Dry matter production and botanical composition of three pasture species and their seed mixtures after an autumn sowing using two overall sowing rates

---

A Dissertation
submitted in partial fulfilment
of the requirements for the Degree of
Bachelor of Agricultural Science with Honours

at

Lincoln University
by
Lachlan John Wood

Lincoln University
2017

Abstract of a Dissertation submitted in partial fulfilment of the requirements for the Degree of Bachelor of Agricultural Science with Honours

Dry matter production and botanical composition of three pasture species and their seed mixtures after an autumn sowing using two sowing rates

by

Lachlan John Wood

Pastoral agriculture in New Zealand is based around the use of white clover and perennial ryegrass. Diploid perennial ryegrass, white clover and plantain were grown in monocultures, two-species mixes and three-species mixes at Lincoln University, Canterbury New Zealand. The dry matter production, botanical composition during two harvests on the 4th of August and the 13th of September 2017 were measured. Ten seed mixes were repeated at two overall sowing rates equivalent to 1000 and 2000 seeds/m^2 and at two overall levels of N, 0 and 100 kg N/ha, respectively. A statistical analysis was performed on the data that was obtained from the measurements and a special cubic model was created. This analysis showed that pasture mixes with two species showed better estimated yields than the three-species pasture mixes. Ryegrass and white clover mixes and white clover and plantain mixes showed significant results ($P<0.05$) when induced as a two-species pasture mix. White clover had the highest weed content of >80% in the monocultures compared with ryegrass and plantain which outcompeted weeds, only producing a weed content between 5-20%. White clover did not perform as a monoculture with the weeds and soil temperature following an autumn sowing not allowing for good establishment. A three-species pasture mix showed a decrease in production compared with the two-species mixes ($P<0.05$). Sowing rate had a significant effect on the yield of the first harvest ($P=0.003$), possibly due to large amounts of germinating seeds that suppress the weed population and result in more herbage especially in mixes containing ryegrass and plantain. The second harvest showed less significance from the sowing rate as pasture mixes that included white clover began to establish better, growing more herbage ($P=0.189$). There was no statistical effect of the botanical composition of the pastures from the sowing rate. N had an overall effect on yield, having the greatest effect on perennial ryegrass across both harvests. Overall increasing sowing rate and N levels had an increase in yield benefit but no composition effect.

Keywords: Diploid perennial ryegrass, white clover, plantain, monoculture, sowing rate, nitrogen, leaf area index, yield, establishment.

Acknowledgements

I am very grateful for the opportunity to do an honours dissertation in my final year of study. I have learnt a lot in the past four years of my degree and am very appreciative of all the lecturers and tutors who have shared their knowledge.

Firstly, I would like to thank Dr Alistair Black for suggesting and organising to start the project in such a short period of time. I am very grateful for the countless time you have supported me, guiding and explaining crucial points throughout the year. Thank you for always taking the time out of your busy schedule to look over my work and give me feedback on how I can improve my skills practically and with written work, it was very helpful and I have gained a wealth of knowledge.

Thanks to the staff of the Field Research Centre, especially Daniel Dash and Dave Jack who helped set up the trial and helped with pest control. I appreciate the help, it has not gone unnoticed.

I would also like to thank the PLSC 321 students Ben, Dougal, Dustin, Jaden, Josh, Reuben, Ryan and William for all the help with harvesting and separations. Without your help the project would have been more difficult for me. Also to Philippine, the French intern, your efforts are appreciated also.

Many thanks to all the people I have had the pleasure of meeting at university and to those who I have made friends with, you have made university very enjoyable and far better experience.

Finally, I would like to thank my family for always encouraging me to do my best and never give up. Your support has really driven me throughout my university career, especially in my final year, for which I am extremely grateful.

Table of Contents

List of Tables

Table of Plates

List of Figures

Chapter 1

Introduction

Throughout New Zealand's pasture-based agriculture the traditional pasture mix of perennial ryegrass (*Lolium perenne* L.) and white clover (*Trifolium repens L.*) is commonly used. More recently plantain (*Plantago lanceolata* L.) has been included in this mix to increase the nutritive value of the pastures. With intensive farming coming more predominant in NZ agriculture, with farmers looking for a high quantity and quality herbage across the year, has largely driven this change. The right seed rates are therefore important to ensure that all species have the ability to establish and contribute to the pastoral system. Seed companies make recommendations for what they think the best seed rate is, as they want to sell their products. However, there is currently little-known science in New Zealand of how seed companies come up with these rates.

Alexander (1933) proposed that sowing mixed pasture swards can have several advantages over monocultures. The first is some of the plants are sure to suit the conditions, the grazing season is lengthened; by mixing early and late flowering grasses thus giving greater annual production. The pasture has a higher nutritive value and greater palatability. Mixtures will also give a better-balanced food source and therefore a diet of higher nutritive value for grazing livestock. Better occupation of the soil can be obtained by deep and shallow rooted grasses. Better pasture longevity; if mixtures properly proportioned the more permanent grass will fill the gaps left by the temporary types. Properly proportioned mixtures reduce the weed proportion in the swards as there are less gaps for weeds to grow in. Ryegrass is commonly more competitive than white clover making it susceptible to be out-competed (Martin and Field, 1984). With ryegrass establishing faster, competing for space by tillering it creates a canopy, shading and reducing the light reaching the white clover. White clover is an important part of the pasture mix, due to its ability to fix N. It is therefore crucial to ensure it is able to have a strong establishment. One solution by Brougham (1969) was to reduce the sowing rate of ryegrass, allowing easier establishment of white clover. He found this to be true in a range of ryegrass species and locations across New Zealand, with all cases having increased establishment of white clover with reduced sowing rates of ryegrass.

Seed companies make recommendations of seed mixes (Table 1.1), as they want to sell their product but what they base this recommendation on in unknown. Seeds mixes tend to be dominated by ryegrass with usually more than 18 kg/ha for diploid perennial ryegrass. They also consist of numerous species in the mix often 2 – 5 species. The mixes are generally ryegrass and clover mixes, often with two cultivars of the same species of cultivar to extend the growing season and some mixes include herb species.

The use of diverse pastures that contain at least three pasture species may provide further advantages through increasing dry matter (DM) production and biological N fixation (Goh and Bruce, 2005). Typical sowing rates for perennial ryegrass are 10-20 kg/ha and white clover 2-3 kg/ha (Charlton, 1991). The addition of pasture herbs such as plantain are commonly sown at a rate of 1-3 kg/ha (Turner, 1992). By adding more clover into the mixes can increase nitrogen levels in the soil by biologically fixing atmospheric N, as a result a reduced amount of fertiliser is required (Black *et al.*, 2009). This is evidence that clovers are an important aspect of a pasture mixture, a common problem is not having enough white clover produced, as it is less competitive than ryegrass and plantain.

Table 1.1. 2017 Sheep and beef pasture seed mix recommendations from Agricom, Agriseeds and PGG Wrightson Seeds.

Seed Company	Suggested seed mix (per ha)	Total (ha)
Agricom	'Request' perennial ryegrass 18 kg 'Tribute' white clover 5 kg 'Relish' red clover 4 kg 'Choice' chicory 2 kg 'Tonic' plantain 1 kg	**30 kg**
Agriseeds	'Shogun' hybrid ryegrass 30 kg 'Weka' white clover 1.5 kg 'Apex' white clover 1.5 kg 'Tuscan' red clover 4 kg	**37 kg**
Agriseeds	'Bealey' ryegrass 30 kg 'Weka' white clover 2 kg 'Apex' white clover 2 kg 'Tuscan' red clover 4 kg	**37kg**
PGG Wrightson Seeds	'Rely' perennial ryegrass 18 kg 'Tekapo' cocksfoot 2 kg 'Bounty' white clover 3 kg 'Tahora II' white clover 2 kg	**25 kg**

The main objective of this experiment was to determine the most effective seed mix and if applying N affected the establishment of the three common pasture species perennial ryegrass, white clover, plantain and their mixes in a Canterbury environment. This dissertation includes a review of previously published literature on pasture mixes, and how formulation of these mixes affects the weed suppression and yield of the pastures. A field experiment was conducted at Lincoln University where each of the three species were grown separatel y and in mixtures of all possible combinations and tested at two overall sowing rates. The ecosystems functions were DM production, weed suppression and leaf area index (LAI). Measurements of these were taken from two separate harvests in August and September 2017, over a 7-month period after an autumn sowing (31st of March 2017) to analyse the effects of species competition and to determine the best seed mix and sowing rate to allow each of the species to establish.

Chapter 2
Review of the literature

2.1 Introduction

Pasture is the foundation for agriculture and livestock production in New Zealand. The vast mass of pastures in New Zealand are dependent on perennial ryegrass and white clove. These species are well adapted to New Zealand climatic conditions and are proven to perform well under stock grazing. However, perennial ryegrass-white clover pastures often fail during key times when they are required for finishing livestock. This results in poorer yields and performance, which impacts the farmer financially. Many farmers now are adapting other species into their pasture mixes such as plantain, to be used as a specialist finishing pasture mix due to the high palatability of either this species or as a complex mix with other high-quality pasture species.

This review covers the rationale behind using three species pasture mixes as opposed to monocultures of pasture species. The physiological, production and competitiveness of the three-species used in the field experiment for this dissertation, diploid perennial ryegrass, white clover and plantain were assessed. Sowing rate will also be assessed along with the effect of N fertiliser on yield, botanical composition and competition of the three species and their mixes.

2.2 Pasture mixes

Early seed mixes in New Zealand were developed using a traditional British method by broadcasting multispecies seed mixtures after bush burns during colonisation of New Zealand (Charlton, 1991). Bush-burn methods and broadcast 'shotgun' sowing of up to 20 species in the hope that some would establish was a common practise (Harris, 1968). Therefore, the past experiences that have had over time with successful pasture and competition between species have formulated the simplified, high quality pasture mixes today. This allows the farmers to target specific species which can provide quick establishment and suppress unsown species, thus producing a high quality and quantity of pasture that can be produced all year round which improves livestock production (Harris, 2001).

Alexander (1933) proposed that sowing mixed pasture swards can have several advantages over monocultures. The first is some of the plants are sure to suit the conditions, the grazing season is lengthened - by mixing early and late flowering grasses thus giving greater annual production. The pasture has a higher nutritive value and greater palatability. Mixtures will also give a better-balanced food source and therefore a food of higher nutritive value for grazing livestock. Better occupation of the soil can be obtained by deep and shallow rooted grasses. Better pasture longevity – if mixtures properly proportioned the more permanent grass will fill the gaps left by the temporary types. Properly proportioned mixtures reduce the weed proportion in the swards as there are less gaps for weeds to grow in. The knowledge obtained from years of research have led to adoption of pasture mixes and new species have been developed and released since the 1970s, as they have become more popular they have formed more complex pasture mixes.

When selecting for new species and cultivars for a specific pasture mix there are some factors that are considered for the pasture mix to be successful. Charlton (1991) stated that these basic principles should be considered before deciding what species or cultivar to choose. The first factor is the compatibility of the species. This is how compatible the species is with other sown species in the mixture, its rate of establishment and how competitive the species is against unsown species (weeds). The second principle is the intended use of the pasture which can become versatile in a livestock production system. If the pasture was intended to finish livestock, for slaughter then it would need to be of high quality and possibly in short rotation. The third principle is environmental factors. Does the species suite the soil type, fertility, and nutrient status? These principles are the fundamental basis of how farmers determine which pasture species and cultivars to select. Therefore, plenty of consideration should be taken in which species should be used in a seed mix for what specific purpose the farmer wants to achieve, because all species interact differently with each other.

Clovers form a significant relationship with grass and herb species in New Zealand pasture mixes due to their ability to biologically fix N. This has the bonus of increasing soil N available to other plant species, increasing livestock production. The other method of increasing the amount of N available to plants is via N fertiliser application. The amount of N fixed by ryegrass-white clover pasture can range significantly usually between 0 to 350 kg N/ha/yr (White and Hodgson, 2000). This should be another important factor in determining what species to select as clover has clear benefits for pastures.

The optimal perennial ryegrass seed rate as determined by Brougham (1953) was 20 kg/ha with regard to establishment, weed suppression, yield and grass-clover balance. This sowing rate will give 1000 seeds/m^2 based of ryegrass seed having a thousand-seed weight of 2 g. Seed weight can fluctuate depending on species and whether or not it is treated. Coated ryegrass can have a thousand-seed weight of 2.4 g but not always. Clovers are a crucial aspect of the diversity of species in a pasture and integrate greater pasture quality by N fixation. It is vital to adjust the ryegrass seed rate in order to not suppress the clover by shading. Brougham (1953) in autumn 1952 planted

‘Grasslands Manawa’ ryegrass across seven rates between 0 to 67 kg/ha with white clover added to the mix at a constant rate of 3.4 kg/ha. The higher ryegrass seed rates initially had the greatest yield due to fast establishment. The clover was suppressed at these rates and yields began to decrease due to poor N fixation. The common sowing rate for New Zealand for ryegrass-white clover pastures is 20 kg perennial ryegrass and 5 kg clover/ha (Harris, 2001). This gives approximately the same number of seeds per same unit area.

2.3 Diploid perennial ryegrass

Perennial ryegrass is the most versatile and commonly sown temperate grass species used in pastures in New Zealand. Perennial ryegrass suites a wide range of New Zealand climates, growing well in a range of conditions, is easily established and is compatible with white clover (Charlton and Stewart, 1999). Perennial ryegrass adapts well to a range of medium to high soil fertility. Growth is impacted when temperature decreases. Ryegrass starts growing at 5˚C and reaches its optimum at 18˚C (White and Hodgson, 2000). This means ryegrass will not tolerate extreme conditions such as cold temperatures or hot-dry conditions.

Perennial ryegrass is very competitive and very fast at establishing, competing aggressively against other species for light, nutrients and space when sown in a mix. A well-established perennial ryegrass pasture will tolerate heavy grazing and treading, persisting for as long as 20 years. Perennial ryegrass is naturally a diploid, containing 2 sets of 7 chromosomes in each cell (Stewart *et al.*, 2014). Diploid perennial ryegrass has a small tiller size but compensates by dense tillering, allowing for fast establishment and can outcompete other species. Diploid perennial ryegrass suites more extensive pastoral systems where there is less control of the grazing management. The optimal diploid ryegrass seed rate as determined by Brougham (1953) was 20 kg/ha with regard to establishment/weed suppression, yield and grass-clover balance.

Annual dry matter yields of diploid perennial ryegrass in New Zealand commonly range between 10 to 25 t DM/ha in soils of high fertility and animal production is typically high with a digestibility of 75-85% (White and Hodgson, 2011). However, in regions where rainfall is highly seasonal and summers are warm and dry, growth and persistence is particularly poor (Turner *et al.*, 2006).

2.4 White clover

White clover is arguably the most beneficial forage legume in New Zealand. White clover has been sown into New Zealand pastures since colonisation of New Zealand. It is adapted to most New Zealand conditions and is a vital component of many pasture mixes. White clover is a prostate perennial plant which produces stolon's. Stolon's are produced from nodes on stems that eventually produce clonal units that become independent of the original plant (White and Hodgson, 2000). White clover is susceptible to drought conditions due to its shallow root system, climates under 600 mm of rain often contribute to poor production of white clover (White and Hodgson, 2000). White clover ceases growth at 8-9˚C and reaches optimum growth rates at 25˚C. This is why white clover has poor development during the winter months and sowing usually is done in spring (White and Hodgson, 2000). White clover is very important in pasture mixes as it can biologically fix N via the rhizobia bacteria in the root nodules. White clover is arguably the most beneficial forage legume in New Zealand.

The amount of N fixed by ryegrass-white clover can significantly differ, usually between 0 to 350 kg N/ha/yr (White and Hodgson, 2000). N fixation increases as proportions of clover increases in the pasture. White clover yields have been recorded between 150 – 5040 kg DM/ha/yr in pasture mixtures with various clover percentages of 2 – 39%. White clover yields have been recorded as high as 7 t DM/ha (Ball *et al*., 1978). White clover is suited to mix well with most pasture species but prefers an open sward where it does not need to compete with other plants for sunlight, which allows white clover to thrive. White clover is usually sown into pasture mixes at a rate of 2-5 kg/ha. This is equivalent to 250-600 seeds/m^2 (Stewart *et al.,* 2014; White and Hodgson, 2000). Perennial ryegrass and white clover have been the most common New Zealand pasture mix. Trends now suggest that popularity is increasing by using white clover with forage herbs such as plantain.

2.5 Plantain

The use of plantain incorporated into a pastoral system has grown in popularity due to its high palatability (Stewart, 1996). Plantain has greater tolerance to drought conditions compared to ryegrass/clover mixes (Nie *et al.,* 2008) which is favourable as some climates in New Zealand are prone to drought. Plantain is very versatile compared to other species and can grow in varied levels of pH levels ranging from 4.2-7.8 (Stewart, 1996), making it suitable for a range of areas in New Zealand. Plantain can also dominate against shallow rooted grasses in low fertility (Stewart, 1996). For prolonged growth into the summer, Sithamparanathan *et al.,* (1986) stated that establishing herbs into an existing pasture needed to be heavily grazed to reduce existing vegetative competition.

A high rate of seed 10 kg/ha was recommended to establish a significant plantain sward in the pasture to be beneficial (Sithamparanathan *et al.*, 1986). Fraser and Rowarth (1996) recommended sowing 'Grasslands Lancelot' plantain at 8 kg/ha. This information is supported by Kemp (2012) who recommended plantain is sown at 8-10 kg/ha when sown as a pure species, or 1-3 kg/ha when sown in a ryegrass and clover.

The other option farmers tend to use is to sow plantain in a pure sward or part of a herb/clover mix, which typically is used for finishing young livestock over the summer period. This method will require a herbicide spray to the existing pasture (Sithamparanathan *et al.*, 1986) to ensure there is no grass competing with the mix between emergence and establishment. The mixes are traditionally sown as 50/50 plantain to clover based on seed count. White clover is added as a base legume, recommended to give additional yield and N fixation in all pastures (Hume and Fraser, 1985). White clover is typically sown at 2-5 kg/ha. When sown as a pure sward, the crude protein content of plantain can be low (<15%) (Lee *et al.*, 2015), potentially limiting animal production. However, the clover content in a herb/clover mix is likely to ameliorate this potential problem (Sinhadipathige *et al.,* 2012). Therefore, the herb and clover mix is more suitable as a specialist summer and winter active perennial forage for lamb finishing than a pure plantain sward.

After the seed has been sown and starting to germinate and establish, proper care needs to be taken to ensure the survival of the species. Before grazing takes place, plantain needs to establish a firm root structure, root dry matter percentage needs to be under 10% of total dry matter and have a minimum of 6 fully developed leaves (Powell *et al.,* 2007). This will reduce plant loss and will increase persistence over many seasons. Plantain grows from a crown and cannot be grazed too low. Plantain is not tolerant to set stocking and should be rotationally grazed to maintain persistence, the plantain should last 4-5 years (Stewart *et al.,* 2014).

2.6 Plant competition

Plant competition occurs naturally between plants. This is when they compete for limited resources such as nutrients, light, water and space. Plant competition has significant impacts on the composition and persistence of mixed pastures. However, in monocultures intraspecific competition is between plants of the same species. Interspecific competition is between species in mixed pastures, where the competition is of different species plants competing with each other for resources (e.g. ryegrass/white clover). Intraspecific competition is far more aggressive than interspecific, mainly due to the same species are competing exactly for the same resources (Haynes, 1980). Intraspecific competition is correlated significantly with plant density and plant density increases as sowing rate increases (Harris, 1990). High seeding rates increase competition between developing seedlings for light, water and nutrients, reduce plant size (Harris, 1990) and potentially survival. When the seedlings are young there is little competition as there generally is ample space, nutrients, water and light for each seedling. As the plants develop they expand their leaves and develop new leaves and stems, thus creating more competition for available light and increasing each individual plant's requirements for resources. This begins to shade other plants and in severe cases will outcompete the less developed plants. Competition plays a crucial role in plant ecosystems. This allows for species to succeed over each other until a steady state is reached.

Interspecific competition is very important to understand in how pasture mixes establish and then interact with each other to form a persistent mix of the sown species. Perennial ryegrass has the ability to rapidly germinate, emerge and develop canopy of leaves, resulting in outcompeting clover for light and space in pasture mixes. The leaves of ryegrass are tall and erect which shades out white clover which has small leaves growing prostrate. As a result, pasture mixes can be dominated by ryegrass with low proportions of clover. This is a good example to highlight the importance of considering the compatibility of pasture species before sowing them in a mix.

Plantain is likely to be similar to perennial ryegrass with its rapid rate of establishment (Blom, 1978). Although being an erect broad leaf species, may cause further shading on white clover than ryegrass. In regard to this dissertation, it is highly likely that the pasture mixtures containing perennial ryegrass will dominate the other two species. White clover will likely make up a low proportion of each mix, due to its slow establishment from an autumn sowing and being suppressed by plantain and perennial ryegrass. Sowing in autumn, when soil temperatures are below 14°C, using ryegrass sowing rates greater than 10 kg/ha may optimise ryegrass establishment at the expense of slower establishing species (Cullen 1958; Hurst *et al.*, 2000; Moot *et al.*, 2000).

2.7 Sowing rate

Perennial ryegrass sowing rates ranging between 1 to 20 kg/ha have little effect on yield through its ability to produce tillers and eventually, filling any gaps between plants in the long term (Hill *et al.*,1999). The most common sowing rate for diploid perennial ryegrass is between 8 and 11 kg/ha, where increased plant populations at germination can lead to intraspecific competition which may reduce establishment of the plants (Hill *et al.*, 1999). Increasing sowing rate to over 20 kg/ha leads to more competition for space, light, nutrients and water as a result producing smaller, weaker plants which results in increased variation across the paddock, impacting yield (Hare, 1990). An autumn sowing trial conducted by Black *et al.* (2002) showed that clover species did not affect yield at first harvest but increasing ryegrass sowing rate did. This was due to the clovers slow establishment after the autumn sowing and perennial ryegrass' ability to tiller. This result is consistent with Moot *et al.* (2000) who recommended white clover based pastures be sown when autumn soil temperatures at 20 mm soil depth are at least 14°C. The substitution of clover with winter annual weeds in pastures sown in autumn, when air and soil temperatures were declining rapidly, supports this conclusion (Moot *et al.*, 2000).

2.8 Nitrogen

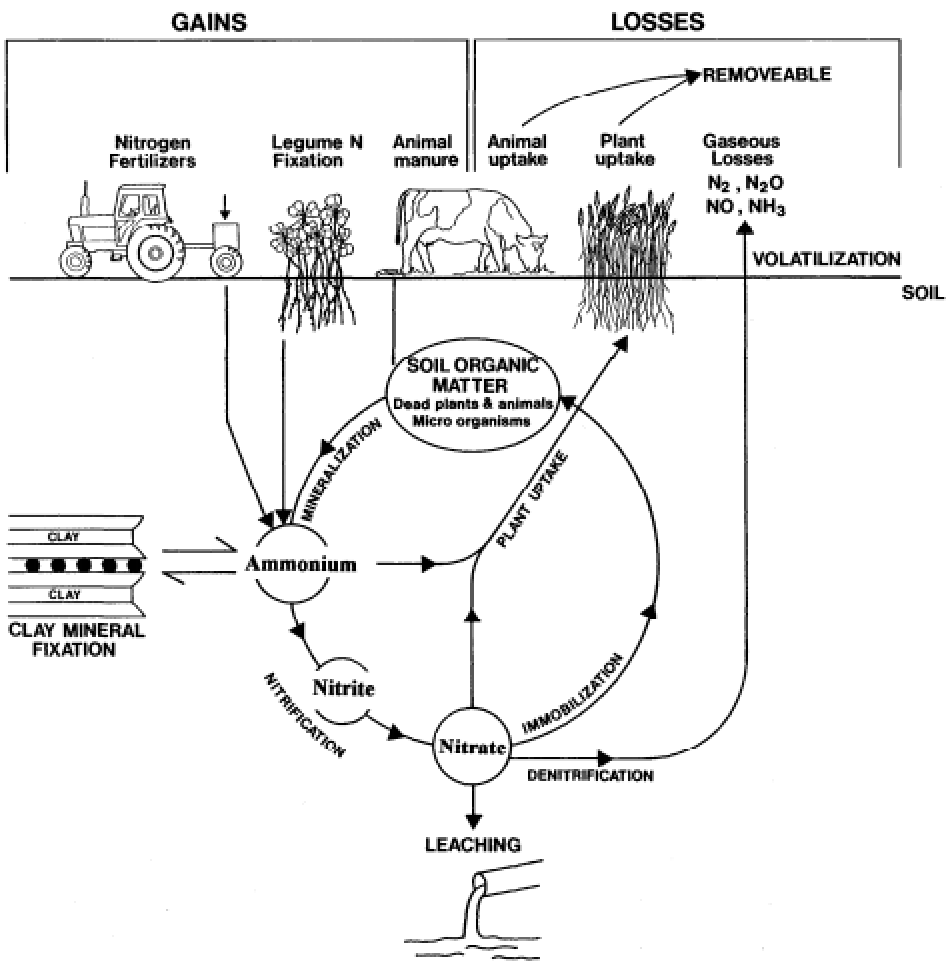

Figure 2.1. The nitrogen cycle in grazed pasture systems (from McLaren and Cameron, 1996).

Nitrogen fertiliser is commonly used to increase the production of pastures containing perennial ryegrass and white clover (Ball and Field, 1982). Nitrogen fertiliser initially increases the protein content in pasture, increasing the rate of photosynthesis, increasing the production of the pasture (Ball and Field, 1982). Leaf area increases due to an increase in cell elongation, producing longer thinner leaves and increasing the number of tillers. This is due to the plants ability to intercept more light. Although the ryegrass plants increase tiller populations over time this is decreased as intraspecific competition causes a decline in tillers (Woledge, 1988). As tillering increases, this causes interspecific competition and can give perennial ryegrass greater resources to shade and outcompete white clover. This can decrease the production and persistence of the pasture over time. Plant demand for N is greatest in late winter and early spring (Cookson *et al.*, 2001). This could be due to little N fixation by clover as the ground temperature is low during the winter months and the high rainfall contributes to increased N leaching.

White clover growth and N fixation are often compromised by N fertiliser. This could possibly due to the clover being reliant on the provided N instead of fixing its own N (Ball and Field, 1982). Stewart (1984) suggested a clover content of at least 30% is needed to make any significant impact on a pasture's N economy, quality of feed and animal performance.

2.9 Tillering

A tiller is described as the primary growth unit of a mature grass plant. Tiller buds are formed in the axil of the main stem leaves and grow out to produce tillers (Hunt and Field, 1976). Hunt and Field (1976) noted when tiller densities are sufficient to induce competition between tillers, differences in tiller density tend to be compensated by differences in tiller growth. Daughter tillers that are produced from the main stem develop their own root systems and can become independent from the main plant. This leaf and tiller continues to grow until specific environmental requirements are reached to shift the plant into the reproduction phase (Williamson, 2008). Tillers increase in number in spring and turnover of tillers is accelerated by N (Hunt and Mortimer, 1982). Typically, the birth of new tillers and death of old tillers are the main contribution for pasture persistence for perennial ryegrass as they influence the tiller population density. The number of vegetative tillers is very important during the reproductive phase of perennial ryegrass plants persistence through the summer period as more tillers are needed to maintain the plants survival (Matthew and Sackville-Hamilton, 2011). Populations of tillers are dynamic with the birth of new tillers and the death of others creating a balance in tiller population densities (Korte *et al.*, 1985; Matthew and Sackville-Hamilton, 2011). The expansion in growth of tiller numbers is usually in spring and after reproductive growth.

A study by Lee *et al* (2013) found that 8 months post sowing at a Canterbury site, perennial ryegrass plants had greater tillering after sowing at 6 and 12 kg/ha compared to 18-30 kg/ha (Table 2.1). There was an average of 24 tillers per plant for the 6 kg/ha sowing treatment whereas there were only 10 tillers per plant for the highest seeding rate (30 kg/ha). There was no significant difference in plant survival among the sowing rate treatments at the Canterbury site. However, it was expected they would due to the plants known to self-thin in times of high competition for resources (Lee *et al.*, 2013).

Table 2.1. Average sowing rate effects on perennial ryegrass tillers per plant 8 months post-drilling in Canterbury (adapted from Lee *et al.*, 2013).

	Seeding Rate (kg/ha)						
	6	12	18	24	30	SED	P-value
Tillers per plant	24	18	13	13	10	1.3	<0.001

2.10 Leaf area index

The interception of solar radiation by leaves of plants is a main driver of photosynthesis and plant development. Plants require chlorophyll in their leaves (green colour) to absorb photosynthetically active radiation (PAR) and use it for photosynthesis and growth. As plants develop they grow bigger leaves which spread out, increasing green leaf area and increasing light interception. Canopy leaf area is quantified as the leaf area index (LAI) which is the green leaf area per unit area of ground (Rattray *et al.*, 2007). The LAI increases as plants develop as the plants spreads out and covers more area with green leaves. Perennial ryegrass does this by tillering. 100% light interception is usually unobtainable as even high LAI has gaps in the canopy. For this reason, 95% light interception is defined as the critical leaf area index (LAIcrit).

A study by Brougham (1957) investigated the relationship between leaf area and light interception in regrowth of pure and mixture swards of pasture species (Figure 2.2). The LAIcrit for perennial ryegrass and white clover is usually at 3.3-4.5 (Rattray *et al.*, 2007). In comparison a pure perennial ryegrass sward will has a LAIcrit of around 6-7. White clover has a LAIcrit of 3.5 (Rattray *et al.*, 2007). No literature was found for critical LAI for plantain. The differences in LAIcrit could possibly be due to the orientation of the leaves towards the sun. Perennial ryegrass has long, erect and thin leaves, whereas leaves of white clover are horizontal, making them more effective at intercepting light than perennial ryegrass.

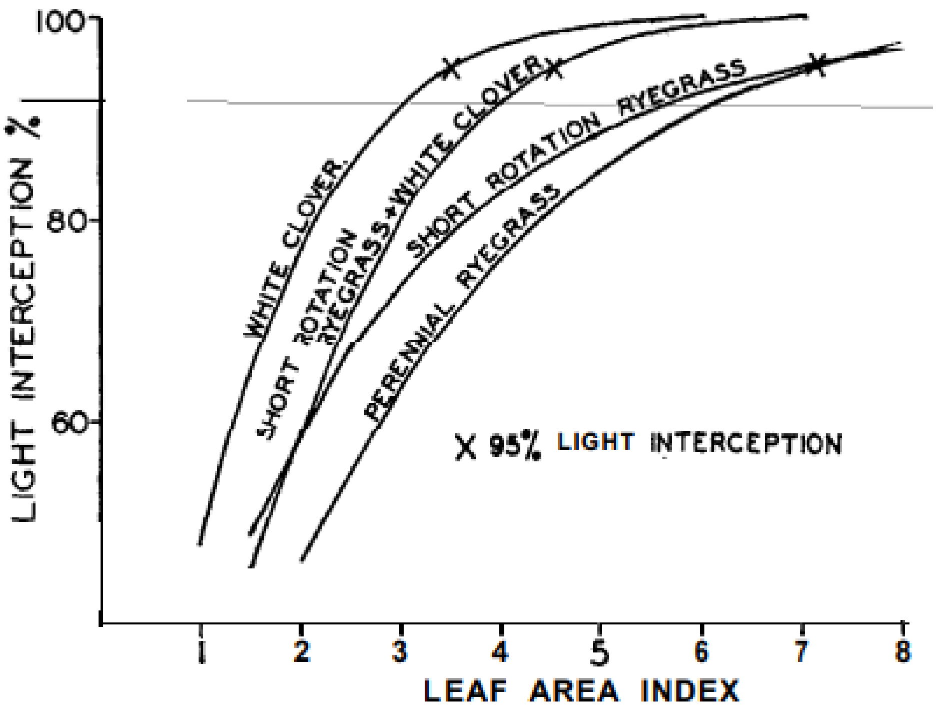

Figure 2.2. The relationship between light interception and leaf area index for four pure stands of white clover, perennial ryegrass, short rotation ryegrass and a mixture of short rotation ryegrass and white clover. Critical leaf area indices are presented and are represented by X (From Brougham, 1957).

2.11 Weed suppression

The ability to rapidly establish pasture is crucial for weed suppression as weeds can significantly reduce DM production and the quality of the feed. They compete for resources such as water, space, nutrients and light (Masters and Mitchell, 2007). Including multiple species in a pasture mix inhibits the establishment and growth of weeds (Masters and Mitchell, 2007). Average weed percentage was consistently greater in monocultures compared to mixtures (Sanderson *et al*., 2013). This demonstrates that mixtures have an ability to suppress weeds. Sowing perennial ryegrass as a monoculture has a greater ability to suppress weeds compared to ryegrass-clover mixes, which means that there is evidence for species effects for suppressing weeds. Higher proportions of white clover have negative effect on weed suppression (Sanderson *et al*., 2013). Species with different tillering rates and high tiller survival have greater expansion at the basal zone of the pasture, therefore occupying space and excluding unsown species (Harris, 2001). Mwangi *et al* (2007) stated that grasses produce a suppressive effect on invasive weed species, whereas legumes produce a facilitative effect on invasive weed species. This could be due to the role clovers play in the N cycle. This provides evidence that weed invasiveness is correlated with available nitrogen in the soil (Dukes, 2001). Pasture mixes tend to use more resources, having more closure in their canopies, intercepting more light and up taking more available nitrogen in the soil than the monocultures (Mwangi *et al*., 2007). This helps explain why pasture mixes are greater at suppressing unsown species than all pure swards.

2.12 Conclusion

To conclude this literature review, there are many factors that impact the production and persistence of species in a pasture mix. Sowing rate has an impact on pasture establishment as faster establishing plants will dominate in their mixes, especially from an autumn sowing. Perennial ryegrass is evident from the studies to be the most dominant in regard to its ability to compete for nutrients, space, water and light. White clover is slower to establish and can be outcompeted against by other sown species and weeds, especially at higher overall sowing rates. Plantain has similar establishment as perennial ryegrass by producing large erect leaves. Pasture mixes tend to use more resources, having more closure in their canopies, intercepting more light and up taking more available N in the soil, therefore having greater weed suppression compared to monocultures.

There is extensive literature on the interactions between perennial ryegrass, white clover and plantain. However, there is little evidence around LAI of these pasture species.

The aim of this dissertation was to compare how sowing rate impacts the pasture production and how species interact with each other and how proportions are impacted. Also, if N application has any effect on the sowing rate and seed mixes with regard to the proportions of each species and their yield.

Chapter 3
Materials and Methods

3.1 Experimental design

Three pasture species were selected for a mixture experiment: perennial ryegrass, white clover and plantain. A single commercially available cultivar was chosen for each species: 'Rely', 'Quartz' and 'Tonic', respectively. The selection of species and cultivars related to their ability to produce high quality pasture under irrigated conditions in mid Canterbury, New Zealand. Stewart *et al.* (2014) described their basic characteristics. 'Rely' perennial ryegrass was a diploid cultivar with; AR37 endophyte (*Epichloë festucae var. lolii* formally known as *Neotyphodium lolii*), a mid-season heading date, fine leaves and dense tillers. 'Quartz' white clover had a medium leaf size and high stolon density. 'Tonic' plantain was characterised by its erect and large leaves. The ryegrass and plantain seed had SUPERSTRIKE treatment, which was a coating of insecticide and fungicide. The white clover seed had SUPERSTRIKE CLOVER treatment, which was a lime-based coating with molybdenum, insecticide and rhizobia (*Rhizobium leguminosarum bv. trifolii*). All seed was obtained from PGG Wrightson Seeds, Kamihia, Christchurch.

Ten seed mixtures varying in species richness from one to three species and in species relative abundance were created using a simplex centroid design (Cornell, 2002) in the statistical software package MINITAB 17®. There were three monocultures, three two-species mixtures (½ of each of two species), one three-species even mixture (⅓ of each species) and three three-species mixtures dominated in turn by each species (⅔ of one species and ⅙ of each of the other species), as illustrated by the triangle diagram in Figure 3.1. The proportions of the species in a mixture summed to one.

The 10 mixtures were repeated at two levels of overall abundance based on seed count: 1000 and 2000 seeds/m^2. These two sowing rates were within the range of total sowing rates recommended for mixtures of perennial ryegrass, white clover and plantain by seed companies such as Agricom, Agriseeds and PGG Wrightson Seeds in New Zealand (Table 1.1). The data from these treatments were used to test whether there was an effect of sowing rate on the species identity and interaction effects i.e., are the species and mixture effects consistent across the range of sowing rate recommended by the seed industry in New Zealand?

The average 1000 seed weights were 2.4, 1.4 and 1.9 g, and the average germination percentages were 98, 93 and 95% for perennial ryegrass, white clover and plantain, respectively. The sowing rates were not adjusted for germination percentage. The equivalent sowing rates in kilograms of seed/ha of each species and in total are listed for the 10 mixtures at the seed count of 1000 seeds/m^2 in Table 3.1. The sowing rates for the high seed count of 2000 seeds/m^2 were double those amounts in the table.

Table 3.1. Sowing rates in kilograms of seed/ha of 10 seed mixtures varying in sown proportions of perennial ryegrass (χ_1), white clover (χ_2) and plantain (χ_3) at an overall level of abundance of 1000 seeds/m^2.

Mixture	Component			Total
	χ_1	χ_2	χ_3	
Pure	24.40	0.00	0.00	24.40
Pure	0.00	14.03	0.00	14.03
Pure	0.00	0.00	18.84	18.84
Binary	12.20	7.02	0.00	19.22
Binary	12.20	0.00	9.42	21.62
Binary	0.00	7.02	9.42	16.44
Ternary	8.13	4.68	6.28	19.09
Ternary	16.27	2.34	3.14	21.75
Ternary	4.07	9.35	3.14	16.56
Ternary	4.07	2.34	12.56	18.97

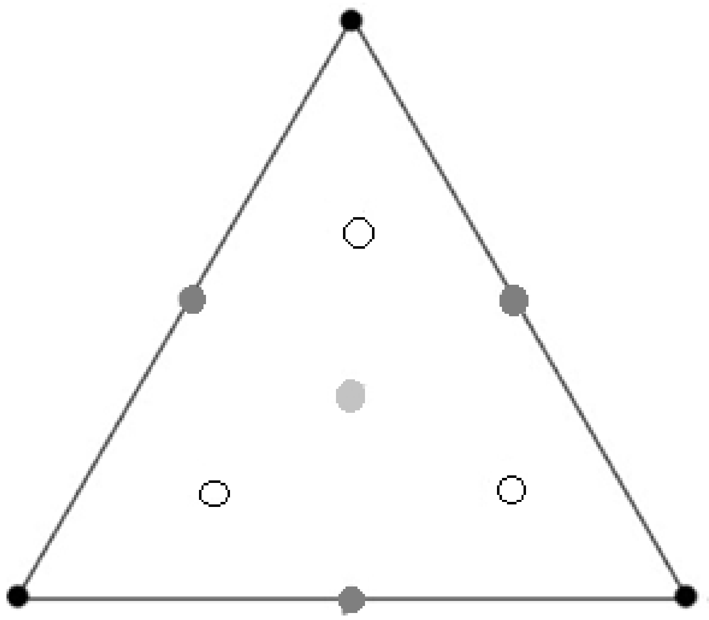

Figure 3.1. Three-species simplex centroid design with three monocultures (black circles), three two-species mixtures (½ of each of two species; dark grey), one three-species even mixture (⅓ of each species; light grey) and augmented with three three-species mixtures dominated in turn by each species (⅔ of one species and ⅙ of each other species; white).

The 20 mixture-amount combinations were repeated at two levels of applied N fertiliser: none and 100 kg/ha. The N fertiliser was urea (46% N) and there were two applications; 50 kg N/ha after sowing on the 1st of April 2017 and 50 kg N/ha on the 10th of August 2017 after the first harvest. The data from the N treatments were used to test whether the species identity, species interaction and overall abundance effects were consistent across contrasting levels of soil N availability.

The 10 mixtures, two levels of abundance and two levels of N were assigned to plots according to a split-split plot randomised complete block design with three replicate blocks. The two levels of N (0 and 100 kg N/ha) were randomly assigned to a main plot (21 m x 12 m) within each replicate, the two levels of abundance (1000 and 2000 seeds/m^2) were randomly assigned to a subplot (21 m x 6 m) within each main plot, and the 10 mixtures were randomly allocated to a sub-subplot (2.1 m x 6 m) within each subplot. A 2 m wide space was allowed between each subplot and around the perimeter of the experiment. There were 120 sub-subplots in total.

Table 3.2. Ten pasture mixes and their species proportions used for the pasture experiment at Lincoln, Canterbury 2017.

Pasture Mix	Species	RG	WC	P
1	RG	1	0	0
2	WC	0	1	0
3	P	0	0	1
4	RG*WC	½	½	0
5	RG*P	½	0	½
6	WC*P	0	½	½
7	RG*WC*P	⅓	⅓	⅓
8	RG*WC*P	⅔	⅙	⅙
9	RG*WC*P	⅙	⅔	⅙
10	RG*WC*P	⅙	⅙	⅔

The 10 different seeds mixes (the proportions of each species in the mix) were created using the mixture design in the statistical software package Minitab 17 (Table 3.2; Figure 3.1). The two seed rates were based on a plant population basis. A total seed population of 1000 and 2000 seeds per m^2 were used to acquire seed rates for all seed mixtures. This was replicated again at two different N applications. The pasture mixes are coded 1-10 and Table 3.2 gives an indication what proportions are in each mix used throughout this experiment.

3.2 Experimental site, establishment and management

The mixture experiment was carried out in paddock I3 in Iversion Field (Plate 3.1) at Lincoln University, Canterbury, New Zealand (43°38'57.2"S 172°28'00.4"E, 11 m above sea level). The soil was classified as a Wakanui silt loam with 180-350 mm of silt loam topsoil overlaying varying textual layers ranging from clay loam to sandy loams. Below 2 m in the soil profile was stony gravels and stones (Cox, 1978).

The site was previously in perennial ryegrass pasture for 2 years. It was sprayed with WEEDMASTER TS 470 (470 g/L of glyphosate at 2 L/ha) on the 16th of March 2017 and then cultivated by conventional methods into a seedbed suitable for small seeded pasture species.

A soil analysis on the 20th of March 2017 was pH 6, Olsen P 16 mg/L, Ca 7.3 me/100 g, Mg 0.98 me/100 g, K 0.40 me/100 g, Na 0.14 me/100 g and sulphate S 4 µg/g. Superphosphate (9% P, 11% S) was applied at 300 kg/ha on 23rd March 2017. The 20 mixture amount combinations were sown into the 120 sub-subplots on the 31st of March 2017 using a Flexiseeder precision drill with 14 coulters spaced 0.15 m apart. The sowing depth was 10-15 mm. METAREX (50 g/kg of metaldehyde at 8 kg/ha) was applied to control slugs (*Deroceras reticulatum*) on the 16th of May 2017.

At the first harvest on the 3rd of August 2017, the plots were cut to a 40-50 mm above ground using a rotary lawn mower to minimise plant damage. The plots were then grazed in common by sheep on the 20–24th of September 2017. The sheep removed most of the herbage and then the residual was trimmed to 30–40 mm using a rotary lawn mower.

Plate 3.2. Mixture experiment at Lincoln University, prior to harvest in September 2017.

3.3 Measurements

Immediately before the first harvest on the 3rd of August 2017, one 0.2 m^2 quadrat of representative herbage was cut using battery operated clippers to 10–20 mm above ground level in each plot. A subsample of the clippings was separated into the three sown species and weeds. The sown species were then separated into two morphological fractions: live leaf (lamina) material and stem (i.e., pseudostem of perennial ryegrass and petiole of white clover and plantain) plus any dead material. The separated material was dried with the rest of the clippings at 70˚C for at least 48 h in a force draft oven. The dry weights were applied to calculate the herbage yield, botanical composition (proportions of sown species and weeds in the herbage mass) and the yield of live leaf material of each sown species, which was required to estimate leaf area (described below).

Tiller population was measured after the first harvest on the 18th of August 2017 by digging plants from a representative 0.3 m section of a drill row in each plot. The samples were washed to remove the soil and the number of tillers (stems) of each sown species were counted and the number of tillers/m^2 calculated.

A different sampling method was used to measure yield and composition on the 13th of September 2017 before the second harvest. For yield, this method involved mowing a 6 m x 0.4 m (1.98 m^2) strip of herbage down the centre of each plot to 30–40 mm above ground. The clippings were collected and weighed, and a subsample of 200 g was dried at 70˚C for at least 48 h. The proportion of dry matter in the subsample was applied to the sample fresh weight to calculate the yield. For botanical and morphological composition, a second sample of herbage was collected from each plot by taking five random clips in the plot and combining into one sample. A subsample of the clippings was then separated into the three sown species, weeds, and live leaf and stem plus dead material of all the sown species before drying to determine the botanical composition and leaf yield (as above).

To help explain any species identity and interaction effects, and effects of sowing rate and level of applied N, light interception and canopy leaf area were also estimated at each harvest. Canopy leaf area was quantified as leaf area index (LAI), which is the green leaf area per unit area of ground. Light interception and LAI were estimated indirectly using a canopy analyser (Sunscan®, Delta-T Devices). The light sensitive "wand" of the canopy analyser was 1 m long and 15 mm high, and contained 64 photodiodes equally spaced along its length which obtain a reading of photosynthetically active radiation (PAR). This gave a measure of how developed the canopy was at intercepting light by determining how much PAR was wasted through the canopy. The wand was placed at ground level under the leaf canopy to give a reading which was repeated twice in each plot (Plate 3.2). The data received by the wand determined how much light was intercepted by the leaf canopy as a proportion (0-1) with the maximum light interception set for pasture at 0.95. Light interception was calculated as the maximum light interception (0.96) * incident light – transmitted light / incident light. This gave an indication of how much light was being intercepted by the mixture (Rattray *et al.*, 2007).

To test the accuracy of the LAI readings estimated by the canopy analyser LAI was also measured using a 'direct' technique before each harvest. Direct LAI was measured on the 3rd of August and 13th of September 2017 for ryegrass, white clover and plantain. Leaf area was measured to provide data on plant development for sown species and explain differences in yields between pasture mixtures. Plant material was only harvested from the monocultures and the three-species even mixture (mixture 7). This was to reduce the number of plots that were harvested. Harvests from the monocultures provided data for species leaf area when sown alone and in a mixture, which provided estimates of the effects of mixing species on species leaf area. Five plants of each species from each plot were destructively harvested to ensure all leaf area was harvested and plants would remain intact. The plants were cut under the ground with scissors to ensure plants remained intact and all plant material was collected. The leaves of each plant were then separated from the stem/base and placed between two sheets of plastic. A photo was taken of the leaves and the leaf area was determined by using the software programme DIGIMIZER®. Plant leaves were dried in the oven at 70°C for 48 h and weighed. The leaves were weighed (g) and weight were matched to the leaf area of each specific leaf. The specific leaf area was than calculated by dividing the leaf area by the dry weight of each individual leaf. Specific leaf area was then used to calculate the leaf area index (LAI) through the following calculation: LAI = Specific leaf area x Leaf % x the dry matter yield per ha (i.e. kg DM/ha).

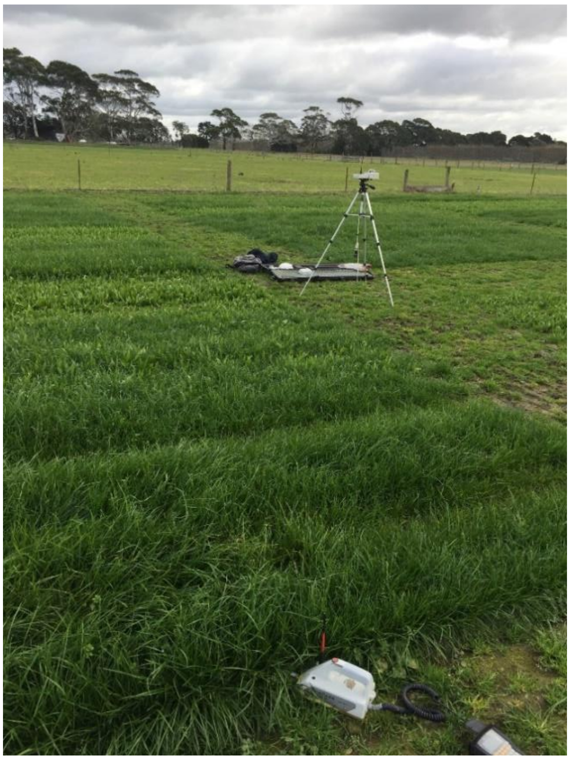

Plate 3.1. Indirect method of calculating LAI and light interception using the Sunscan® wand (bottom centre of photo) inserted level into the base of the pasture to intercept PAR on the 13th of September 2017 at Lincoln, Canterbury.

3.4 Statistical analysis

Yield was analysed using the mixture regression method in Minitab® 17 statistical software. The dry matter yields for each mix were summed up to give an overall production, weed content and species composition from sowing to the last harvest in spring on the 13th of September 2017. The data was analysed to determine if there was an interaction on yield caused by sowing rate and within each sowing rate how nitrogen impacted the yield. These were utilised to determine the optimal seed mixture for yield from sowing to spring. A special cubic model was fitted to the data. This model can separate two-species (pairwise) and multi-species interactions in the mixtures. It had the general form:

$\hat{y} = \beta 1x1 + \beta 2x2 + \beta 3x3 + \beta 12x1x2 + \beta 13x1x3 + \beta 23x2x3 + \beta 123x1x2x3 + R\pm + N\pm + \varepsilon$ **(Model 1)**

Where $\hat{y}$ is the predicted yield response from a mixture; x1, x2 and x3 are the sown proportions of ryegrass, white clover and plantain, respectively; β1 to β3 are estimates of the response of the monocultures; β12 to β23 represent the interaction effects for the combination of two species; β123 is the additional interaction effects for the combination of three species; R represents the high and low sowing rates coded 1 and -1; N represents the two nitrogen applications coded 1 and -1 and ε is a random error term, assumed to be normally and independently distributed with the mean zero and constant variance.

The analysis of variance tested if the estimates were significantly different ($P<0.05$) from zero or not. There were no t-tests or P values for the first three estimates - the predicted yields of the monocultures - because the model does not have an intercept term.

3.5 Climate

The site received 502 mm of rainfall in January to December 2016, 118 mm January to March, 321 mm in April to July 2017 and 109 mm in August to September. Average monthly temperatures range between 17˚C in January and 4˚C in July (CLIFLO, 2017).

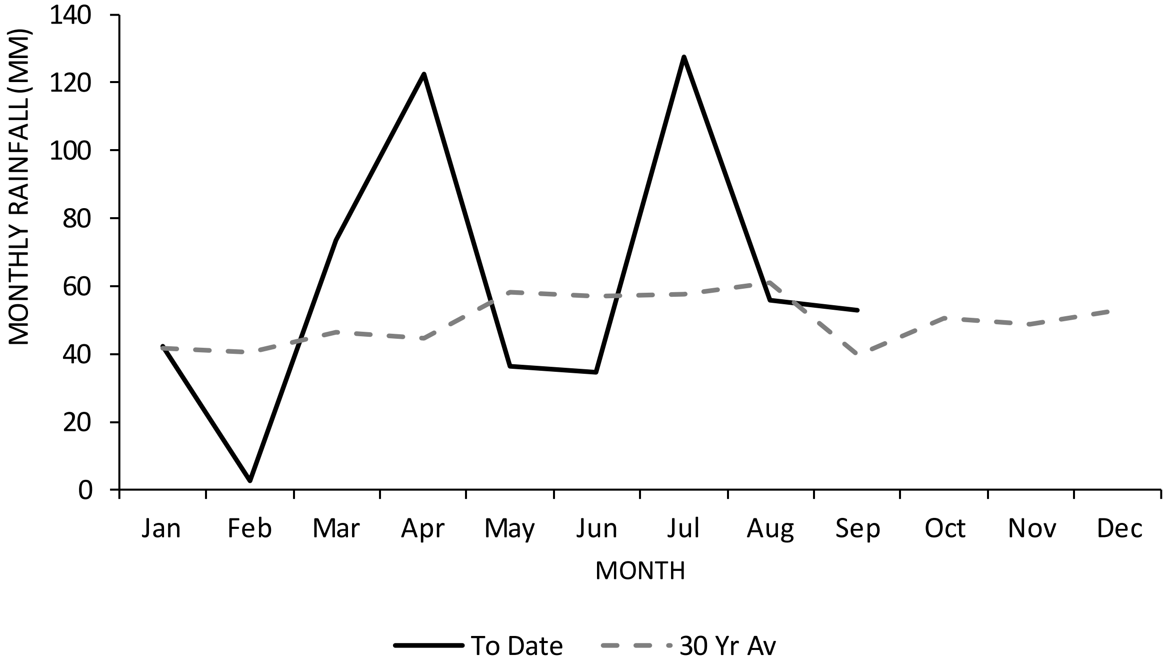

Figure 3.2. 30-year average rainfall (mm) compared to 2017 year to date rainfall (mm) for Broadfields, Lincoln.

The mean monthly soil temperature ranges from 16.6˚C in January decreasing to 4.5˚C in July during the winter months. The temperature starts to increase in August and reaches 9˚C in September.

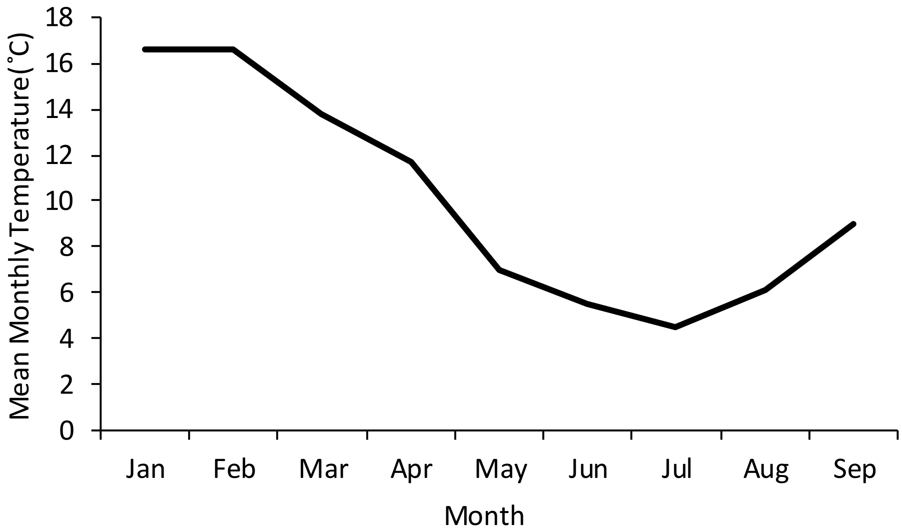

Figure 3.3. Mean average monthly 10 cm depth soil temperature for 2017 at Broadfield, Lincoln.

Chapter 4

Results

Predictions can be made from the special cubic model of mixture yield. In the analysis of variance, the highly significant ($P<0.05$) linear component of the regression indicates that the estimated yields for the monocultures were different to each other. This was backed up by the ANOVA of the actual yields ($P<0.05$). There were species effects on the mixture of two or more species. The greatest was for the ryegrass and white clover two-species mix ($P<0.05$) with ryegrass suppressing the white clover due to faster establishment from an autumn sowing. The ANOVA Tables 4.2, 4.5 and 4.8 show the quadratic component of the model was highly significant ($P<0.05$) indicating that the mean estimated yield for all the two species mixes was greater than the mean of the estimated yield of the monocultures. However, the significant values were only present for two of the two-species mixes ($P=0.021$ and 0.014 for ryegrass and white clover mixes and white clover and plantain mixes, respectively. Those species interactions could be due to the poor establishment of the white clover monocultures over the winter months, being largely dominated by weeds. Thus, there was a benefit of adding two species in a mix. There was a negative difference in adding a third species into the mix, reducing the yield compared to the two-species mixes ($P<0.05$).

Sowing rate had a highly significant effect on the yield of the first harvest ($P=0.007$). This could be since more seed allowed for the faster establishing species such as ryegrass and plantain to establish quicker and suppress the white clover and weeds. Although the sowing rate had an impact on the first harvest, at the second harvest the sowing rate had less significance ($P=0.092$) due to white clover plots beginning to yield more and all plots beginning to tiller and fill in the gaps in the canopy. Overall sowing rate did not have any significance ($P=0.577$) on the overall botanical composition of each species in their mixtures.

Nitrogen had a highly significant effect on the yield of the first harvest ($P=0.000$). This was possibly due to more available soil N. This gives the plant greater access to nutrients to have faster establishment. The second harvest also had a significant difference caused by N ($P=0.001$). This gave the ryegrass the access to nutrients which resulted in the ryegrass tillering and being able to fill in the gaps. Nitrogen also at low sowing rate gave the weeds N which is evidence that weed percentage increased with more available soil N ($P<0.05$).

4.1 August harvest

Ryegrass monocultures yield was greater than white clover and plantain (P<0.05). There was synergistic interaction between ryegrass and white clover and white clover and plantain, such that these mixes yielded more than expected based on monoculture yields (i.e. diversity effects). There was a decrease in yield for the three species mix compared to the average yield of the two species mixes. Ryegrass yield increase with N (P=0.019), but not for white clover and plantain. Species interactions were more consistent across both levels of N and across both sowing rates.

Table 4.1. Estimate regression coefficients and analysis of variance for mixture model of yield (kg DM/ha) for harvest one on the 4th of August 2017 at Lincoln University, Canterbury.

Term	Coef	SE	P
RG	3093	174.5	*
WC	551	174.5	*
P	2068	174.5	*
RG*WC	2054	878.6	0.021
RG*P	571	878.6	0.517
WC*P	2202	878.6	0.014
RG*WC*P	-11855	5790.2	0.043
N	59	174.5	0.737
Rate	61	174.5	0.729
RG*N	581	243.4	0.019
WC*N	75	243.4	0.757
P*N	-226	181.34	0.146
RG*WC*N	-555	878.3	0.529
RG*P*N	-351	878.3	0.69
WC*P*N	368	878.3	0.676
RG*WC*P*N	4041	5790.2	0.487
RG*Rate	142	243.4	0.562
WC*Rate	-117	243.4	0.631
P*Rate	-102	181.34	0.576
RG*WC*Rate	1435	878.3	0.106
RG*P*Rate	1184	878.3	0.181
WC*P*Rate	76	878.3	0.932
RG*WC*P*Rate	-4318	5790.2	0.458
RG*N*Rate	225	174.5	0.201
WC*N*Rate	-132	174.5	0.453
P*N*Rate	-41	174.5	0.813
RG*WC*N*Rate	629	878.3	0.476
RG*P*N*Rate	105	878.3	0.905
WC*P*N*Rate	-112	878.3	0.898
RG*WC*P*N*Rate	-4408	5790.2	0.448

Table 4.2. Parsimonious mixture model of estimate regression coefficients and analysis of variance for mixture model of yield (kg DM/ha) for harvest one on the 4th of August 2017 at Lincoln University, Canterbury.

Term	Coef	SE	P
RG	3093	167.18	*
WC	551	167.18	*
P	2068	167.18	*
RG*WC	2054	841.55	0.016
RG*P	571	841.55	0.499
WC*P	2202	841.55	0.010
RG*WC*P	-11855	5548.07	0.035
N	371	83.32	0.000
Rate	227	83.32	0.007
RG*N	452	172.94	0.010
RG*WC*Rate	1247	692.9	0.075
RG*P*Rate	1277	692.9	0.068
RG*N*Rate	195	119.03	0.104

The non-significant interaction terms were removed from the special cubic model and then analysis was repeated with a more parsimonious model, which has more degrees of freedom for the residual (Table 4.2; R^2Adj=62.06%). This model shows the estimated monoculture yields are still similar as the linear component is significant (P=0.003). But the quadratic and special cubic components remained significant and non-significant with P-values of 0.037 and 0.186, respectively. The presence of the three-species mix term supports the choice of a simplex mixture design. The more parsimonious model is as follows:

$$Y=(t\ DM/ha) = 3.093_{x1} + 0.551_{x2} + 2.068_{x3} + 2.054_{x1x2} + 2.202_{x2x3} - 11.855_{x1x2x3} + 0.227_{S} + 0.371_{N} + \mathcal{E}$$

The actual yields observed of the pasture mixes were compared against a respective estimated pasture yield (Figure 4.1). Ryegrass monocultures at high sowing rate and high N produced an actual yield higher than the estimate (3691 and 4327 kg DM/ha respectively) at a high sowing rate and low N the estimated yield was greater than actual (2949 and 2430 kg DM/ha respectively). At a low sowing rate and at both application of N the estimated and actual yields was similar. Interestingly, white clover and plantain monocultures did not follow suit, producing lower actual yields (419 and 2142 kg DM/ha) at a high sowing rate and high N compared with the estimates (1149 and 2666 kg DM/ha), and similar actual yields when compared with the estimates. All two species mixtures at both sowing rates and N applications appeared to produce similar actual yields when compared to their respective estimates. This gives a good indication that the model is a good fit for this data. Once a third species was included in the mix the estimates and actuals became varied. This indicated that the model was not a good fit for the three-species data. The white clover dominant three species mix (pasture mix 9) showed that it would produce higher actual yield than the estimates across all treatments.

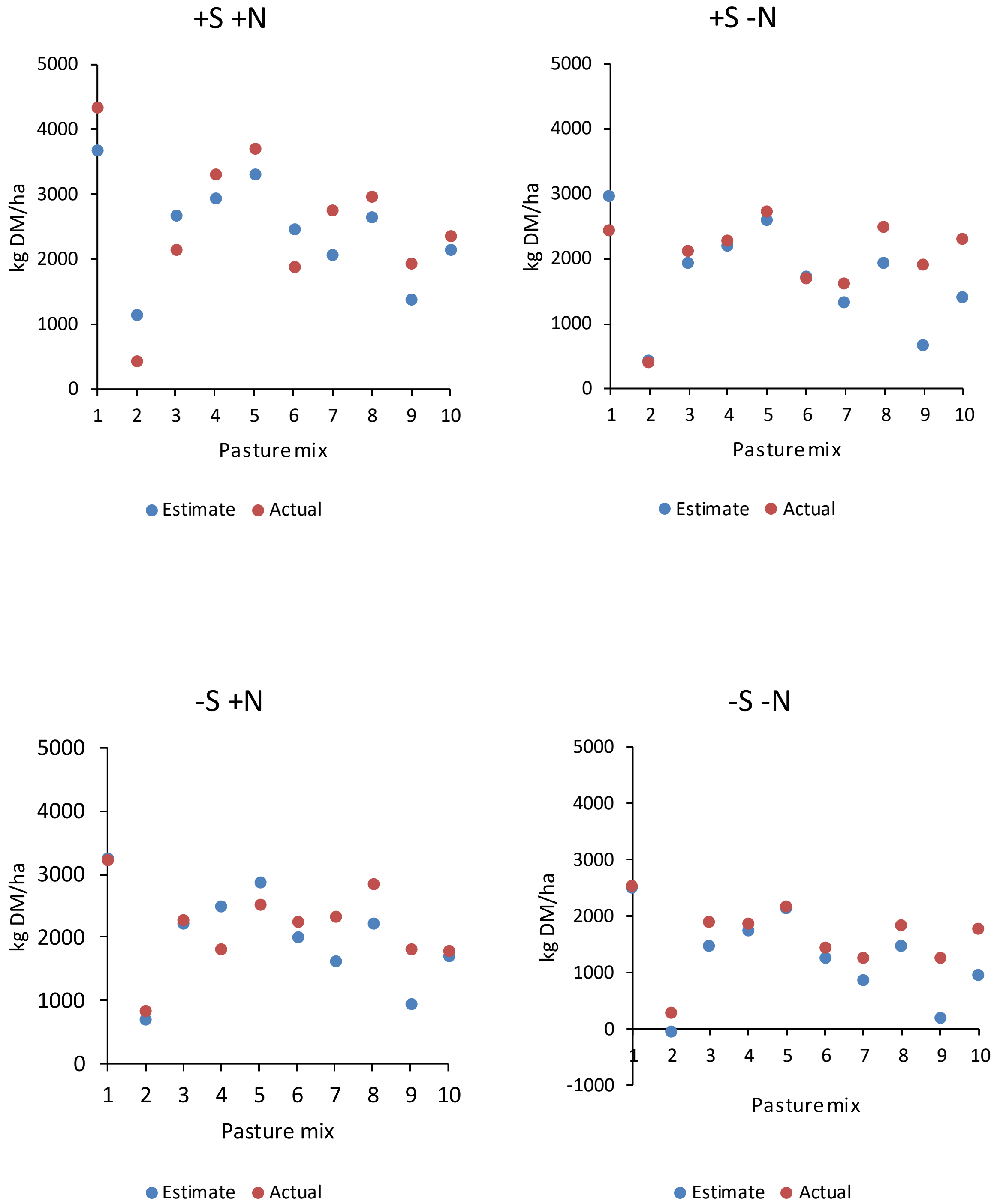

Figure 4.1. Estimated and actual yield (kg DM/ha) from the 4th of August 2017 harvest of the 10 pasture mixes at Lincoln University, Canterbury (+S represents high sowing rate, -S represents low sowing rate, +N represents high level of N and -N represents low level of N).

Ryegrass was the dominant pasture species involved due to its higher yield as shown by the colour distribution of the multiple mixture contour plot charts (Figure 4.2). At a low N application (left hand charts), the ryegrass yielded between 2000-2500 kg DM/ha, and upwards of 3500 kg DM/ha at a high seed rate and high N (right hand charts). The white clover yielded low at both seed rates and N applications between 500-1000 kg DM/ha and some below 500 kg DM/ha, while plantain showed an increase from 1500-2000 kg DM/ha at low rate up to 2000-2500 kg DM/ha at high rate across both N applications. All treatments were consistent across species effects with ryegrass dominating across all mixes. Ryegrass responded more to N than plantain and white clover.

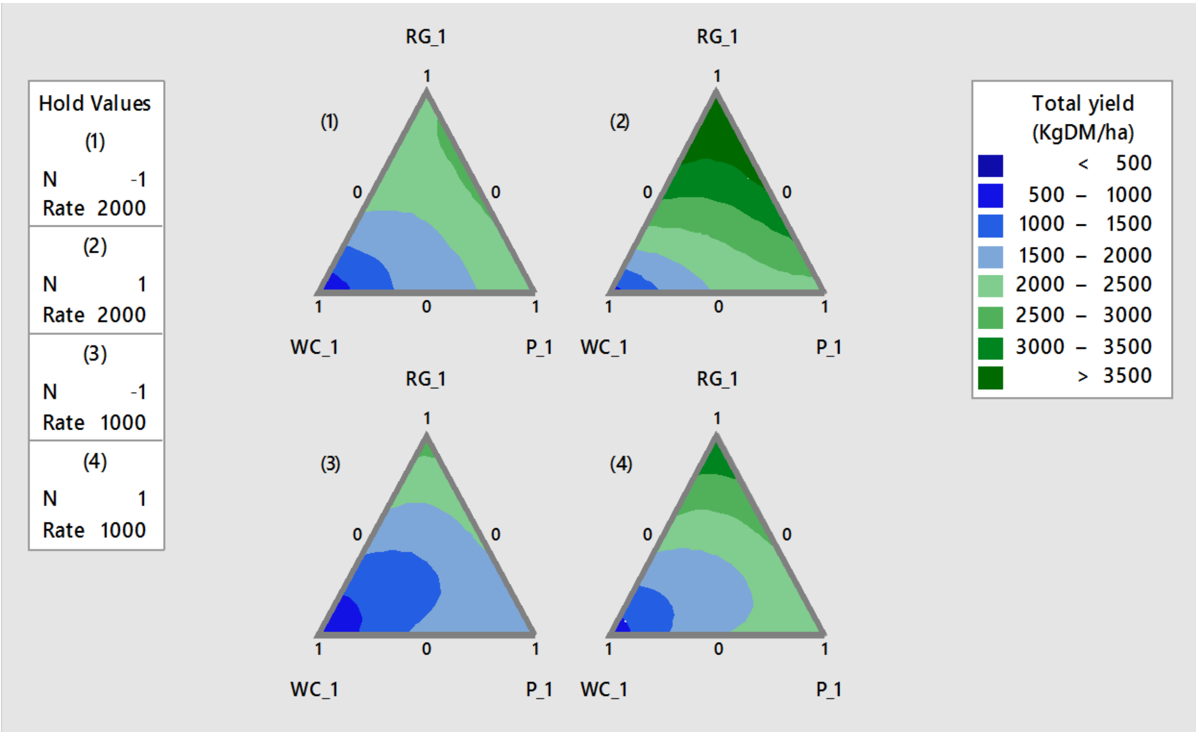

Figure 4.2. Multiple mixture contour plot of yield (kg DM/ha from the 4th of August 2017 harvest in response to mixture proportions of RG, WC and P, at two sowing rates of 1000 and 2000 seeds/m^2 and two levels of N (0 and 100 kg N/ha) at Lincoln University, Canterbury.

Proportions of perennial ryegrass, white clover and plantain in the herbage yield of the two species mixtures and three species mixtures were dominated by perennial ryegrass and plantain (Figure 4.3). White clover proportions were on average 0.011 when sown in a two-species and three-species mixture with perennial ryegrass and/or plantain. The proportion of perennial ryegrass was on average 0.768 when sown in a two-species mixture and 0.548 when sown in a three-species mixture. The proportion of plantain was on average 0.722 when sown in a two-species mixture and 0.432 when sown in a three-species mix. Ryegrass was observed to be the dominant species in almost every pasture mix it is included in, although overtaken by plantain in the plantain dominant mixes.

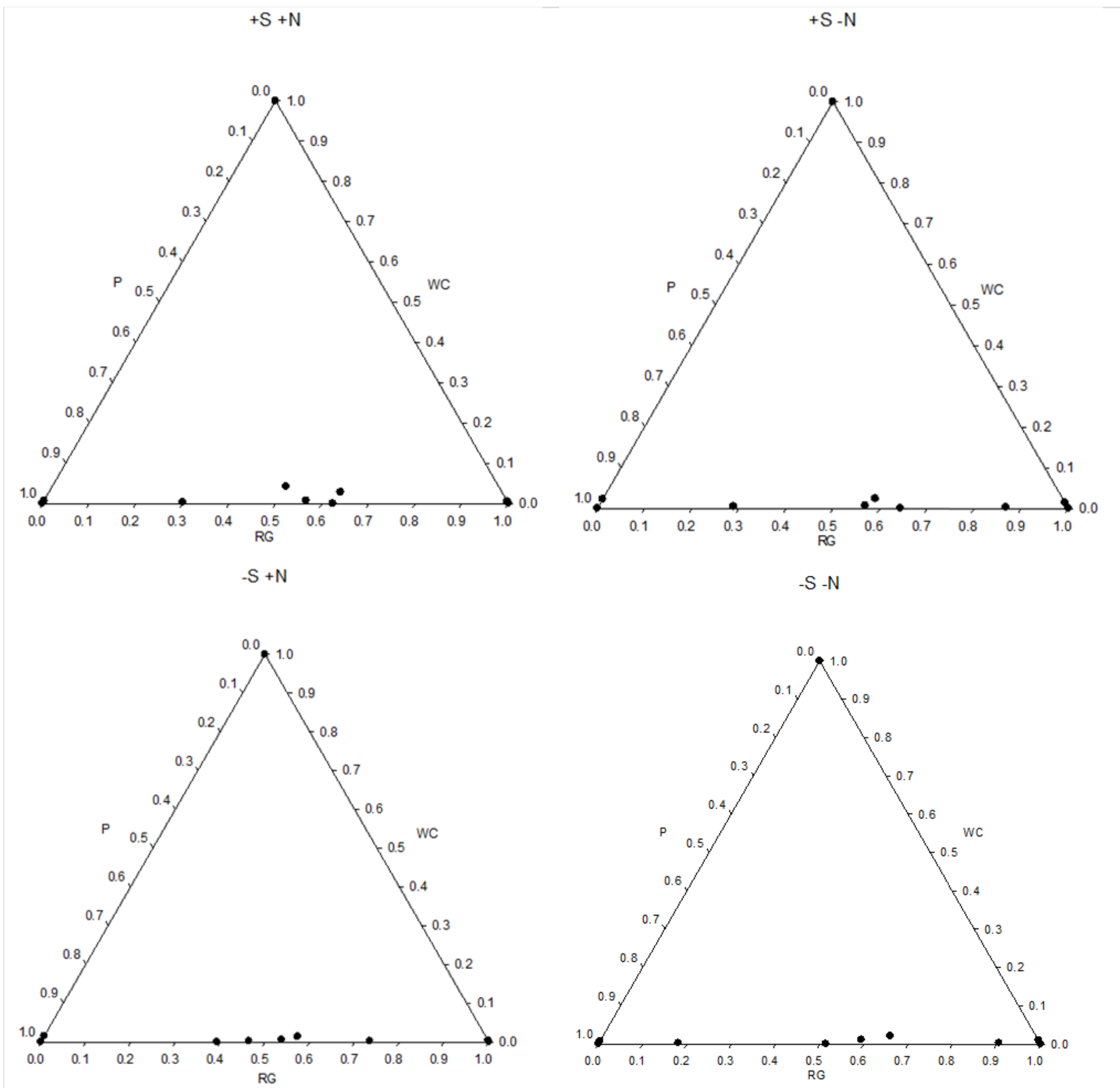

Figure 4.3. Proportions of perennial ryegrass, white clover and plantain in 10 pasture mixtures across two sowing rates and two levels of N applications on the 4th of August 2017 at Lincoln University, Canterbury. (+S represents high sowing rate, -S represents low sowing rate, +N represents high level of N and -N represents low level of N).

When ryegrass was present, weeds were suppressed to less than 20% of the plant material composition (P<0.05) (Figure 4.4). The white clover monoculture had a poor establishment so increased proportions of weeds at both seed rates and N applications, expressing compositions of excess 90% weeds for each sowing rate and N treatment respectively. Species and mixture effects were consistent across both sowing rates and N applications, with a slight increase in weed proportions in the low sowing rate although not significantly different.

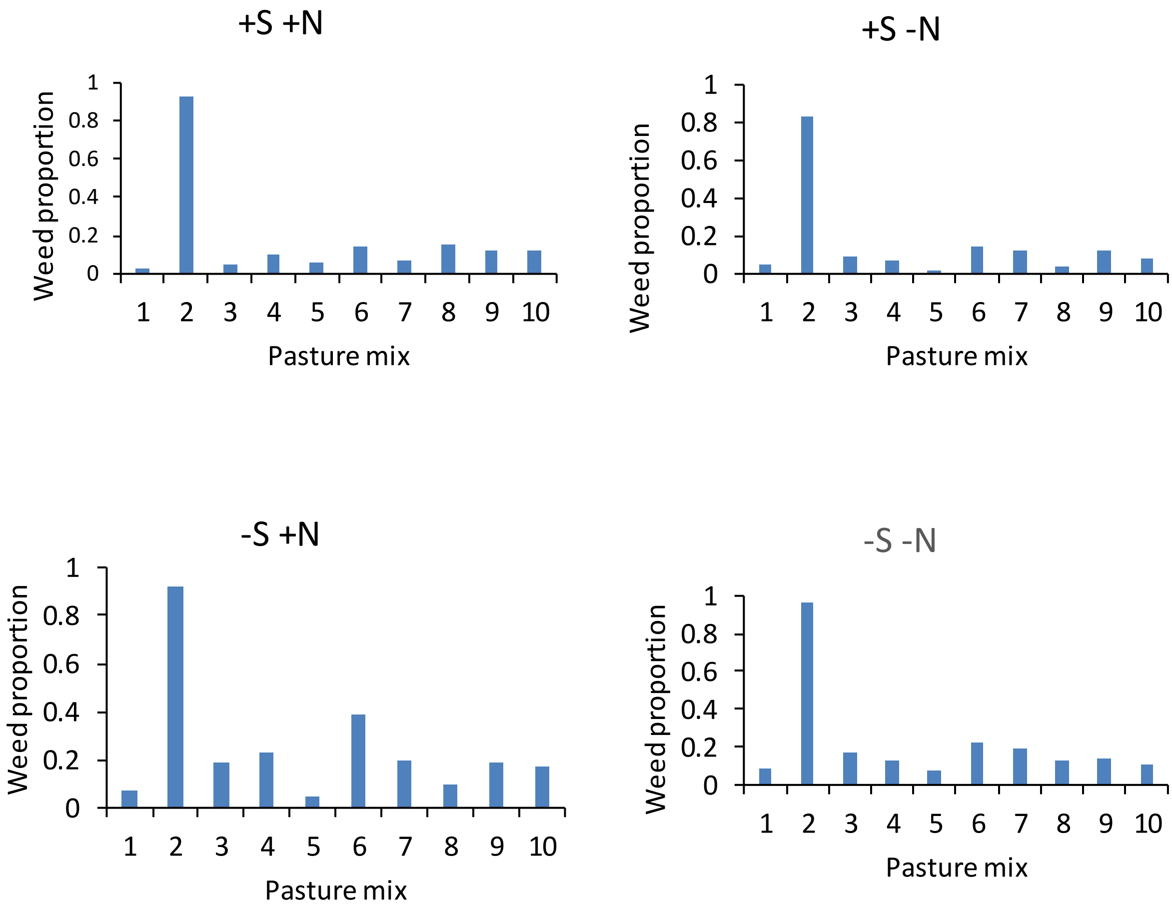

Figure 4.4. Average sum of weed proportion of perennial ryegrass, white clover and plantain and their mixes at the first harvest on the 4th of August 2017, at Lincoln University, Canterbury. (+S represents high sowing rate, -S represents low sowing rate, +N represents high level of N and -N represents low level of N).

The average LAI for the SUNSCAN® ranged between 0.1 to 8.8 over all pasture mixtures (Figure 4.5). An increase in LAI responded with an increase in yield (R^2=0.5918). Perennial ryegrass monoculture had LAI above 2.5 to 8.8 and plantain monoculture ranged between 0.7 to 4.3. White clover monoculture LAI ranged between 0.1 to 1.5. All two and three species mixtures LAI were dominated by ryegrass and plantain with little contribution form clover due to poor establishment across all mixtures. Nitrogen had a significant effect on the ryegrass LAI (P=0.027) due to the ability available N gives ryegrass by producing bigger leaves and dense tillering, this produced canopy closure earlier than the other species.

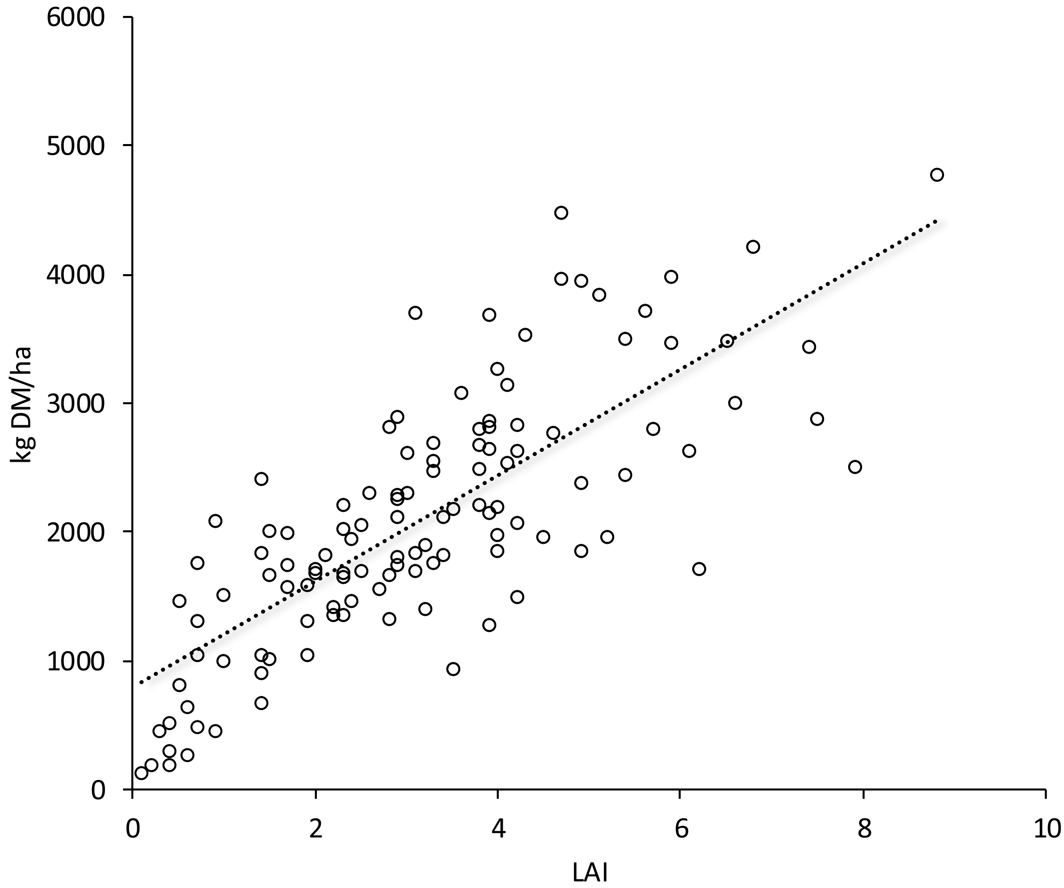

Figure 4.5. Indirect measurement of leaf area index (LAI) using a Sunscan® meter of all the species and their mixtures in relation to total dry matter production on the 4th of August 2017 at Lincoln University, Canterbury.

The direct average LAI for all pasture mixes ranged between 0.01 to 8.35 (Figure 4.6). An increase in LAI responded with an increase in yield (R^2=0.8982). Perennial ryegrass monoculture had LAI above 3.4 to 8.35 and plantain monoculture ranged between 1.5 to 4.76. White clover monoculture LAI ranged between 0.01 to 0.21. All two and three species mixtures LAI were dominated by ryegrass and plantain with little contribution form clover due to poor establishment across all mixtures. Nitrogen had a significant effect on the ryegrass LAI (P=0.048) due to the ability available N gives ryegrass by producing bigger leaves and dense tillering, this produced canopy closure earlier than the other species. The correlation between using direct and indirect measurements is 0.75 (Figure 4.7), this indicates that using direct method or indirect methods are viable methods for estimating the LAI of the pasture swards.

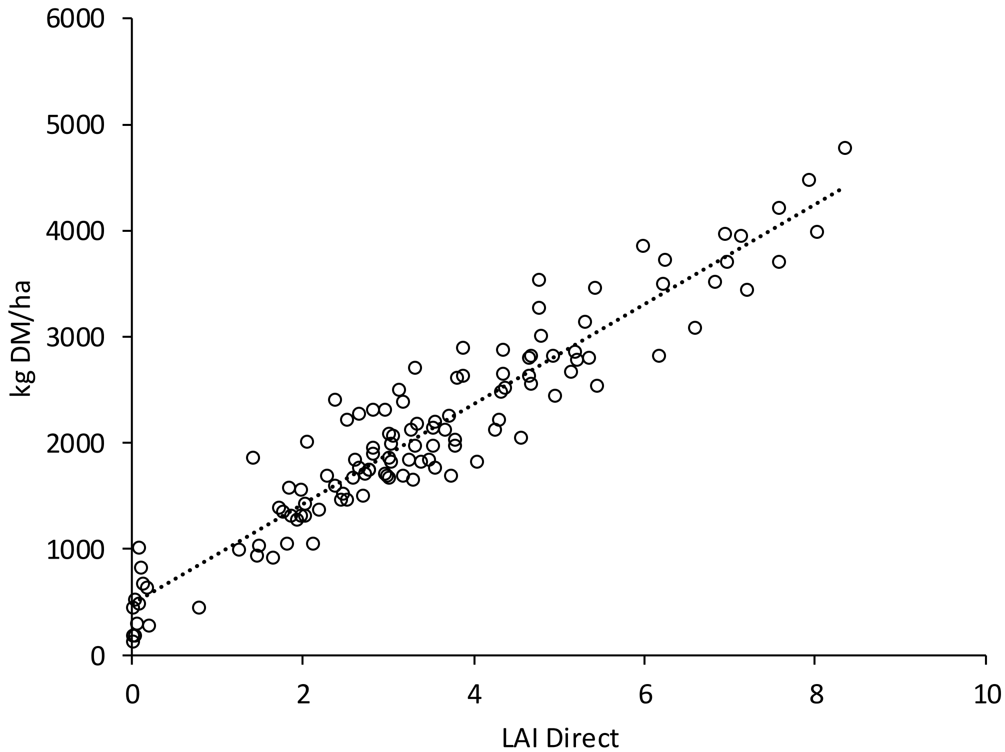

Figure 4.6. Direct measurements of leaf area index (LAI) of all the species and their mixes in relation to total dry matter production on the 4th of August 2017 at Lincoln University, Canterbury.

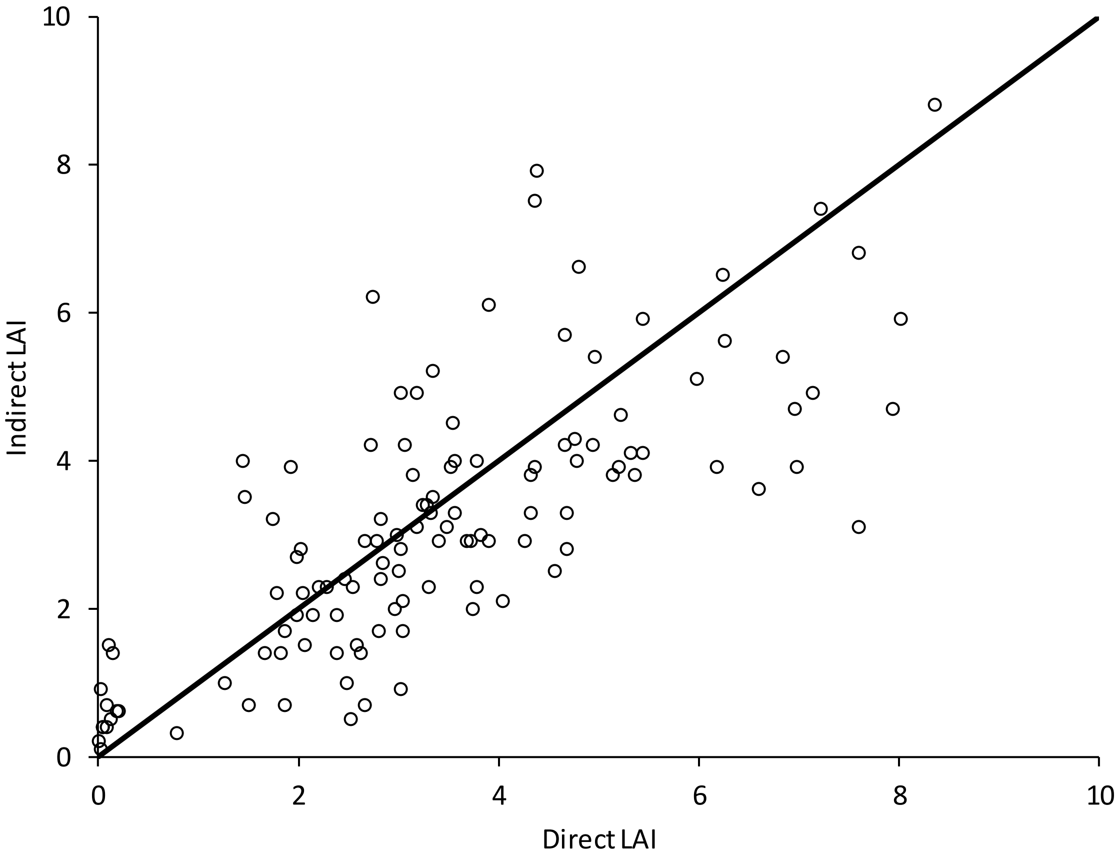

Figure 4.7. Correlation between direct leaf area index (LAI) measurements and indirect leaf area index (LAI) methods on the 4th of August 2017 at Lincoln University, Canterbury.

As LAI increases so too does light intercepted using the indirect method. When the pasture mix reached 4 LAI it would be intercepting maximum light (0.95 I/Io). This was consistent across all pasture mixes and their treatments (R^2=0.9985). The white clover monocultures and two species mixtures with plantain did not reach this critical level and as a result did not reach canopy closure, causing the weed population to increase as not all the light is being intercepted by the sown species.

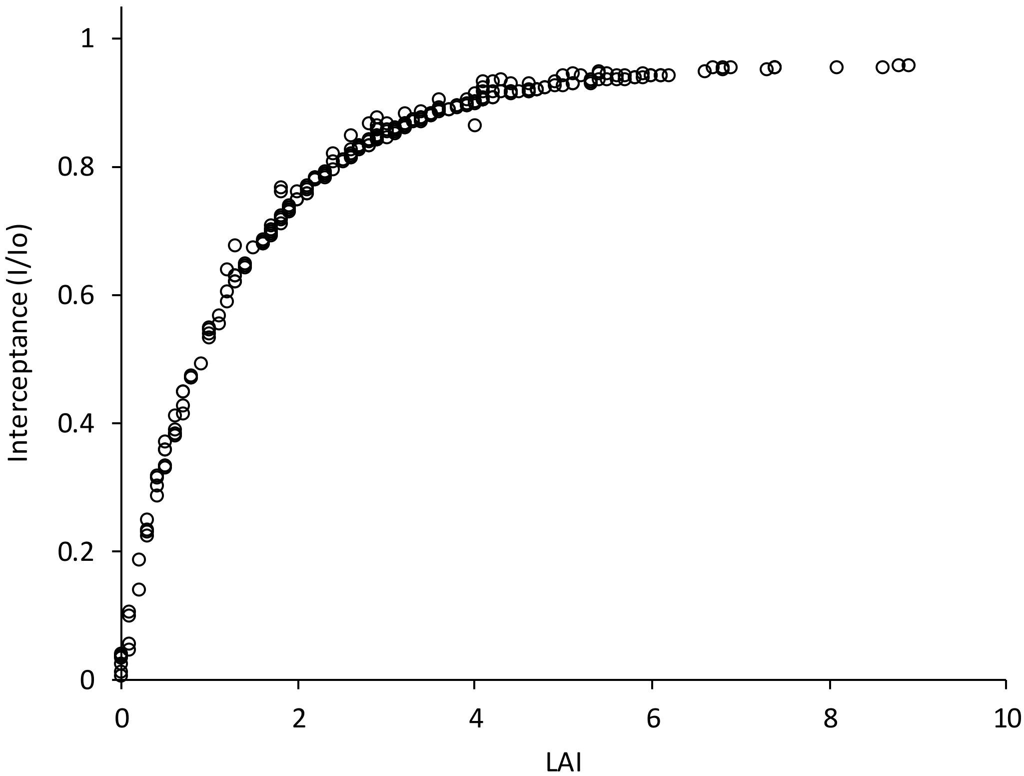

Figure 4.8. Relationship of light interception and indirect leaf area index (LAI) of ryegrass, white clover and plantain and their mixtures on the 4th of August 2017 at Lincoln, Canterbury.

Figure 4.9 describes the proportions of the data in Figure 4.8 by as measuring the LAI for the all the monoculture plots for each species. It is obvious that white clover monoculture did not reach the critical LAI 0.95 due to poor/slow establishment and competition with weeds (R^2=0.6329), plantain also was the 'mid-range' for the LAI for this experiment although not reaching the critical LAI was close (R^2=0.7532). Ryegrass had significant impact on the LAI for this trial, the ability to use available N and tiller to compensate in gaps allowed for canopy closure at critical LAI to be reached, giving it the optimum resources to be the dominate species (R^2=0.9485).

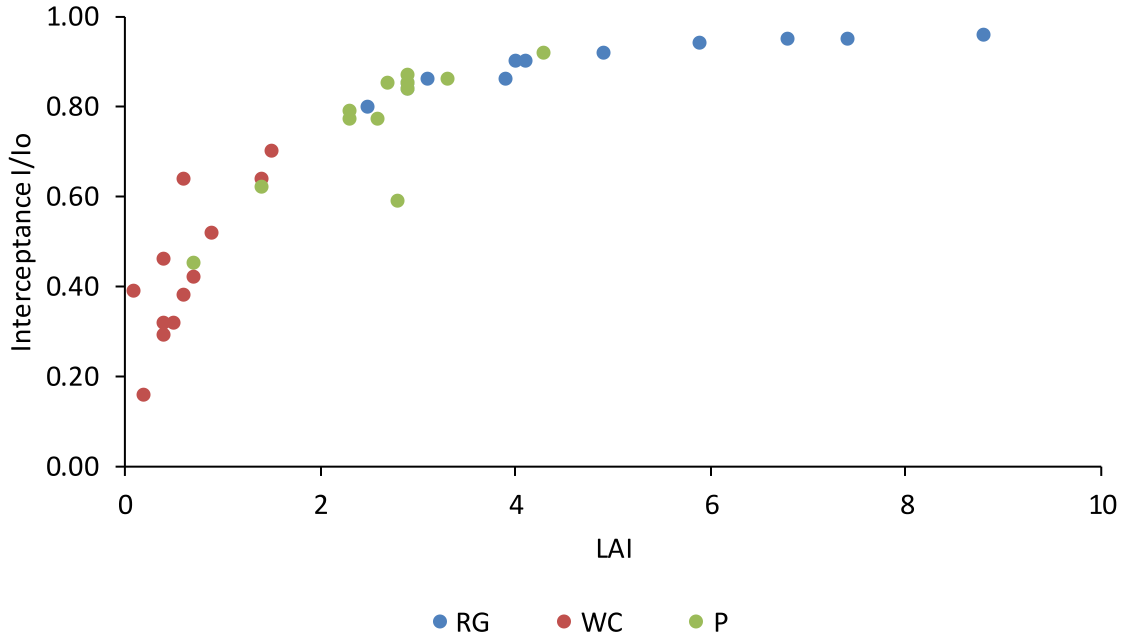

Figure 4.9. Relationship of light interception and the LAI of ryegrass, white clover and plantain monocultures on the 4th of August 2017 at Lincoln, Canterbury.

4.2 September harvest

White clover and plantain two species mix was no longer significant compared to the first harvest due to the weeds significantly growing in the white clover monoculture.

Table 4.3. Estimate regression coefficients and analysis of variance for mixture model of yield (kg DM/ha) for harvest two on the 13th of September 2017 at Lincoln University, Canterbury.

Term	Coef	SE	P
RG	3075	173.8	*
WC	2115	173.8	*
P	2442	173.8	*
RG*WC	2738	874.7	0.002
RG*P	1493	874.7	0.091
WC*P	1300	874.7	0.141
RG*WC*P	-8895	5766.9	0.126
Rate	180	173.8	0.303
N	118	173.8	0.498
RG*Rate	103	242.4	0.672
WC*Rate	153	242.4	0.529
P*Rate	-34	175.85	0.848
RG*WC* Rate	-823	874.7	0.349
RG*P*Rate	-609	874.7	0.488
WC*P*Rate	659	874.7	0.453
RG*WC*P*Rate	13847	5766.9	0.018
RG*N	222	242.4	0.363
WC*N	-17	242.4	0.944
P*N	-57	175.85	0.749
RG*WC*N	-947	874.7	0.282
RG*P*N	-945	874.7	0.283
WC*P*N	-127	874.7	0.885
RG*WC*P*N	3312	5766.9	0.567
RG*N*Rate	67	173.8	0.7
WC*N*Rate	-290	173.8	0.099
P*N*Rate	100	173.8	0.568
RG*WC*N*Rate	1923	874.7	0.03
RG*P*N*Rate	202	874.7	0.817
WC*P*N*Rate	808	874.7	0.358
RG*WC*P*N*Rate	-9998	5766.9	0.086

Table 4.4. Parsimonious mixture model of estimate regression coefficients and analysis of variance for mixture model of yield (kg DM/ha) for harvest two on the 13^{th} of September 2017 at Lincoln University, Canterbury.

Term	Coef	SE	P
RG	3075	168.51	*
WC	2115	168.51	*
P	2442	168.51	*
RG*WC	2738	848.51	0.002
RG*P	1493	848.26	0.081
WC*P	1300	848.26	0.128
RG*WC*P	-8895	5592.27	0.115
N	232	68.75	0.001
Rate	94	55.12	0.092
RG*WC*P*N	12300	4437.43	0.007
WC*N*Rate	-193	147.36	0.193
RG*WC*N*Rate	1816	737.44	0.015
RG*WC*P*N*Rate	-5029	4370.54	0.252

The non-significant interaction terms except rate and N were removed from the special cubic model and then analysis was repeated with a more parsimonious model, (Table 4.5; R^2Adj=41.33%). This model shows the estimated monoculture yields are still similar as the linear component was significant (P=0.003). But the quadratic and special cubic components remained significant and non-significant with P-values of 0.037 and 0.186, respectively. The presence of the three-species mix term supports the choice of a simplex mixture design. The more parsimonious model is as follows:

$$Y= (t\ DM/ha) = 3.075_{x1} + 2.115_{x2} + 2.442_{x3} + 2.735_{x1x2} - 8.895_{x1x2x3} + 0.094_{S} + 0.232_{N} + \varepsilon$$

Species effects are starting to become more apparent in the second harvest. This is due to the species being well established and competing against each other. Clover was yielding greater due to warmer spring temperatures. Ryegrass and plantain mixes (5) started to yield similar to the ryegrass monocultures, this is a beneficial increase in yield due to two species interacting. At low sowing rate and no N application the ryegrass and white clover mix (4) also had a greater yield compared to the monocultures ($P<0.05$), this could possibly due to the clover being able to biologically fix N and provide the ryegrass with available N which promotes growth. The ryegrass monoculture in this treatment would have no/little available N. Three-species mixtures with the addition of N across both sowing rates had an increase in yield similar to the two-species mixes (Figure 4.10) which provides evidence that N has the ability to promote growth in all species ($P<0.05$). Without the addition of N the three-species mixes yield was poorer than the two-species mixes ($P<0.05$). Figure 4.10 compared to Figure 4.1 demonstrates that the modelled data are becoming a better fit for the actual data.

Ryegrass monocultures at high sowing rate and high N produced an actual yie ld higher than the estimate (3401 and 3958 kg DM/ha respectively) all other treatments actual yields were similar to the estimated respectively. Interestingly, white clover had increased yield from the first harvest. This is due to increasing temperatures increasing in the spring, although the bulk is still dominated by weeds. All estimates were similar to actual yields for both white clover and plantain treatments. All two species mixtures at both sowing rates and N applications appear to produce similar actual yields when compared to their respective estimates, this gives a good indication that the model is a good fit for this data. Except for ryegrass and white clover mix which produced a lower actual yield compared to estimated, at high sowing rate and low N (2610 and 3142 kg DM/ha, respectively). The three-species mixtures again produced varied results with the bulk of the data producing higher actual yields compared to estimates, this indicates the model is not a good fit for the three-species data. White clover again still contributed lower yields to the total yield for harvest two due to its poor ability to establishment, but had increased yield from harvest one due to the temperature increasing.

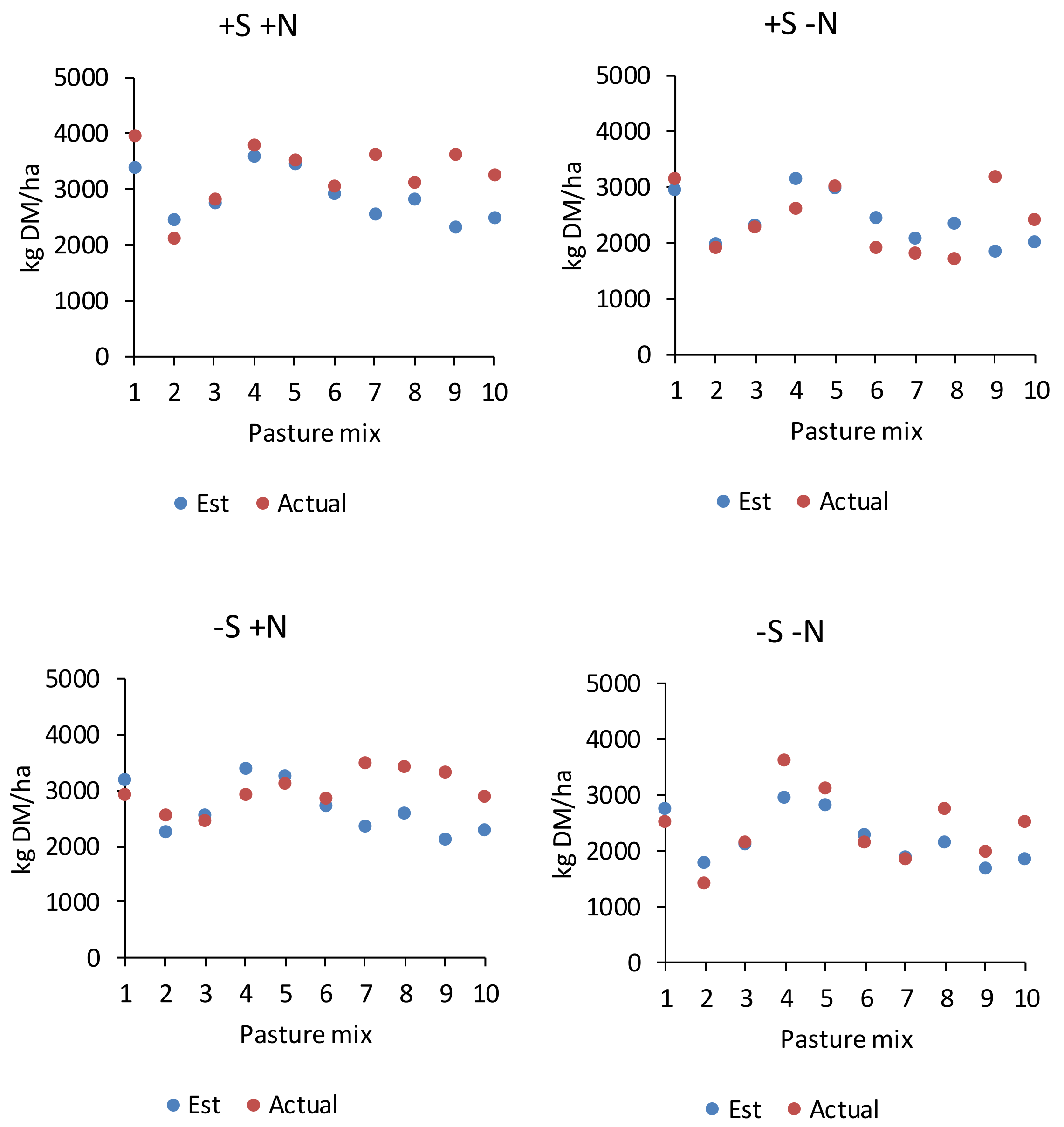

Figure 4.10. Estimated and actual yield (kg DM/ha) from the 13th of September 2017 harvest of the 10 pasture mixes at Lincoln University, Canterbury. (+S represents high sowing rate, -S represents low sowing rate, +N represents high level of N and -N represents low level of N).

Ryegrass was the dominant pasture species involved due to its higher yield as shown by the colour distribution of the multiple mixture contour plot charts (Figure 4.11). At a low N application (bottom charts), the ryegrass yielded between 2000-2500 kg DM/ha, and upwards of 3500 kg DM/ha at a high seed rate and high N (right hand charts). The white clover yielded low at both seed rates and N applications between <2000 kg DM/ha, while plantain showed an increase from 2000 kg DM/ha at low rate up to 2500 kg DM/ha at high rate across both N applications. Sowing rates were consistent across N applications. Species effects are becoming more apparent in this harvest with ryegrass and white clover mixes starting to demonstrate a benefit in yield once adding the two species. This results in an addition of 500 kg DM/ha compared to the monocultures of each.

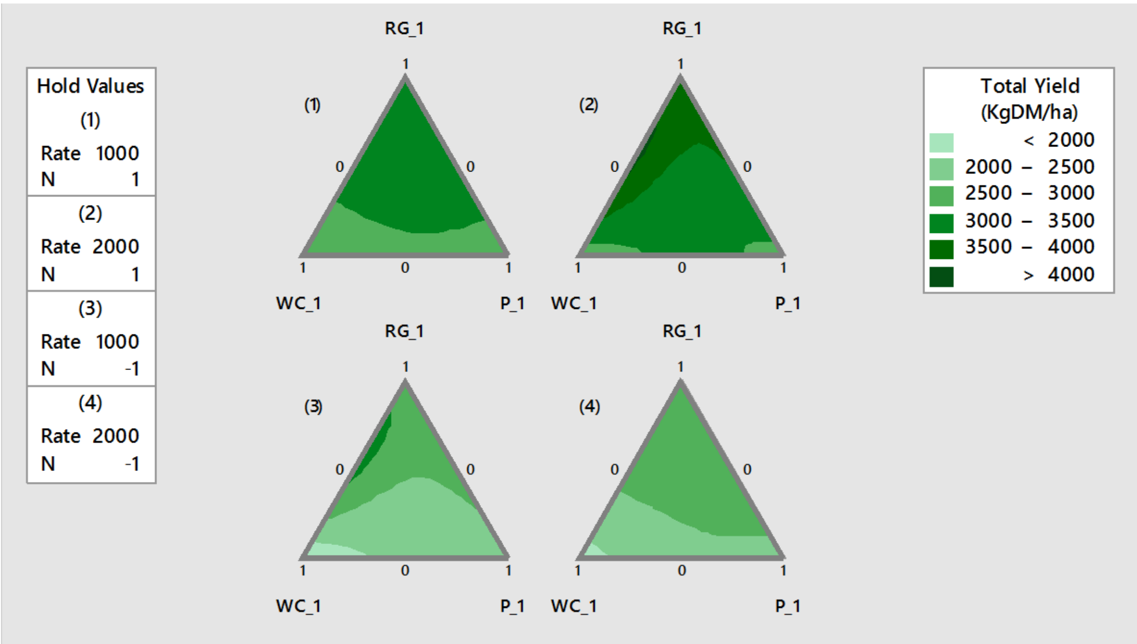

Figure 4.11. Multiple mixture contour plot of yield (kg DM/ha) on the 13th of September 2017 harvest in response to mixture proportions of RG, WC and P, at two sowing rates of 1000 and 2000 seeds/m^2 and two levels of N (0 and 100 kg N/ha) at Lincoln University, Canterbury.

Proportions of perennial ryegrass, white clover and plantain in monocultures, two species mixtures and three species mixtures were dominated by perennial ryegrass and plantain (Figure 4.12). White clover proportions were on average 0.04 when sown in a two-species and 0.025 three-species mixture with perennial ryegrass and/or plantain, this has increased from 0.01 in the August harvest. The proportion of perennial ryegrass was on average 0.81 when sown in a two-species mixture and 0.64 when sown in a three-species mixture which also has increased in proportion since the August harvest. The proportion of plantain has decreased from the first harvest and was on average 0.65 when sown in a two-species mixture and 0.34 when sown in a three-species mix. Ryegrass was observed to be the dominant species in almost every pasture mix it is included in, although overtaken by plantain in the plantain dominant mixes.

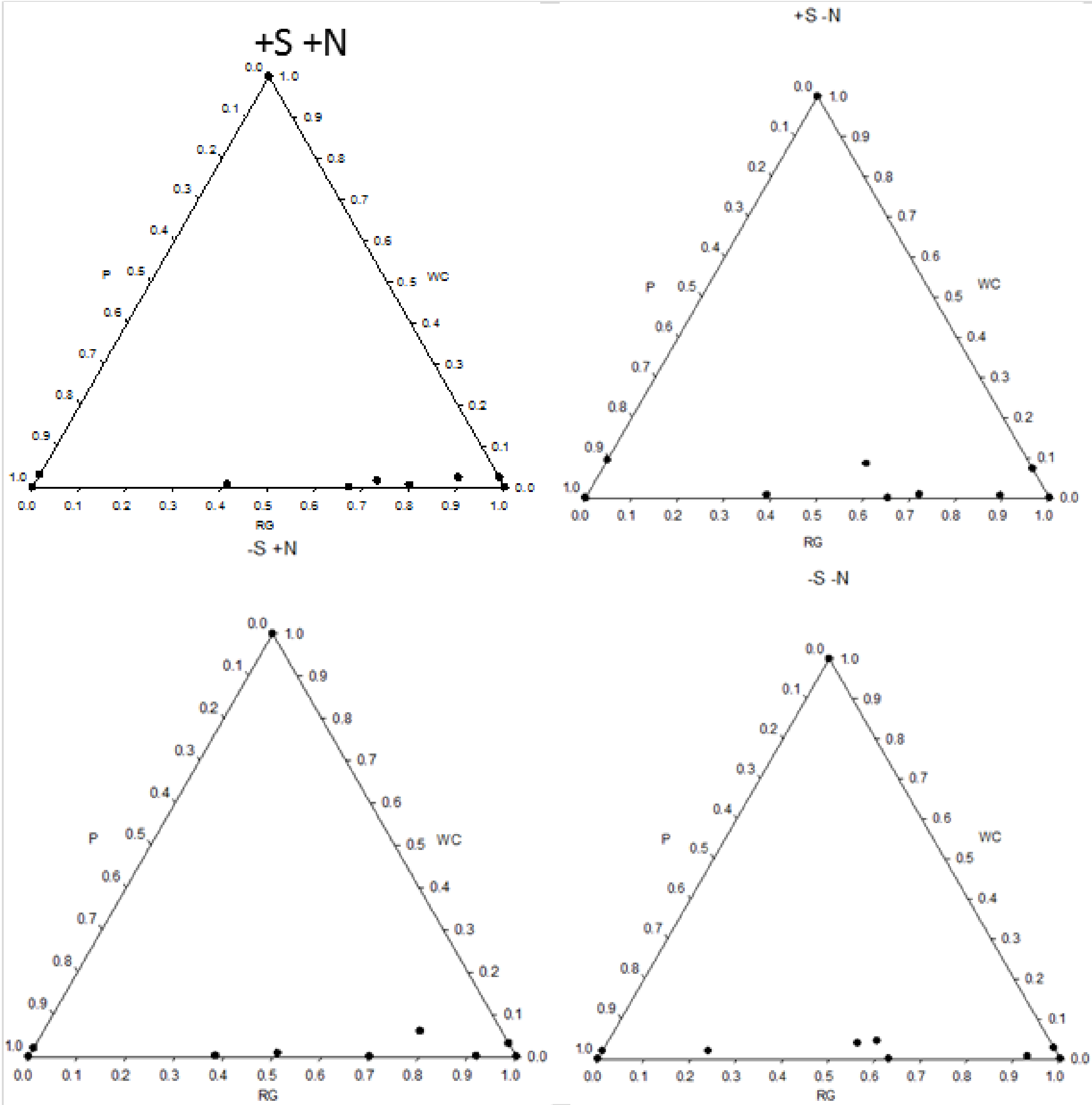

Figure 4.12. Proportions of perennial ryegrass, white clover and plantain in 10 pasture mixtures across two sowing rates and two levels of N applications on the 13th of September 2017 at Lincoln, Canterbury. (+S represents high sowing rate, -S represents low sowing rate, +N represents high level of N and -N represents low level of N).

When ryegrass was present, weeds were suppressed to less than 20% of the plant material composition (P<0.05) except for the white clover dominant mix (8) which had 53.6% of weeds (Figure 4.13). The white clover monoculture was heavily outcompeted by weeds at both seed rates and N applications due to its poor establishment, expressing compositions of excess 86% weeds for each sowing rate and N treatment respectively. Species and mixture effects were consistent across both sowing rates and N applications

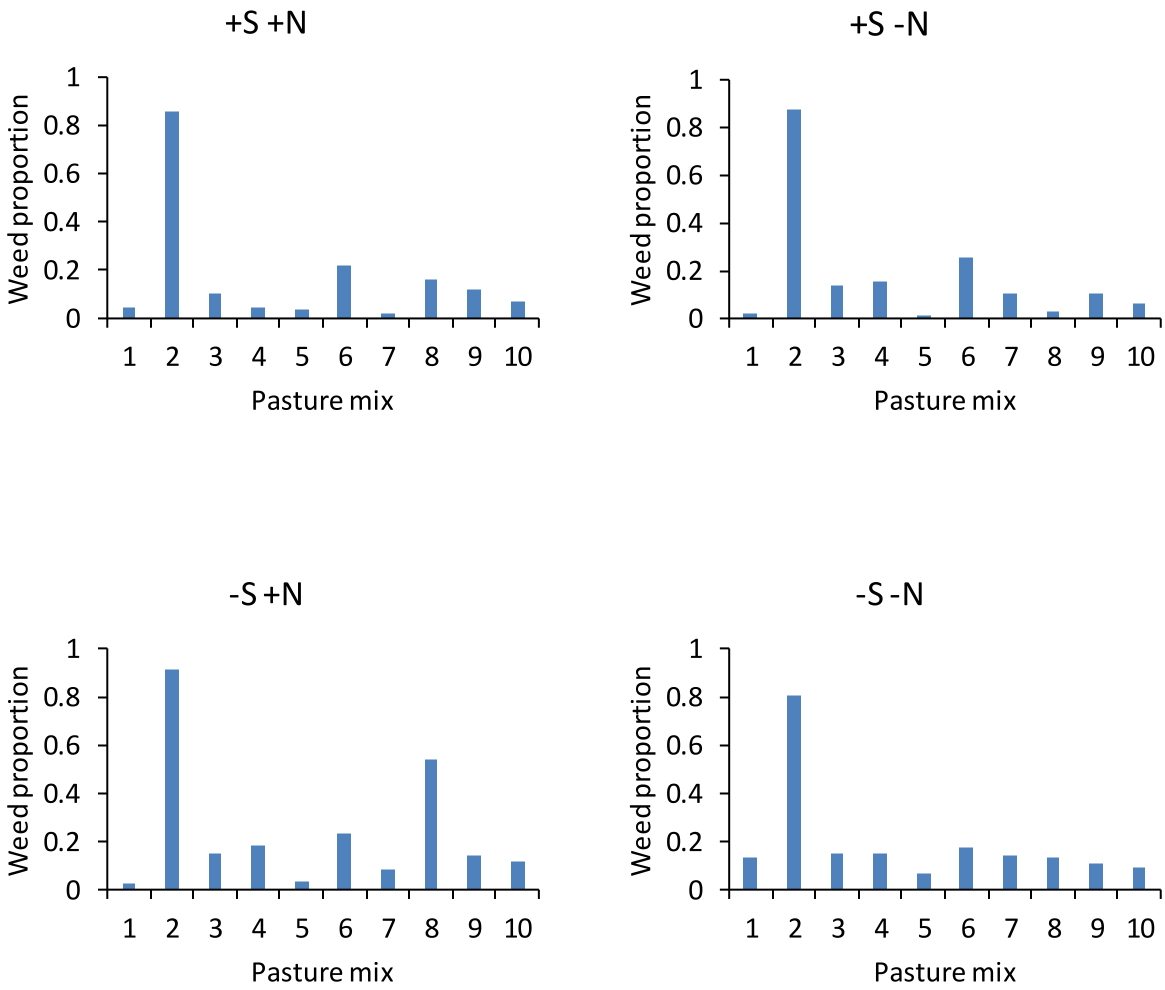

Figure 4.13. Average sum of weed proportion of perennial ryegrass, white clover and plantain and their mixes at the second harvest on the 13th of September 2017, at Lincoln University, Canterbury. (+S represents high sowing rate, -S represents low sowing rate, +N represents high level of N and -N represents low level of N).

The average LAI for the Sunscan® ranged between 0.4 to 5.9 over all pasture mixtures (Figure 4.14). An increase in LAI responded with an increase in yield (R^2=0.5784). Perennial ryegrass monoculture had LAI above 1.4 to 5.3 and plantain monoculture ranged between 0.8 to 4. White clover monoculture LAI ranged between 0.5 to 4.5. All two and three species mixtures LAI were dominated by ryegrass and plantain with little contribution form clover due to poor establishment across all mixtures. Nitrogen had a significant effect on the ryegrass LAI (P=0.002) due to the ability available N gives ryegrass by producing bigger leaves and dense tillering, this produced canopy closure earlier than the other species. Sowing rate did not have a significant effect on indirect LAI.

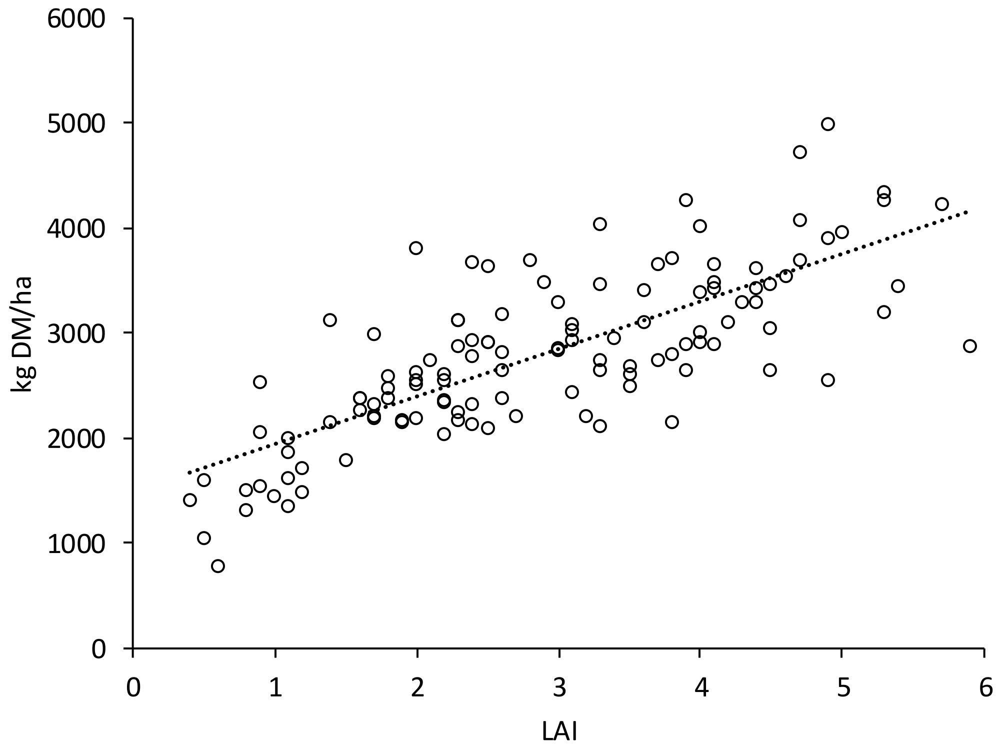

Figure 4.14. Indirect measurement of leaf area index (LAI) using a Sunscan® meter of all the species and their mixtures in relation to total dry matter production (kg DM/ha) on the 13th of September 2017 at Lincoln University, Canterbury.

The direct average LAI for all pasture mixes ranged between 0.01 to 5.43 (Figure 4.15). An increase in LAI responded with an increase in yield (R^2=0.7114). Perennial ryegrass monoculture had LAI above 2.14 to 4.53 and plantain monoculture ranged between 1.49 to 3.86. White clover monoculture LAI ranged between 0.2 to 1.19. All two and three species mixtures LAI were dominated by ryegrass and plantain with little contribution form clover due to poor establishment across all mixtures. Nitrogen or sowing rate did not have a significant effect on the direct LAI. The correlation between using direct and indirect measurements is 0.59 (Figure 4.16) which was decreasing from the first harvest on the 4th of August 2017. This indicates that using direct method or indirect methods are viable methods for estimating the LAI of the pasture swards.

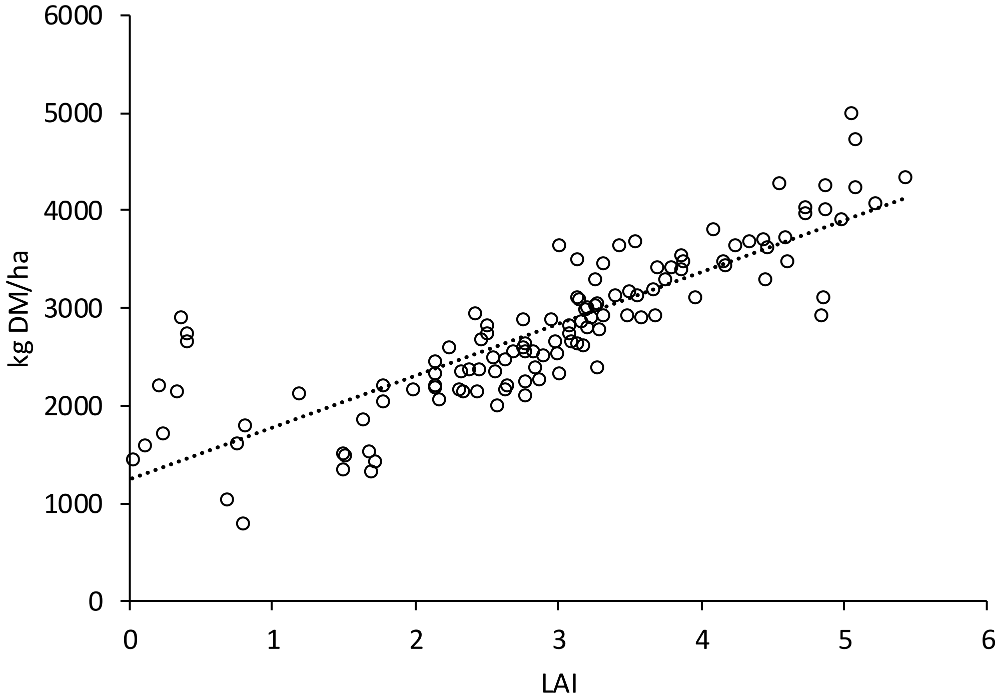

Figure 4.15. Direct measurement of leaf area index (LAI) of all the species and their mixtures in relation to total dry matter production (kg DM/ha) on the 13th of September 2017 at Lincoln University, Canterbury.

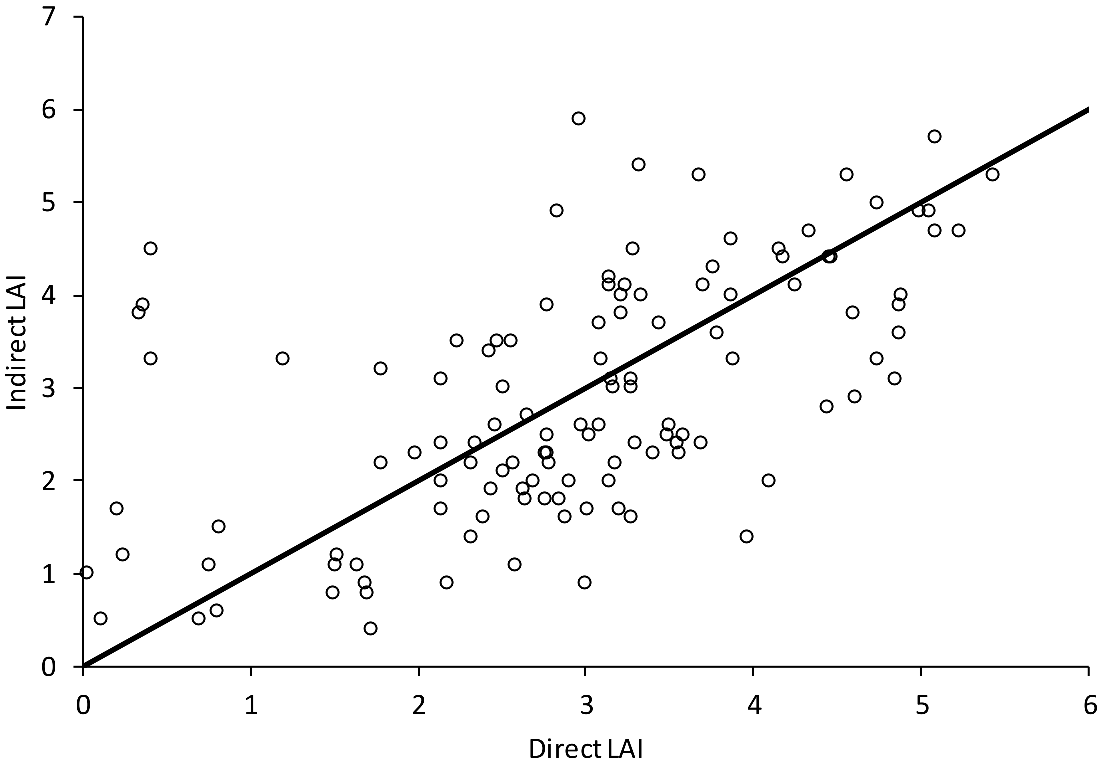

Figure 4.16. Correlation between direct leaf area index (LAI) measurements and indirect leaf area index (LAI) methods on the 13th of September 2017 at Lincoln University, Canterbury.

As LAI increases so too does light intercepted using the indirect method. When the pasture mix reached 4 LAI it would be intercepting maximum light (0.95 I/Io). This was consistent across all pasture mixes and their treatments (R^2=0.9985). The white clover monocultures and two species mixtures with plantain did not reach this critical level (0.95) and as a result did not reach canopy closure, causing the weed population to increase as not all the light is being intercepted by the sown species.

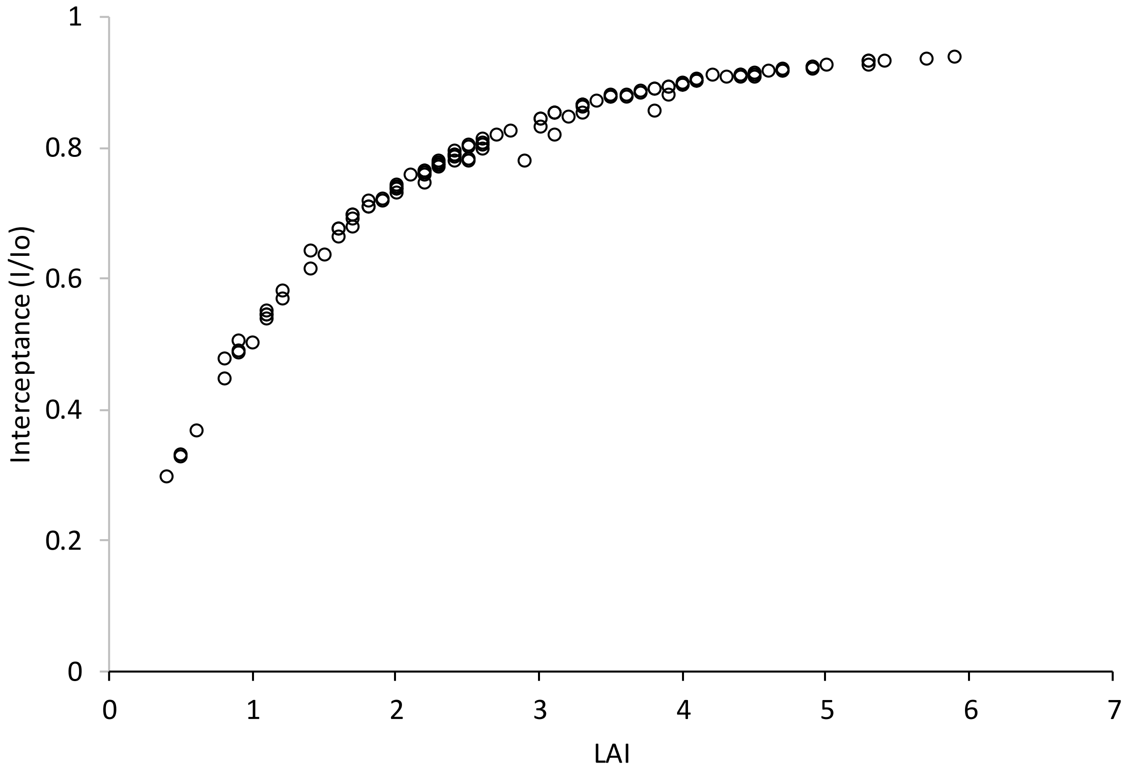

Figure 4.17. Relationship of light interception and leaf area index (LAI) of ryegrass, white clover and plantain and their mixtures on the 13th of September 2017 at Lincoln University, Canterbury.

Figure 4.18 describes the proportions of the data in Figure 4.17 by as measuring the LAI for the all the monoculture plots for each species. White clover has increased the LAI due to greater growth from warmer temperatures and higher weed growth (R^2=0.9883), plantain also was again in the 'mid-range' for the LAI for this experiment although not reaching the critical LAI was close (R^2=0.9949). Ryegrass had significant impact on the LAI for this trial, the ability to use available N and tiller to compensate in gaps allowed for canopy closure at critical LAI to be reached, giving it the optimum resources to be the dominate species (R^2=0.9846) although all pasture mixes are beginning to perform higher LAI.

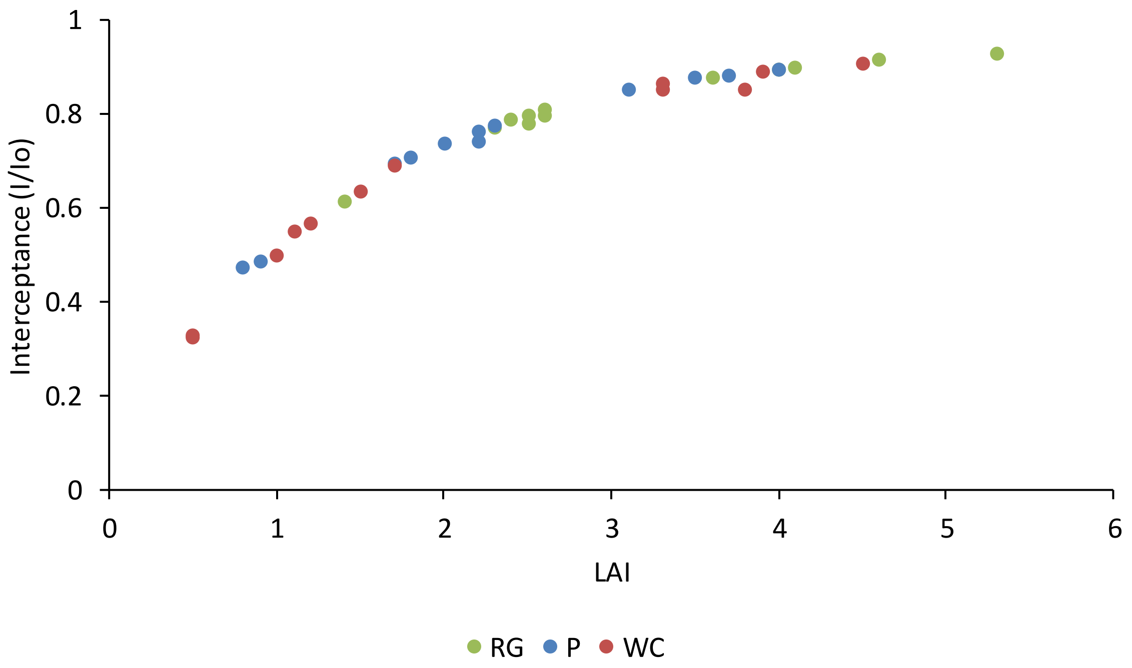

Figure 4.18. Relationship between light interception and the LAI of ryegrass, white clover and plantain monocultures on the 13th of September 2017 at Lincoln University, Canterbury.

4.3 Accumulated yield

Table 4.5. Estimate regression coefficients and analysis of variance for mixture model of total yield (kg DM/ha) for 2017 at Lincoln University, Canterbury.

Term	**Coef**	**SE**	**P**
RG	5859	249.4	*
WC	2485	332.7	*
P	4619	311.4	*
RG*WC	-3841	11618.8	0.742
RG*P	-170	1401.7	0.904
WC*P	-310	16852.3	0.985
RG*WC*P	-56973	71362.6	0.427
Rate	177	311.4	0.571
N	251	311.4	0.421
RG*Rate	354	386.6	0.362
WC*Rate	-250	455.7	0.584
P*Rate	-268	283.8	0.347
RG*WC* Rate	1810	11618.8	0.877
RG*P*Rate	-505	1401.7	0.720
WC*P*Rate	-5008	16852.3	0.767
RG*WC*P*Rate	-43414	71362.6	0.544
RG*N	496	386.6	0.203
WC*N	230	455.7	0.615
P*N	-242	280.3	0.389
RG*WC*N	-4197	11618.8	0.719
RG*P*N	783	1401.7	0.578
WC*P*N	8729	16852.3	0.606
RG*WC*P*N	-62378	71362.6	0.384
RG*N*Rate	169	249.4	0.5
WC*N*Rate	-365	332.7	0.276
P*N*Rate	3	311.4	0.991
RG*WC*N*Rate	9625	11618.8	0.41
RG*P*N*Rate	535	1401.7	0.704
WC*P*N*Rate	-6628	16852.3	0.695
RG*WC*P*N*Rate	-106666	71362.6	0.138

Table 4.6. Parsimonious mixture model of estimate regression coefficients and analysis of variance for mixture model of total yield (kg DM/ha) for 2017 at Lincoln University, Canterbury.

Term	Coef	SE	P
RG	5911	228.6	*
WC	2486	324.3	*
P	4655	270.2	*
RG*WC	-10926	7690.3	0.158
RG*P	-851	1270.5	0.504
WC*P	-4938	8054.6	0.541
RG*WC*P	-23541	40110.9	0.558
Rate	293	104.4	0.006
N	576	104.7	0.000

The non-significant interaction terms were removed from the special cubic model and then analysis was repeated with a more parsimonious model, which has more degrees of freedom for the residual (Table 4.8) (R^2 Adj=50.36%). This model shows the estimated monoculture yields are still similar as the linear component is significant (P=0.003). But the quadratic and special cubic components remained significant and non-significant with P-values of 0.037 and 0.186, respectively. The presence of the three-species mix term supports the choice of a simplex mixture design. The more parsimonious model is as follows:

$$Y=(\text{kg DM/ha}) = 5.911_{x1} + 2.486_{x2} + 4.655_{x3} - 23.541_{x1x2x3} + 0.293_{xs} + 0.576_{xN} + \mathcal{E}$$

The actual yields observed of the pasture mixes compared against a respective estimated pasture yield (Figure 4.19) Ryegrass monocultures at high sowing rate and high N produced an actual yield higher than the estimate (6780 and 8586 kg DM/ha respectively) across all other treatments estimated values and actual yields were similar). All two species mixtures at both sowing rates and N applications appear to produce similar actual yields when compared to their respective estimates, this gives a good indication that the model is a good fit for this data. Once a third species is included in the mix the estimates and actuals become varied, this indicates the model is not a good fit for the three-species data. There was no benefit of adding two or more species in a mix compared to the monocultures of each species.

All two-species mixes and three species mixes were consistently performing under each treatment. N application increased the yield across both sowing rates. All two-species mixes that included clover had poor estimates to the actual yields, this is due to the poor establishment and being dominated by weeds. Ryegrass and plantain mixes (5) started to yield similar to the ryegrass monocultures, this is a beneficial increase in yield due to two species interacting and being influenced by N. Three-species mixtures with the addition of N across both sowing rates had an increase in yield similar to the two-species mixes (Figure 4-19) which provides evidence that N has the ability to promote growth in all species ($P<0.05$). Without the addition of N the three-species mixes yield was poorer than the two-species mixes ($P<0.05$). The overall modelled data is not a good fit for the actual data from the two harvests in 2017.

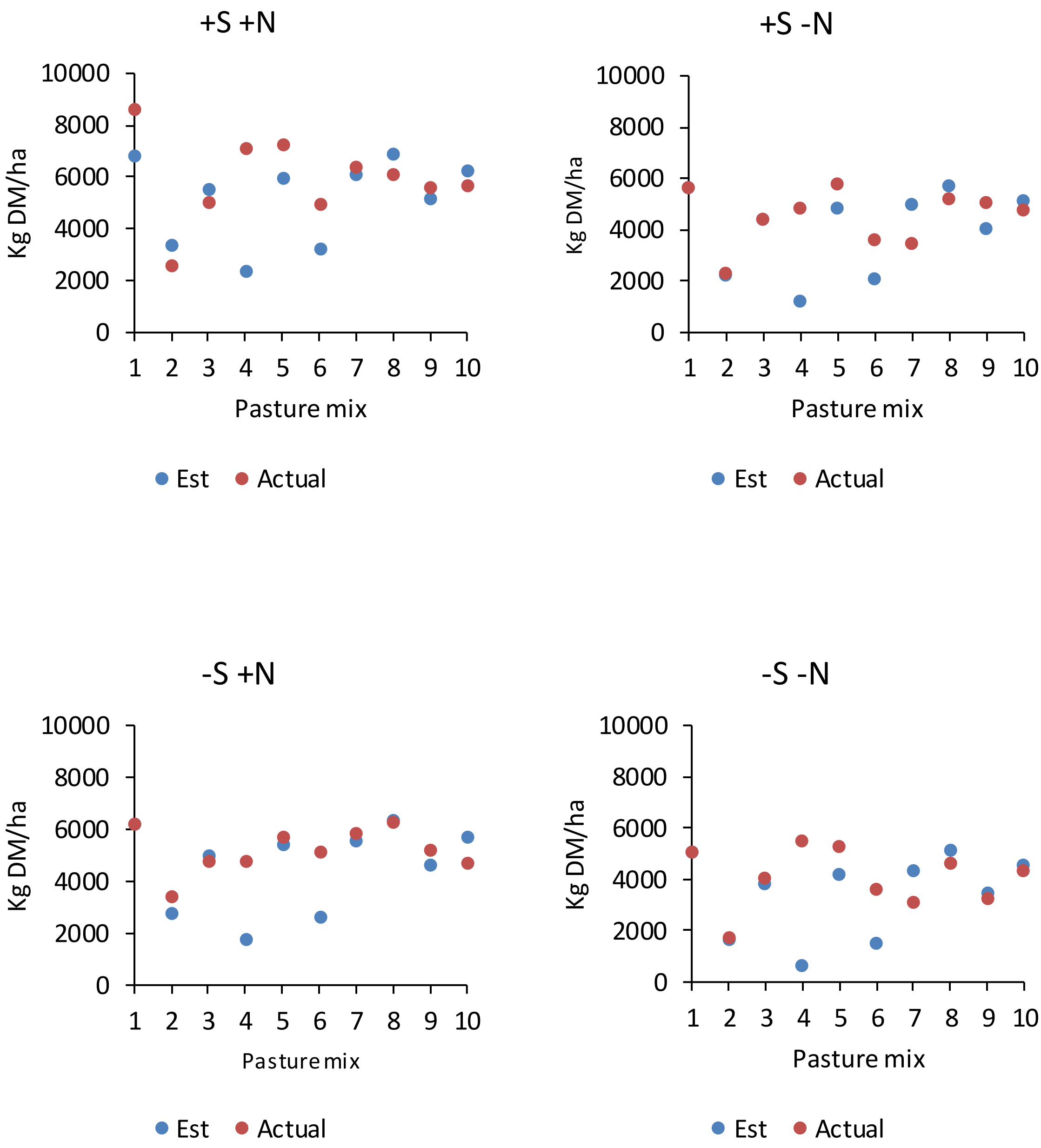

Figure 4.19. Estimated and actual yield (kg DM/ha) for the total yield of the 10 pasture mixes at Lincoln University, Canterbury. (+S represents high sowing rate, -S represents low sowing rate, +N represents high level of N and -N represents low level of N).

Ryegrass was the dominant pasture species involved due to its higher yield as shown by the colour distribution of the multiple mixture contour plot charts (Figure 4.20) At a low N application (bottom charts), the ryegrass yielded between 4000-5000 kg DM/ha, and upwards of 5000 kg DM/ha at high N (top charts). The white clover yielded low at both seed rates and N applications between 1000 and 2000 kg DM/ha, while plantain showed an increase from 3000 kg DM/ha at low rate up to 5000 kg DM/ha at high rate across both N applications. Total yield is consistent across both sowing rates and N applications.

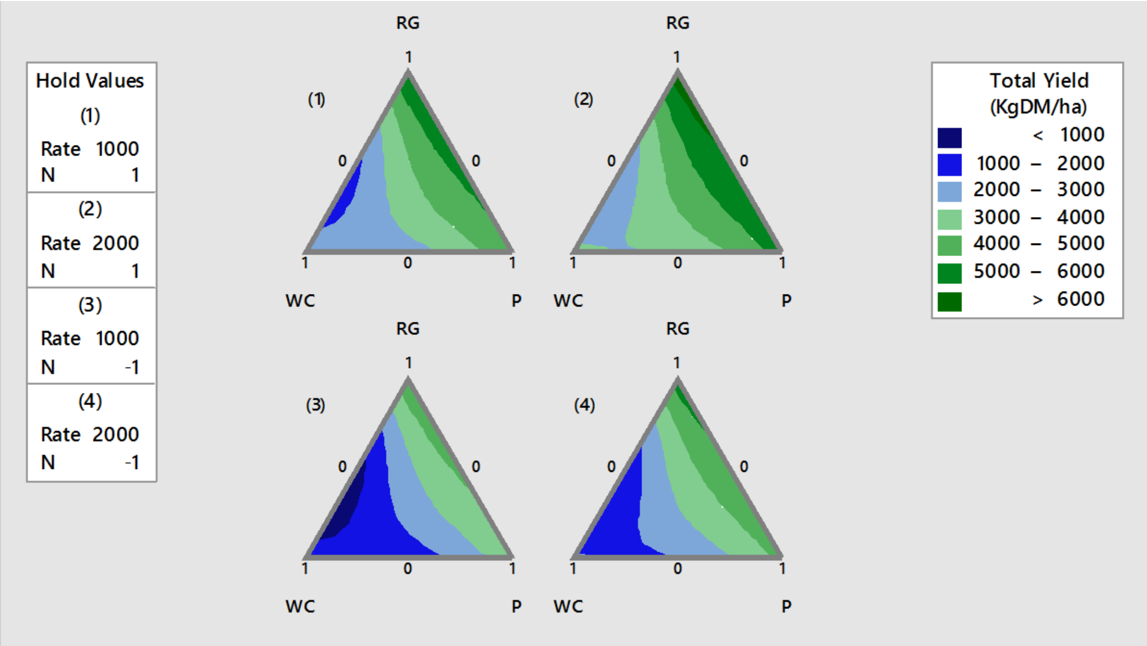

Figure 4.20. Multiple mixture contour plot of total yield (kg DM/ha) in response to mixture proportions of RG, WC and P, at two sowing rates of 1000 and 2000 seeds/m^2 and two levels of N application (0 and 100 kg N/ha) at Lincoln University, Canterbury.

The average tillers per mix was dependent on the species. All mixes that included ryegrass had high tillering rates compared to white clover and plantain. In monocultures ryegrass had a range of 8214 – 8667 tillers/m^2 across all treatments whereas white clover had a range from 1274 – 2600 tillers m^2, plantain ranged between 1052 – 2000 tillers/m^2. When clover was included in a mix the plants did not put any energy into tillering, but into getting a single leaf up into the canopy to intercept light. This can be noted in mixes 4, 6, 8, 9 and 10 9 (Figure 4.21) where there was little tillering per plant. Plantain also did not tiller as much as when included into a mix 5, 6, 7, 8, 9 and 10. Ryegrass has the ability to tiller and fill the gaps. This was evident across all mixes and treatments. Sowing rate had a significant effect on the plantain ability to tiller (P=0.000) as the sowing rate increases the plants per m^2 as a result creates interspecific and intraspecific competition between plants. There was no diversity effect for white clover and plantain as its tiller population appeared to be the same as averages of white clover and plantain monocultures. Species and mixture differences appeared to be consistent across sowing rates and N rates. Ryegrass compensated by tillering in ryegrass and white clover mixes which produced more tiller per m^2 then expected from the two monocultures, hence the interaction effect for ryegrass and white clover yield.

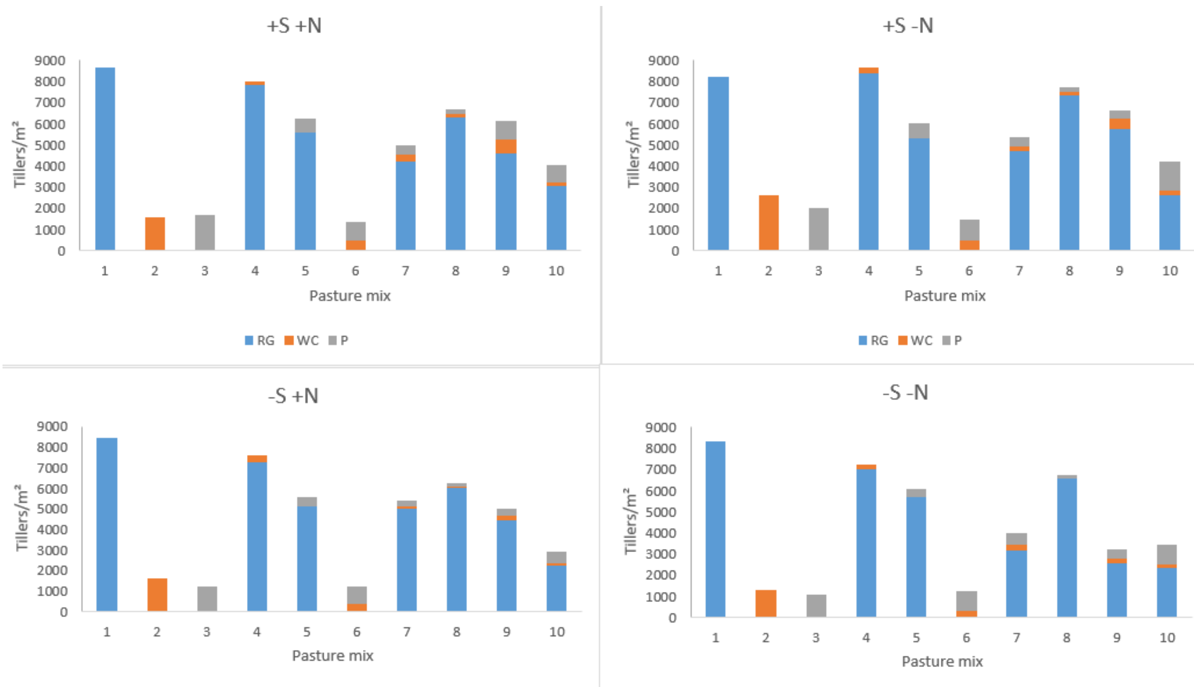

Figure 4.21. Average total tillers per m^2 for ryegrass, white clover and plantain and their mixtures on the 4th of August 2017 at Lincoln University, Canterbury. (+S represents high sowing rate, -S represents low sowing rate, +N represents high level of N and -N represents low level of N).

Average tillers per plant for ryegrass were different (P<0.05) for pasture mixes containing ryegrass compared to ryegrass monoculture (Figure 4.22). The number of tillers per plant ranged from 6.51 to 14.02 for high rate and high N, 6.59 to 12.89 for high rate and low N, 10.55 to 15.69 for low rate and high N and 8.82 to 17.23 for low rate and low N. Mix number 9 which was dominated by white clover seed had poor establishment of clover, as a result produced more gaps in the canopy. Ryegrass responded to this by tillering as ryegrass tillers per plant were significantly greater in this mix across all treatments compared to other mixes. White clover tillers per plant for monocultures across all treatments ranged between 1.83 and 2.34 whereas across all other mixes the clover did not tiller as much ranging from 1 to 1.43. This is due to the clover putting most of its energy into getting a leaf into the canopy to intercept light rather than tillering across its stolon. Plantain reacted similar to white clover by not tillering as much. The tillering per plant across all mixes ranged between 1 to 2.36, sowing rate had an effect on the plantains ability to tiller (P=0.000) as the high sowing rate increases the plants per m^2 as a result creates interspecific and intraspecific competition between plants.

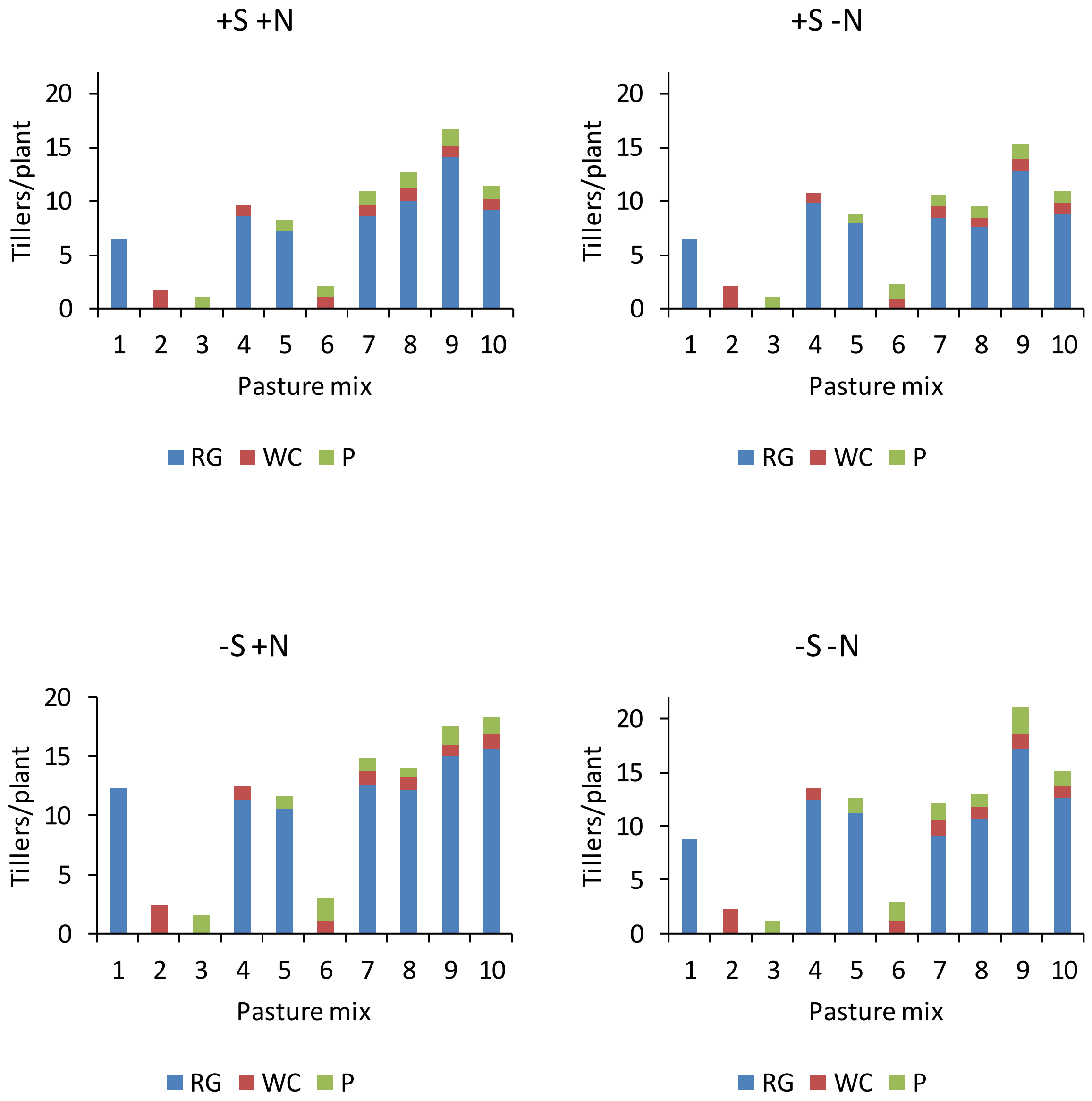

Figure 4.22. Average total tillers per plant for ryegrass, white clover and plantain and their mixtures on the 4th of August 2017 at Lincoln, Canterbury. (+S represents high sowing rate, -S represents low sowing rate, +N represents high level of N and -N represents low level of N).

Plate 4.1. Perennial ryegrass monocultures (mix 1) in order from left to right of high sowing rate high N, high sowing rate low N, low sowing rate high N and low sowing rate low N, on the 13th of September 2017 at Lincoln University, Canterbury.

Plate 4.2. White clover monocultures (mix 2) in order from left to right of high sowing rate high N, high sowing rate low N, low sowing rate high N and low sowing rate low N, on the 13th of September 2017 at Lincoln University, Canterbury. (Note obvious dominance of weeds).

Plate 4.3. Plantain monocultures (mix 3) in order from left to right of high sowing rate high N, high sowing rate low N, low sowing rate high N and low sowing rate low N, on the 13th of September 2017 at Lincoln University, Canterbury.

Plate 4.4. Two-species even mix perennial ryegrass and white clover (mix 4) in order from left to right of high sowing rate high N, high sowing rate low N, low sowing rate high N and low sowing rate low N, on the 13th of September 2017 at Lincoln University, Canterbury.

Plate 4.5. Two-species even mix perennial ryegrass and plantain (mix 5) in order from left to right of high sowing rate high N, high sowing rate low N, low sowing rate high N and low sowing rate low N, on the 13th of September 2017 at Lincoln University, Canterbury.

Plate 4.6. Two-species even mix white clover and plantain (mix 6) in order from left to right of high sowing rate high N, high sowing rate low N, low sowing rate high N and low sowing rate low N, on the 13th of September 2017 at Lincoln University, Canterbury.

Plate 4.7. Three-species even mix perennial ryegrass, white clover and plantain (mix 7) in order from left to right of high sowing rate high N, high sowing rate low N, low sowing rate high N and low sowing rate low N, on the 13th of September 2017 at Lincoln University, Canterbury.

Plate 4.8. Three-species mix dominated by ⅔ of perennial ryegrass and ⅙ of each white clover and plantain according to seed rate (mix 8) in order from left to right of high sowing rate high N, high sowing rate low N, low sowing rate high N and low sowing rate low N, on the 13th of September 2017 at Lincoln University, Canterbury.

Plate 4.9. Three-species mix dominated by ⅔ of white clover and ⅙ of each perennial ryegrass and plantain according to seed rate (mix 9) in order from left to right of high sowing rate high N, high sowing rate low N, low sowing rate high N and low sowing rate low N, on the 13th of September 2017 at Lincoln University, Canterbury.

Plate 4.10. Three-species mix dominated by ⅔ of plantain and ⅙ of each perennial ryegrass and white clover according to seed rate (mix 10) in order from left to right of high sowing rate high N, high sowing rate low N, low sowing rate high N and low sowing rate low N, on the 13th of September 2017 at Lincoln University, Canterbury.

Chapter 5
Discussion

5.1 Species effects

Accumulated DM yields of perennial ryegrass, white clover and plantain and their mixes over the 7-month establishment period, from sowing on the 31st of March 2017 to their final harvest on the 13th of September 2017. The yields varied, ranging from 2529 to 8586 kg DM/ha for high sowing rate and high N, for high sowing rate and no N yields ranged between 2294 and 5783 kg DM/ha, low sowing rate and high N yields ranged between 3404 and 6275 kg DM/ha and for low sowing rate and low N ranged between 1710 and 5465 kg DM/ha (Figure 4.19). The monocultures of each species (mixes 1-3) yield provided a model which could explain yields when sown in multiple species mixes (mixes 4-10). The ryegrass had the highest proportion in all mixes, this was due to the rapid germination, emergence and seedling growth of perennial ryegrass, which give it a competitive advantage over species like white clover (Brougham, 1953). Plantain also has fast establishment which comes close to that of perennial ryegrass, which could explain the high proportions of plantain in the seed mixes compared to clover (Blom, 1978). White clover was fast to emerge (150˚Cd) similar to perennial ryegrass (160˚Cd) (Moot *et al*., 2000), this is evidence that temperature was the greatest limitation to the growth and establishment of the white clover when the temperature dropped below 8˚C over the winter period (Figure 3.4). As expected the high seed rates of perennial ryegrass initially had the greatest yield due to fast establishment, the white clover was suppressed at these rates but future research could result in yields beginning to decrease due to poor N fixation (Brougham, 1953).

From the first harvest on the 4th of August 2017 to the second harvest on the 13th of September 2017, species effects caused by interspecific competition between plant species became more apparent. Ryegrass had increased proportions across all mixes, this could be due to ability ryegrass has to tiller, especially in leading into spring when the temperature starts to increase and more mineralisation of N occurs (Hunt and Mortimer, 1982). This caused the ryegrass mixes to have greater yields than the other mixes, suppressing the yields of white clover and plantain. The intraspecific competition between plants of the same species had little effect on the establishment of each pasture mix.

This directly relates to the ability of the plants to intercept light for photosynthesis, this is measured by calculating the LAI of the monocultures and their mixes. The direct LAI was ranged between 0.01 and 8.35 for all plots on the 4th of August 2017 harvest (Figure 4.6). The LAI correlated significantly with increase in yield (R^2=0.8982). Perennial ryegrass monocultures had LAI above 3.4 to 8.35 and plantain monocultures ranged between 1.5 to 4.76. White clover monocultures LAI ranged between 0.01 to 0.21. The indirect LAI ranged between 0.1 to 8.8 over all pasture mixtures (Figure 4.5). An increase in LAI responded with an increase in yield (R^2=0.5918). Perennial ryegrass monoculture had LAI above 2.5 to 8.8 and plantain monoculture ranged between 0.7 to 4.3. White clover monoculture LAI ranged between 0.1 to 1.5. The correlation between using the direct and indirect methods were 0.75 (Figure 4.7), this demonstrates that there is a significant relationship between the two methods. Therefore, the use of the direct method of determining the LAI validates the use of the indirect method. Further research needs to be studied in relation to using the indirect method to determine LAI as the Sunscan® device is programmed for forage crops and not pasture crops. Further study could replicate a model to fit the programme for more accurate readings and predictions of LAI. Ryegrass and plantain provided the majority of the LAI of the mixes. The LAI critical level as mentioned by Rattray *et al* (2007) was reached in the ryegrass monocultures of 3.3-4.5 but not in the plantain or white clover monocultures (Figure 4.9). During this period of 5 months after sowing it is assumed that the pasture mixes were well established, white clover was not as they were most likely limited by soil temperature which over these months was on average below 8˚C (Figure 3.4) which was getting to the point where white clover stops growing (8-9˚C) (White and Hodgson, 2000).

The perennial ryegrass first leaf during establishment is generally larger than that of white clover and the second shoot of ryegrass appears earlier than white clover. Therefore, ryegrass can intercept more light earlier, giving the plant a more competitive advantage. These effects may not have been as evident if the plots had been spring sown when the temperature will have been warmer resulting in more favourable conditions for white clover. This explains the difference in LAI and light interceptance which is caused by the different species and their mixes. Figure 4.9 describes how each species monoculture causes the LAI to change, the light interceptance for white clover did not reach over 0.7 I/Io (light interceptance), with its lowest reading at 0.15 I/Io. Perennial ryegrass obviously was far more aggressive and had greater LAI which resulted in a larger light interceptance ranging between 0.81 I/Io and 0.95 I/Io which was the same critical LAI and light interceptance as Brougham (1957) found (Figure 2.2).

The 13th of September 2017 indirect average decreased from the 4th of August 2017 LAI ranging between 0.4 to 5.9 over all pasture mixtures (Figure 4.14). An increase in LAI responded with an increase in yield (R^2=0.5784). Perennial ryegrass monoculture had LAI above 1.4 to 5.3 and plantain monoculture ranged between 0.8 to 4. White clover monoculture LAI ranged between 0.5 to 4.5. The direct LAI had decreased from the first harvest for all pasture mixes ranging between 0.01 to 5.43 (Figure 4.15). An increase in LAI responded with an increase in yield (R^2=0.7114). Perennial ryegrass monoculture had LAI above 2.14 to 4.53 and plantain monoculture ranged between 1.49 to 3.86. White clover monoculture LAI ranged between 0.2 to 1.19. The correlation between using direct and indirect measurements is 0.59 (Figure 4.16) which still provided evidence that the direct method had validated the indirect method. The white clover monocultures began to increase LAI as temperature was beginning to increase reaching 9˚C during September 2017 (Figure 3.4) which is above the point where white clover starts growing (8-9˚C) (White and Hodgson, 2000).

Mixes with high proportions of ryegrass and plantain in the mix provided the greatest yield (Mixes 1, 3, 4, 5, 7, 8, 9 and 10; Figure 4.19). Due to the rapid establishment of perennial ryegrass and seedling growth resulted in the highest yield of the monocultures and all the mixes (5061-8586 kg DM/ha) (Figure 4.19). The plantain monocultures yield in the monocultures and mixes were consistently high too compared to the white clover monoculture due to their fast establishment. White clover had very low contribution to total yi eld as a direct result of poor establishment in all the mixtures.

These mixes had the greatest weed suppression (<20%) except for mix 8 in low sowing and high N availability which had 53.6% weeds which is significantly greater than all seed mixes. White clover monoculture had poor weed suppression (>80%) (Figure 4.13) across all treatments due to slow establishment and poor seedling vigour which offers little competition against aggressive weed species that establish quickly and shade clovers (Lee, 1985). The main weeds present were Annual poa (*Poa annua*), Stagger weed (*Stachys arvensis*), Chickweed (*Stellaria media*), Twin cress (*Lepidium didymium*), Broad leaved dock (*Rumex obtusifolius*) and Groundsel (*Senecio vulgaris*), respectively.

In monocultures perennial ryegrass had the greatest tillering with a range of 8214 – 8667 tillers/m^2 across all treatments which was similar to a study by Lee *et al* (2013) which indicated perennial ryegrass tillered more at lower sowing rates compared to higher sowing rates, th is increased intraspecific competition and perennial ryegrass responded by increasing tillering to fill in the gaps (Williamson, 2008). It is generally accepted that there is more competition in monocultures as the plants are competing for the same resources (Haynes ,1980). White clover had a range from 1274 – 2600 tillers m^2 and plantain ranged between 1052 – 2000 tillers/m^2. When clover was included in a mix the plants did not put any energy into tillering, but into getting a single leaf up into the canopy to intercept light (Harris and Clark, 1996). This can be noted in mixe s 4, 6, 8, 9 and 10 (Figure 4.22) where there was little tillering per plant. Plantain also did not tiller as much as when included into a mix 5, 6, 7, 8, 9 and 10. Sowing rate had a significant effect on plantains ability to tiller as the sowing rate increases the plants per m^2 as a result creates interspecific and intraspecific competition between plants.

Average tillers per plant for perennial ryegrass were different for pasture mixes compared to ryegrass monoculture (Figure 4.22). The number of tillers per plant ranged from 6.51 to 14.02 for high rate and high N, 6.59 to 12.89 for high rate and low N, 10.55 to 15.69 for low rate and high N and 8.82 to 17.23 for low rate and low N which is similar to a study by Lee *et al* (2013). Mix number 9 which was dominated by white clover seed had poor establishment of white clover, as a result produced more gaps in the canopy. Ryegrass responded to this by tillering as ryegrass tillers per plant were significantly greater in this mix across all treatments compared to other mixes (Williamson, 2008). White clover tillers per plant for monocultures across all treatments ranged between 1.83 and 2.34 whereas across all other mixes the clover did not tiller as much ranging from 1 to 1.43. This is due to the clover putting most of its energy into getting a leaf into the canopy to intercept light rather than producing growth buds across its stolon. Plantain reacted similar to white clover by not tillering as much. The tillering per plant across all mixes ranged between 1 to 2.36, sowing rate had an effect on the plantains ability to tiller as the high sowing rate increases the plants per m^2 as a result creates interspecific and intraspecific competition between plants.

5.2 Species richness

Based on tiller populations per m^2 and tillers per plant on the 4th of August 2017 (Figure 4.21 and Figure 4.22) the proportions of each species sown in the mixtures were well represented of the proportion of each species present in the plant population albeit white clover. The results indicated trends towards increased perennial ryegrass populations and decreases in the plantain and white clover populations across all pasture mixes. This was consistent across both sowing rates and N applications, due to perennial ryegrasses ability to rapidly establish and tiller to fill the gaps. Tillers increase in number in spring and turnover of tillers is accelerated by nitrogen (Hunt and Mortimer, 1982), which could have had an impact on the timing of the harvest for tillers as this was done after the first harvest in August 2017 when temperatures are increasing, resulting in more available N and increasing in number of tillers. Tillering should also be counted perhaps in months prior to winter to give an indication of population and tiller development over the year, this could give a greater understanding of the species richness and development. Perennial ryegrass has the ability to produce daughter tillers which grow their own root system and become independent from main plant (Williamson, 2008) made it difficult to determine what was actually a ryegrass plant as during separation, especially in the high sowing rates where the plants were smaller and had higher tiller density. This caused the tillers when pulled apart during sorting the roots would break and make it hard to distinguish between a tiller and an individual plant.

There was an advantage of including more than one species in a pasture mix with regard to yield and weed suppression, but there was no greater effect of includi ng three species in a mix (Figure 4.19). The mean total yield for the 6-month establishment period, from sowing on the 31st of March 2017 to their final harvest on the 13th of September 2017. The yields varied, ranging from 2529 to 8586 kg DM/ha for high sowing rate and high N, for high sowing rate and no N yields ranged between 2294 and 5783 kg DM/ha, low sowing rate and high N yields ranged between 3404 and 6275 kg DM/ha and for low sowing rate and low N ranged between 1710 and 5465 kg DM/ha (Figure 4.19).

Intraspecific competition in the monocultures resulted in the plants competing for the same resource such as space, nutrients and light which as a result produced more plants and less tillering per m^2 resulting in smaller plants which was evident in a trial by Beaumer and de Wit, (1968). The results from this trial indicated that two-species mixtures would yield greater than those of the three-species mixtures. This was not similar to the results found by Sanderson *et al*. (2005) as they found that mixtures that contained three to nine species would produce greater yield than those that contained two-species. However, they only tested a two-species mixture of cocksfoot (*Dactylis glomerata*) which is known for low DM yield and summer persistency. Therefore, for the pasture mix to produce greater DM and supress weeds effectively it would need to include either ryegrass or plantain or both ryegrass and plantain at high proportions.

5.3 Relative abundance

The varied proportions of species in their respective seed mixes had no effect on total dry matter production and weed suppression (Figure 4.3 and Figure 4.12). Expectations were that if species were sown at equal proportions than they would have equal proportions of the yield in the mix (Kirwan *et al.*, 2007), or if one species was sown dominantly by seed population than it would remain the dominating species in that species mix yield. However, this was not evident as all sown species appeared to perform differently in their respective mixtures compared to their monocultures. White clover yielded less than expected in each mix than it did as a monoculture, this was due to poor establishment due for the autumn sowing. The results were similar to Sanderson *et al.* (2013) which found that no pattern of greater yield for mixtures with increasing species evenness. The results also agreed with Sanderson *et al.* (2013) who also found that there was no difference in weed suppression between mixtures that were dominated by one species and mixtures with an equal proportion of species. This may have been due to weeds being suppressed by species diversity (Frankow-Lindberg *et al.*, 2009). The weed suppression results demonstrated that all species mixtures across all treatments had good weed suppression (<20% weeds) except for mix 8 in low sowing and high N availability which had 53.6% weeds which was significantly greater than all seed mixes. This was due to the availability of N from the soil for the weeds and the fact that lower sowing rate allowed for competition for light and space to increase the proportion of weeds, this was similar to the findings of Dukes (2001). Therefore, for the pasture mix to supress weeds effectively it needed to include either high proportions of ryegrass or plantain or both ryegrass and plantain. The results indicated that there was no evidence that total yield and weed suppression were greater for mixtures of even proportions or of mixes that had in one dominating species in relation to all three-species mixes.

The results could be more significant if the trial had been spring sown which would result in a greater proportion of white clover in each mix. This would have had a greater impact on total yield and weed suppression and perhaps the proportions of each species in terms of yield would have been similar to that of the sown seed mix.

5.4 Practical implications

The results of this study indicated that sowing diploid perennial ryegrass, white clover and plantain in different pasture mixes, in an autumn sowing (late March) will result in high proportions of ryegrass and plantain and lower proportions of white clover in relation to total yield. This was consistent across both sowing rates and N applications. Autumn was not favourable for the establishment of clovers, resulting in poorer yields, this could likely improve performance by sowing in spring when temperatures are warmer. When selecting pasture seed mixes perennial ryegrass and plantain should consist of at least 50% of the total seed mixture to achieve high yields. This was consistent across both rates, although increasing sowing rate increases the proportions of perennial ryegrass, in the long term this will most likely affect the overall quality of the pasture as clover persistence was weakened resulting in less biologically fixed N being provided to the perennial ryegrass.

Increasing species richness (increasing amounts of species in the mix) in the pastures mix did not result in increased yields. Adding white clover into the mix to add biologically fixed N to increase the persistence of the pasture is beneficial, but is not crucial for high DM production.

There is also the cost increase of doubling the sowing rate to consider from 20 kg/ha to 40 kg/ha for (mix 7) three-species even mix. Perennial ryegrass seed costs $3.80 kg at 8.13 kg/ha, white clover seed costs $9.50 kg at 4.68 kg/ha and plantain seed costs $18.75 kg at 6.28 kg/ha which total costs $193.10 kg/ha more than the low sowing rate (Cridge Seeds, 2017). If this mix only produces on average an extra 152.5 kg DM/ha regardless of N application (Figure 4.19) from the increase in sowing rate at 20c kg DM this would only return and extra $30.50/ha to the farmer so economically this is not viable for the farmer. The farmer would need to increase that yield to 966 kg DM/ha just to break even.

The model generated from this experiment in the first two harvests on the 4th of August and the 13th of September 2017 across the establishment phase was not a good fit for the actual data. This was due to the variation between the estimate data values and actual data values of weeds, perennial ryegrass, white clover and plantain. The model may become a better fit to the data by the continuation of the experiment, as the species start to have more significant effects on each other in their respective seed mixes.

5.5 Future research

Continuing this experiment would be valuable to gain long term data on the performance of the pasture mixtures. This would be interesting to measure how the botanical composition changes with continued harvesting and competition between plants with increasing age of the pasture. Harvesting over the summer period would give a good indication of the white clover performance as this is when the plant is most active and has the greatest production. The yield for plantain over this period would also be of interest as this is when the plant is most active. The long-term persistence of perennial ryegrass, white clover and plantain in their pasture mixes is another key aspect for the continuation of this experiment, especially for plantain which has been reported to have a relatively short-lived persistence in pastures (4 years) (Stewart *et al.*, 2014).

The continuation of this experiment would result in a greater understanding of how the sowing rate has any effect on the total DM production of each mixture. N too would also be of interest to investigate if it increases the production for multiple species mixes. There is also the potential to analyse the nutritional value (metabolizable energy) of the pasture from further harvests to see how the nutritional value of each mix is affected over time. Repeating this experiment with a spring sowing and lower sowing rates would highly benefit the proportion of white clover due to the higher temperatures increasing the establishment rate and less interspecific competition. Livestock performance could also be investigated on each of the pasture mixes across both rates to see how animal performance responds to each treatment.

Chapter 6

Conclusions

- Perennial ryegrass and white clover, white clover and plantain two-species mixes both performed greater than the averages of both monocultures, this is due to the poor establishment of the white clover monoculture. Perennial ryegrass and plantain two-species mixes did not perform greater than the averages of both monocultures due to the large yields of the perennial ryegrass monoculture >5061 kg DM/ha.

- There was no yield benefit of including more than two-species in a pasture mix due to the interspecific competition caused by perennial ryegrass suppressing both white clover and plantain.

- Perennial ryegrass and plantain had greater suppression of weeds than white clover because of their ability to rapidly establish after an autumn sowing.

- White clover monocultures were dominated by weeds and suppressed by perennial ryegrass and plantain in their respective mixes.

- Sowing rate and nitrogen level had no effect on weed suppression or species proportions due to the competitiveness and ability to establish for perennial ryegrass and plantain except for low sowing rate and high nitrogen which increased weed proportion in mix 8 (⅔ of perennial ryegrass and ⅙ of each white clover and plantain) which had an increase in weed.

- The evenness of the proportions of species in their pasture mixes had no effect on dry matter production or weed suppression.

- There was no benefit to tillering caused by increase in sowing rate and N application.

- The special cubic model was a valuable for predicting the yield of the monocultures and their two-species mixes, but not for their three-species mixes as there was no benefit of including another species into the mix. The three-species mixes yield was less of the two-species mixes and was varied due to the poor performance of the white clover in each mix.

- Further research of the perennial ryegrass, white clover and plantain and their respective mixes performance is required into late spring and summer following establishment to determine further species effects, species richness and changes to species abundance.

References

Alexander, R. E. (1933). Pure versus mixed pastures for grazing. In *Proceedings of the New Zealand Grassland Association, 2.*

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.

Ball, P. R., & Field, T. R. O. (1982). Responses to nitrogen as affected by pasture characteristics, season and grazing management. *Nitrogen fertilisers in New Zealand agriculture*, 45-64.

Baeumer, K., & De Wit, C. T. (1968). *Competitive interference of plant species in monocultures and mixed stands, 363*, 20.

Black, A. D., Moot, D. J., & Lucas, R. J. (2002). Seedling development and growth of white clover, caucasian clover and perennial ryegrass grown in field and controlled environments. In *Proceedings of the New Zealand Grassland Association, 64,* 197-204.

Black, A.D.; Laidlaw, A.; Moot, D.; O'Kiely, P. (2009). Comparative growth and management of white and red clovers. *Irish Journal of Agricultural and Food Research,* 149-166.

Blom, C. W. P. M. (1978). Germination, seedling emergence and establishment of some *Plantago* species under laboratory and field conditions. *Acta botanica neerlandica*, *27*(5/6), 257-271.

Brougham, R.W. (1953). Seeding rates of short rotation ryegrass. In *Proceedings of the New Zealand Grassland Association, 14,* 172-181.

Brougham, R.W. (1957). Some factors that influence the rate of growth of pasture. In *Proceedings of the New Zealand Grassland Association, 19,* 109-116.

Charlton, J.F.L. (1991). Some basic concepts of pasture seed mixtures for New Zealand farms. *Proceedings of the New Zealand Grasslands Association*, *53*, 37-40.

Charlton, J.F.L, Stewart A.V. (1999). Pasture species and cultivars used in New Zealand - a list. *Proceedings of the New Zealand Grassland Association, 67,* 147-166.

Cliflo National Climate Database. (2017). The *National Climate Database: September 2017*. Retrieved from *https://cliflo.niwa.co.nz/pls/niwp/wgenf.genform1*

Cookson, W. R., Rowarth, J. S., & Cameron, K. C. (2001). The fate of autumn-, late winter-and spring-applied nitrogen fertilizer in a perennial ryegrass (*Lolium perenne* L.) seed crop on a silt loam soil in Canterbury, New Zealand. *Agriculture, ecosystems* and *environment*, *84*(1), 67-77.

Cornell, J.A. (2002). Experiments with mixtures: Designs, models, and the analysis of mixture data. 3rd Edition. Wiley, Chichester, England. 305.

Cox, J.E. (1978). Soils and agriculture of part Paparua County, Canterbury, New Zealand. New Zealand Soil Bureau Bulletin No. 34. New Zealand Department of Scientific and Industrial Research, Wellington, New Zealand. 128.

Cridge Seeds New Zealand. (2017). *Seed price list: October 2017.* Retrieved from *http://www.cridgeseeds.co.nz/prices.aspx*

Cullen, N.A. (1958). Pasture establishment studies at Invermay Research Station. *Proceedings of the New Zealand Grassland Association, 20*, 138-147.

Dukes, J.S. (2001). Biodiversity and invasibility in grassland microcosms. *Oecologia, 126*, 563-568.

Frankow-Lindberg, B.; Brophy, C.; Collins, R.; Connolly, J. (2009). Biodiversity effects on yield and unsown species invasion in a temperate forage ecosystem. *Annals of Botany, 103*, 913-921.

Fraser, T. J., and Rowarth, J. S. (1996). Legumes, herbs or grass for lamb performance. *New Zealand Grassland Association, 58,* 49-52.

Goh, K.M.; Bruce, G.E. (2005). Comparison of biomass production and biological nitrogen fixation of multi species pastures (mixed herb leys) with perennial ryegrass-white clover pasture with and without irrigation in Canterbury, New Zealand. *Agriculture, Ecosystems and Environment, 110*, 230-240.

Harris, W. (1968). Pasture seeds mixtures, competition and productivity. In: *Proceedings of the New Zealand Grassland Association, 30,* 143-153.

Harris, W. (1990). Pasture as an ecosystem. In 'Pastures: Their Ecology and Management.' (Ed. RHM Langer.) (Oxford University Press: Auckland, New Zealand), 75-131.

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,* 149-158.

Harris, W. (2001). Formulation of pasture seed mixtures with reference to competition and succession in pastures. *Competition and succession in pastures*, 149-174.

Hare, M. D. (1990). Establishment, spacing density and grazing effect. *Rowarth, JS Management of grass seed crops. Grasslands Research and Practice Series*, *5*.

Haynes, R. J. (1980). Competitive aspects of the grass-legume association. *Advances in agronomy*, *33*, 227-261.

Hill, M. J., Hampton, J. G., & Rowarth, J. S. (1999). Herbage seeds. *New Zealand Pasture and Crop Science*, 249-262.

Hume, D.E., Fraser, T.J. (1985). Establishing and managing cultivars in arable dryland pastures. *Grassland Research* and *Practice Series, 3*, 45-50.

Hunt, W. F., & Field, T. R. O. (1976). Growth Characteristics of Perennial Ryegrass. *Proceedings of the New Zealand Grassland Association*, *40*, 104-113.

Hunt, W. F., & Mortimer, B. J. (1982). A demographic analysis of growth differences between Nui and Ruanui ryegrass at high and low nitrogen inputs. In *Proceedings of the New Zealand Grassland Association, 43,* 125-132.

Hurst, R.G.M.; Black, A.D.; Lucas, R.J.; Moot, D.J. (2000). Sowing strategies for slow-establishing pasture species on a North Otago dairy farm. *Proceedings of the New Zealand Grassland Association, 62,* 129-135.

Kemp, P.D.; Kenyon, P.R.; Morris, S.T.; Somasiri, S.C. (2013). Plantain (*Plantago lanceolata*) in herb and legume pastures increases lamb growth relative to perennial ryegrass and white clover. *Proceedings of the 22nd International Grassland Congress,* 561-562.

Korte, C., Watkin, B., & Harris, W. (1985). Tillering in 'Grasslands Nui' perennial ryegrass swards. Effect of cutting treatments on tiller appearance and longevity, relationship between tiller age and weight, and herbage production. *New Zealand Journal of Agricultural Research*, *28*(4), 437-447.

Lee, W.O. (1985). Weed control. *Clover Science and Technology*, 295-308.

Lee, J. M., Thom, E. R., Chapman, D. F., Wynn, K. T., Waugh, D., & Rossi, L. (2013). Ryegrass seeding rate alters plant morphology and size–possible implications for pasture persistence *Science, 41*, 359-366.

Lee, J.M.; Hemmingson, N.R.; Minneé, E.M.K.; Clark, C.E.F. (2015). Management strategies for chicory (*Cichorium intybus* L.) and plantain (*Plantago lanceolata* L.): Impact on dry matter yield, nutritive Characteristics and plant density. *Crop and Pasture Science, 66,* 168-183.

Masters, R.A.; Mitchell, R. (2007). Weed management. *Forages: The science of grassland agriculture*, 395-409.

Matthew, C., & Sackville-Hamilton, N. R. (2011). Analysing persistence of grass swards in terms of tiller birth and death. *Pasture Persistence – Grassland Research and Practice, 15,* 63 - 68.

McLaren R.G. & Cameron K.C. (1996). Soil Science, Oxford University Press, Auckland, 302.

Moot, D.J.; Scott, W.J.; Roy, A.M.; Nicholls, A.C. (2000). Base temperature and thermal time requirements for germination and emergence of temperate pasture species. *New Zealand Journal of Agricultural Research, 43,* 15-25.

Mwangi, P.N.; Schmitz, M.; Scherber, C.; Roscher, C.; Schumacher, J.; SCHERER-LORENZEN, M.; Weisser, W.W.; & Schmid, B. (2007). Niche pre-emption increases with species richness in experimental plant communities. *Journal of Ecology 95*, 65-78.

Nie, Z., Miller, S., Moore, G., Hackney, B., Boschma, S., Reed, K., Mitchell, M., Albertsen, T., Clark, S., & Craig, A., (2008). Field Evaluation of Perennial Grasses and Herbs in Southern Australia. *Australian Journal of Experimental Agriculture, 48,* 424-435.

Powell, A. M., Kemp, P. D., Jaya, I. K. D., & Osborne, M. A. (2007). Establishment, growth and development of plantain and chicory under grazing. *Proceedings of the New Zealand Grassland Association, 69*, 41–45.

Rattray, P.V.; Brookes, I.M.; Nicol, A.M. (2007). Pasture and supplements for grazing animals. New Zealand society of animal production, Hamilton, New Zealand.

Sanderson, M. A., Soder, K. J., Muller, L. D., Klement, K. D., Skinner, R. H., & Goslee, S. C. (2005). Forage mixture productivity and botanical composition in pastures grazed by dairy cattle. *Agronomy Journal*, *97*(5), 1465-1471.

Sanderson, M.A.; Brink, G.; Stout, R.; Ruth, L. (2013). Grass–legume proportions in forage seed mixtures and effects on herbage yield and weed abundance. *Agronomy Journal, 105*, 1289-1297.

Sinhadipathige, S.C.S.; Kenyon, P.R.; Kemp, P.D.; Morris, S.T.; Morel, P.R.H. (2012). Can herb-clover mixes increase lamb liveweight gains in spring. *Proceedings of the New Zealand Grasslands Association, 74,* 137-142.

Sithamparanathan, J., Macfarlane, M. J., and Richardson, S. (1986). Effect of treading, herbicides, season, and seed coating on oversown grass and legume establishment in easy North Island hill country. *New Zealand journal of experimental agriculture*, *14*(2), 173-182.

Stewart, T.A. (1984). Utilising clover in grass based animal production systems. *Occasional Symposium of the British Grassland Society, 16,* 93–103.

Stewart, A. V. (1996). Plantain (*Plantago lanceolata*)-a potential pasture species. *Proceedings of the Conference-New Zealand Grassland Association, 58,* 77-86.

Stewart, A.V.; Kerr, G.; Lissaman, W.; Rowarth, J. (2014). Pasture and forage plants for New Zealand. fourth Edition. *New Zealand Grassland Association*, Dunedin, New Zealand.

Turner, J.D. (1992). High quality pasture fed for southern New Zealand - a consequence of cultivar choice and their subsequent management. *Proceeding of the New Zealand grasslands association, 54*, 91-94.

Turner L.R., Donaghy D.J., Lane P.A., Rawnsley R.P. (2006). Effect of defoliation interval on water-soluble carbohydrate and Nitrogen energy reserves, regrowth of leaves and roots, and tille r number of cocksfoot (*Dactylis glomerata* L.) plants. *Australian Journal of Agricultural Research, 57,* 243-249.

Williamson, M. L. (2008). Differential responses of tillers to floral induction in perennial ryegrass (*Lolium perenne* L.): implications for perenniality: a thesis presented in partial fulfilment of the requirements for the degree of Master of Science in Plant Biology at Massey University, Palmerston North, New Zealand.

White, J., Hodgson, J. 2000. New Zealand pasture and crop science. Oxford University Press.

White J., Hodgson J 2011. New Zealand Pasture and Crop Science. White J, Hodgson J ed.

Woledge, J. (1988). Competition between grass and clover in spring as affected by nitrogen fertiliser. *Annals of applied biology*, *112*(1), 175-186.