regions of New Zealand: implications for ryegrass persistence. *New Zealand Journal of Agricultural Research, 57*(1), 14-29. doi:10.1080/00288233.2013.863785

Turkington, R., & Harper, J. L. (1979a). The growth, distribution and neighbour relationships of *Trifolium repens* in a permanent pasture: I. Ordination, pattern and contact. *The Journal of Ecology*, 201-218.

Turkington, R., & Harper, J. L. (1979b). The growth, distribution and neighbour relationships of *Trifolium repens* in a permanent pasture: II. Inter-and intra-specific contact. *The Journal of Ecology*, 219-230.

Turkington, R., & Jolliffe, P. A. (1996). Interference in *Trifolium repens - Lolium perenne* mixtures: Short- and long-term relationships. *Journal of Ecology, 84*(4), 563-571. doi:10.2307/2261478

Ulyatt, M. J. (1970). Evaluation of pasture quality under New Zealand conditions. *Proceedings of the New Zealand Grassland Association, 32*, 61-68.

van Bysterveldt, A. (2005). Lincoln University dairy farm, now a cropping farm? *Proceedings of the South Island Dairy Event (SIDE)*.

van Loo, E. N., Schapendonk, A., & Devos, A. L. F. (1992). Effects of nitrogen supply on tillering dynamics and regrowth of perennial ryegrass populations. *Netherlands Journal of Agricultural Science, 40*(4), 381-400.

Verbyla, A. P., Cullis, B. R., Kenward, M. G., & Welham, S. J. (1999). The analysis of designed experiments and longitudinal data by using smoothing splines. *Journal of the Royal Statistical Society: Series C (Applied Statistics), 48*(3), 269-311. doi:10.1111/1467-9876.00154

VSN International. (2013). GenStat for Windows 16th Edition [Computer software]. Hemel Hempstead, UK: VSN International.

VSN International. (2014). GenStat for Windows 17th Edition [Computer software]. Hemel Hempstead, UK: VSN International.

Waghorn, G. C., & Clark, D. A. (2004). Feeding value of pastures for ruminants. *New Zealand Veterinary Journal, 52*(6), 320-331. doi:10.1080/00480169.2004.36448

Wakelin, S. A., Eslami, Y., Dake, K., Dignam, B. E. A., & O'Callaghan, M. (2016). Cost of root disease on white clover growth in New Zealand dairy pastures. *Australasian Plant Pathology, 45*(3), 289-296. doi:10.1007/s13313-016-0411-x

Walker, T., Orchiston, H. D., & Adams, A. (1954). The nitrogen economy of grass legume associations. *Grass and Forage Science, 9*(4), 249-274.

Watkinson, J., & Perrott, K. (1990). A new soil test for sulphate and mineralisable organic sulphur. *Proceedings of the New Zealand Fertiliser Manufacturers' Research Association Conference*, 188-198.

Whitehead, D. C. (1970). *The role of nitrogen in grassland productivity. A review of information from temperate regions*. Farnham Royal, United Kingdom: Commonwealth Agricultural Bureaux
Whitehead, D. C. (1995). *Grassland nitrogen*. Wallingford, United Kingdom: CAB International.
Widdup, K. H., & Barrett, B. A. (2011). Achieving persistence and productivity in white clover. *Grassland Research and Practice Series, 15*, 173-180.
Widdup, K. H., & Turner, J. D. (1983). Performance of 4 white clover populations in monoculture and with ryegrass under grazing. *New Zealand Journal of Experimental Agriculture, 11*(1), 27-31.
Wilkins, P. W. (1991). Breeding perennial ryegrass for agriculture. *Euphytica, 52*(3), 201-214. doi:10.1007/bf00029397
Williams, T. A., Abberton, M. T., Evans, D. R., Thornley, W., & Rhodes, I. (2000). Contribution of white clover varieties in high-productivity systems under grazing and cutting. *Journal of Agronomy and Crop Science, 185*(2), 121-128. doi:10.1046/j.1439-037x.2000.00424.x
Williams, T. A., Abberton, M. T., Thornley, W., & Rhodes, I. (2001). Relationships between the yield of perennial ryegrass and of small-leaved white clover under cutting or continuous grazing by sheep. *Grass and Forage Science, 56*(3), 231-237. doi:10.1046/j.1365-2494.2001.00269.x
Wilman, D., & Asiegbu, J. E. (1982a). The effects of clover variety, cutting interval and nitrogen application on herbage yields, proportions and heights in perennial ryegrass-white clover swards. *Grass and Forage Science, 37*(1), 1-13. doi:10.1111/j.1365-2494.1982.tb01571.x
Wilman, D., & Asiegbu, J. E. (1982b). The effects of variety, cutting interval and nitrogen application on the morphology and development of stolons and leaves of white clover. *Grass and Forage Science, 37*(1), 15-27. doi:10.1111/j.1365-2494.1982.tb01572.x
Wilman, D., Koocheki, A., Lwoga, A. B., Droushiotis, D., & Shim, J. S. (1976). The effect of interval between harvests and nitrogen application on the numbers and weights of tillers and leaves in four ryegrass varieties. *Journal of Agricultural Science, Cambridge, 87*(1), 45-57.
Wilman, D., & Wright, P. T. (1983a). Some effects of applied nitrogen on grass growth in field swards at different times of year. *Proceedings of the XIV International Grassland Congress*, 297-299.
Wilman, D., & Wright, P. T. (1983b). Some effects of applied nitrogen on the growth and chemical composition of temperate grasses. *Herbage Abstracts, 53*(8), 387-393.
Wims, C. M., Lee, J. M., Rossi, L., & Chapman, D. F. (2014a). Variation in the reproductive development of perennial ryegrass ( *Lolium perenne* L.) cultivars. *Proceedings of the New Zealand Grassland Association, 76*, 189-192.
Wims, C. M., Lee, J. M., Rossi, L., & Chapman, D. F. (2014b). Variation in the reproductive development of perennial ryegrass ( *Lolium perenne*) cultivars. *Proceedings of the 25th General Meeting of the European Grassland Federation*, 840-842.
Woledge, J. (1988). Competition between grass and clover in spring as affected by nitrogen-fertilizer. *Annals of Applied Biology, 112*(1), 175-186. doi:10.1111/j.1744-7348.1988.tb02053.x
Woledge, J., Davidson, I. A., & Tewson, V. (1989). Photosynthesis during winter in ryegrass white clover mixtures in the field. *New Phytologist, 113*(3), 275-281. doi:10.1111/j.1469-8137.1989.tb02404.x
Woledge, J., & Pearse, P. J. (1985). The effect of nitrogenous fertilizer on the photosynthesis of leaves of a ryegrass sward. *Grass and Forage Science, 40*(3), 305-309. doi:10.1111/j.1365-2494.1985.tb01756.x
Woodfield, D. R. (1999). Genetic improvements in New Zealand forage cultivars. *Proceedings of the New Zealand Grassland Association, 61*, 3-7.
Woodfield, D. R. (Ed.) (1996). *White clover: New Zealand's competitive edge. Proceedings of a joint symposium between Agronomy Society of New Zealand and New Zealand Grassland Association, Lincoln University, New Zealand, 21 - 22 November 1995*. Palmerston North, New Zealand: New Zealand Grassland Association.
Woodfield, D. R., & Caradus, J. R. (1994). Genetic-improvement in white clover representing 6 decades of plant-breeding. *Crop Science, 34*(5), 1205-1213.
Woodfield, D. R., Clifford, P. T. P., Baird, D. B., Cousins, G. R., Miller, J. E., Widdup, K. H., & Caradus, J. R. (2003). Grasslands Tribute: a multi-purpose white clover for Australasia. *Proceedings of the New Zealand Grassland Association, 65*, 157-162.

Woodfield, D. R., Clifford, P. T. P., Cousins, G. R., Ford, J. L., Baird, I. J., Miller, J. E., . . . Caradus, J. R. (2001). Grasslands Kopu II and Crusader: new generation white clovers. *Proceedings of the New Zealand Grassland Association, 63*, 103-108.

Woodfield, D. R., & Easton, H. S. (2004). Advances in pasture plant breeding for animal productivity and health. *New Zealand Veterinary Journal, 52*(6), 300-310.

Woodward, S. L., Waugh, C. D., Roach, C. G., Fynn, D., & Phillips, J. (2013). Are diverse species mixtures better pastures for dairy farming. *Proceedings of the New Zealand Grassland Association, 75*, 79-84.

Yoda, K. (1963). Self-thinning in overcrowded pure stands under cultivated and natural conditions. *Journal of Biology Osaka City University, 14*, 107-129.