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**Dry matter production and botanical composition of
four pasture species and their seed mixtures
after an autumn sowing**

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

at
Lincoln University
by
Samuel Anderson

Lincoln University
2015

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The majority of New Zealand pastoral agriculture is based around white clover and perennial ryegrass. These pastures are generally well suited to the majority of New Zealand. However these pastures often fail to perform at crucial periods, such as in summer when pasture growth and quality is vital to finish livestock. The inclusion of summer active pasture species with high pasture quality could provide a solution to poor summer production from white clover-perennial ryegrass pastures. A field experiment was conducted to investigate the effect of different pasture mixtures and sowing strategies on the yield and composition of sown species over an establishment period of 7 months from an autumn sowing. The sown species included tetraploid ryegrass, white clover, plantain and red clover were used in a number of pasture mixture combinations. The experiment was conducted at Lincoln University, Canterbury, New Zealand from the 26th of March to the 22nd of September 2015. Accumulated yield from sowing to the 22nd of September was significantly different over the 23 pasture mixtures. Sowing pasture mixtures with proportions, of tetraploid perennial ryegrass and/or plantain of 50% or more results in high accumulated yields between 3000 – 3900 kg DM/ha. White and red clover pure swards produced poor yields <2000 kg DM/ha and had high weed content (0.6 weed proportion of total yield). Pasture mixtures were dominated by ryegrass and plantain even when clover composed 50% of the seed mixture. Alternate drill row sowing produced small improvements in white clover proportions, but accumulated yield was not affected.

Keywords: Tetraploid ryegrass, plantain, red clover, white clover, monoculture, alternate drill row, leaf area index, legume, herb, yield.

Acknowledgements

I would like to thank the entire team at the field service centre. Especially Dave and Shaun for all their help getting the plots prepared and sown.

To my supervisor Alistair, thanks for organising my project and making it so applicable to my background interests in agriculture. Thanks again for all your help and patient throughout the year.

Thanks to Ea for helping with all the sampling and sorting. Without your help I would have been run off my feet. Having some company out in the field made those long days of sampling that much easier.

I would like to thank Aurlie for all the help sorting, weighing and harvesting.

Thanks to my two groups of PLSC 321 students; Aran, Samara, Sean, Dan, Ollie, Bianca, Tom, and Isaac for their assistance with harvesting the plots in August and September and all the long hours spent in the separating room.

Thanks Travis for all your assistance in sowing the plots, and your expertise using imageJ and the NIRS.

I would like to thank Derrick, Jeff and Dick for your helpful feedback on my dissertation throughout the year.

To all my friends, thanks for your support throughout the year, especially my flat mates Ethan, James and Cameron.

To the other plants science honours students Tim, Blair, Steph, Nathan, Steph and Tori thanks for making this whole experience more enjoyable. All those drinks breaks in the tea room definitely helped keep me going throughout the year.

To my family, thanks for all your support this year and over the course of my four years at Lincoln University. I couldn't have done it without you.

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1 Introduction

Intensive lamb finishing systems in New Zealand require high lamb live weight gains to reach target live weights. High live weight gains are essential to reach early markets and gain premiums. Generally lambs are grazed on perennial ryegrass (*Lolium perenne*) and white clover (*Trifolium repens*) pastures. However, these pastures often fail to perform at crucial times when forage quality is vital for lamb growth. This is often due to the pastures having very low clover content. Selective grazing of clover is another cause of poor quality of these pastures due to low clover proportions. Sheep have been reported to have a 70% preference for clover and 30% preferences for ryegrass when offered both perennial ryegrass and white clover (Penning *et al.* 1997). The aggressive competitive nature of perennial ryegrass is another cause of low clover proportions.

One solution to this issue is to sow pasture species with high feed value. Red clover (*Trifolium pratense*), plantain (*Plantago lanceolata*), chicory (*Cichorium intybus*), lucerne (*Medicago sativa*), lotus (*Lotus* sp.), timothy (*Phleum pratense*) and tetraploid perennial ryegrass offer potential options for high quality forages for lamb finishing. The use of these species, in addition with diploid perennial ryegrass and white clover can provide effective means of improving pasture feed quality. The use of specialist finishing pastures also have merits for finishing lambs. Higher lambs growth rates have been reported from herb-clover mixes compared with traditional ryegrass-white clover pastures (Golding *et al.* 2011; Kemp *et al.* 2013; Sinhadipathige *et al.* 2012). 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 & Bruce 2005).

Seed mixtures in New Zealand are generally dominated by perennial ryegrass or other grass species depending on the environment, with relatively little white clover sown. Typical sowing rates for perennial ryegrass are 10-20 kg/ha, white clover 2-3 kg/ha and 2-4 kg/ha for red clover (Charlton 1991). The addition of pasture herbs such as plantain and chicory are commonly sown at a rate of 1-3 kg/ha (Turner 1992). The dominate sowing of perennial ryegrass species is a major contributor to the high ryegrass content in pastures and low clover content.

Species competition is one of the main reasons that most pastures are ryegrass dominated. Ryegrass is more competitive than white clover so tends to dominate pasture mixtures when these two species are sown together (Martin & Field 1984). White clover, however, is highly important due to both its high feed quality and its ability to biologically fix N. A balance of both ryegrass and white clover is desirable to increase production. One potential solution to increase the proportion of clover is to sow ryegrass and clover in alternate drill rows to avoid species competition (Hurst *et al.* 2000).

By separating ryegrass and clover into alternate drill rows, interspecific competition is likely to be reduced and there is potential for improved establishment of clover. This could provide a means of obtaining a balance between ryegrass and clovers, or other species included in the mix such as plantain.

The objective of this dissertation was to examine the effects of common pasture species and their seed mixtures on the DM production, botanical composition and nutritive value of pastures. The project involved a review of published scientific literature on the establishment of common pasture species and pasture seed mixtures. It also required a field plot experiment capable of systematically testing and disentangling effects of pasture species type (species identity), species richness (number of species) and species relative abundance (proportions of each species in seed mixtures), on ecosystem functions as well as effects of spatial separation of species, of plant communities. Here, the plant communities were swards created from seed mixtures with up to four pasture species, namely tetraploid perennial ryegrass, white clover, plantain and red clover. Spatial separation effects were tested by planting a subset of the two and three species mixtures in alternate drill rows. The ecosystems functions were DM production, weed suppression, leaf area index and nutritive value, which are all agronomically important variables for pasture establishment, measured over a 7 month establishment phase after an autumn (March 2015) sowing.

2 Review of the literature

2.1 Introduction

Pasture is highly important in New Zealand for livestock production. The majority of pastures in New Zealand are based on perennial ryegrass and white clover, which are both well adapted to stock grazing and to most of the prevailing climatic conditions. However, these pastures often fail to perform at key times when they are required most for animal production. This results in poor animal growth rates and performance. A potential solution to this is to introduce highly palatable pasture species. They can be used as specialist finishing pastures or even included in a complex pasture mixtures with perennial ryegrass and white clover.

This review will cover the rationale behind using pasture mixtures as opposed to monocultures of pastures species. The physiological and production characteristics of the four species used in the field experiment of this dissertation, namely tetraploid perennial ryegrass, white clover, plantain and red clover were assessed. The nature of competition in pasture mixtures and the rationale behind spatially separating pasture species are covered.

2.2 Pasture mixtures

Seed mixtures in New Zealand pastures were developed based on British practices of sowing multi-species seed mixtures during early years of settlement (Charlton 1991). Early bush-burn practices were commonly followed by the practice of sowing up to 20 species in a seed mixture in the hope that a few would establish (Harris 1968). Sowing this large amount of species was a wise decision, given that there was little knowledge of the climate, topography and soil in New Zealand at the time. Breeding of clovers and ryegrasses in the 1930s led to the use of more simple pastures (Harris 1968). Work by Alexander (1933) suggested sowing mixed pasture swards can have several advantages over pure sown swards. Alexander (1933) stated that by using a mixture:

- 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.

This knowledge led to the increased adoption of pasture mixtures and since the 1970s there has been an increase in the amount of new species released. These new species have become more popular now, which has resulted in the adoption of more complex pasture mixtures.

When selecting species and cultivars for a pasture mixture there are some factors to consider to achieve a successful pasture mixture. Harris (2001) put forward three basic principles to complying pasture seed mixtures. The first principal is matching species and cultivars to the environment where they are going to be sown. This is dependent on the response of the species to temperature, water and nutrient availability. To have a successful pasture mixture it must be able to successfully establish and grow in the selected environment. The second principal is to provide early cover by including species that can quickly occupy ground, which inhibits the establishment of weeds. This highlights the importance of competition in pasture, with plants that can get a foothold in pasture quickly usually having a competitive advantage over plants that establish slowly. The third principal is selecting a mixture with versatility that allows for both the heterogeneity of environmental microsite or ecological niches within the area being sown and for the uncertainty about the course of succession of the species after sowing. With more species and cultivars in the pasture seed mixture, this ensures that at least some of the proportion of the sown species will find a suitable niche for establishment and growth. These principals form the basis for how farmers should be selecting their pasture seed mixtures.

Another aspect in selecting species is the value of biological N fixation from clovers. Clovers can biologically fix atmospheric N and provide an important source of N that would otherwise have to be added via N fertiliser. Clovers fix N via the rhizobium bacteria which are present in the root nodules of clovers. Clovers and rhizobia live in a symbiotic relationship, where the legume provides the rhizobia with energy in exchange for N. The amount of N fixed by ryegrass/white clover pastures ranges from 0 to 350 kg N/ha/yr (White & Hodgson 2000). The amount fixed is dependent on many factors including the clover content in pasture, clover growth rate, soil temperature, nutrient availability and moisture. The biological N fixation ability of clovers has provided the basis for pastoral farming in New Zealand. Andrews *et al.* (2007) reported similar plant DM production and milk production from a perennial ryegrass/white clover pasture, compared with a perennial ryegrass monoculture pasture receiving 200 kg N/ha/yr as fertiliser. The addition of clover into pasture mixtures has clear benefits, not only for biological N fixation but also for their high quality, protein rich feed value for grazing animals (Stewart *et al.* 2014). Clover provides strong basis for addition into pasture seed mixtures.

2.3 Tetraploid perennial ryegrass

Perennial ryegrass is the most important and widely sown temperate grass in New Zealand. Perennial ryegrass grows well in a range of conditions and is easily established. It provides high animal production and is well suited to grazing. Perennial ryegrass prefers medium to high soil fertility. It does not perform well in extremely cold temperatures and is poorly adapted to hot – dry conditions. Growth of perennial ryegrass starts at 5 °C and reaches optimum at 18 °C (White & Hodgson 2000). Perennial ryegrass has fast establishment and is very competitive for resources and will outcompete other grass species when sown in a mixture. Effective recovery after heavy treading and grazing makes ease of grazing management a major advantage over other pasture species. Perennial ryegrass is very persistent in pastures and can last as long as 20 years.

Perennial ryegrass is naturally a diploid, containing 2 sets of 7 chromosomes in each cell. Most cultivars are diploid but plant breeders have bred ryegrass to produce tetraploid cultivars, which contain 2 sets of 14 chromosomes and have a different balance of features (Stewart *et al.* 2014). Tetraploids have got a lower tiller density than diploids, but the size of the tillers are larger. In comparison to diploid cultivars, tetraploids are more palatable to stock so they are preferred by grazing animals (Van Bogaert 1975). Tetraploidy significantly increases nutritive value when compared with similar diploid cultivars (Smith *et al.* 2001). This is due to reductions in neutral detergent fibre (NDF) and increases in the amount of water soluble carbohydrate (WSC) in the herbage, which are linked with increased cell size. Vipond *et al.* (1993) reported tetraploid perennial ryegrass swards had 16% greater overall lamb output than a diploid perennial ryegrass sward. This was due to a 10% higher stocking rate on the tetraploid ryegrass sward as a consequence of improved growth. Rattray *et al.* (2007) reported tetraploid perennial ryegrass having a higher feeding value than diploid perennial ryegrass (83 vs 52 rankings for tetraploid and diploid perennial ryegrass respectively), when both were compared with white clover (100) (Table 2.3). Improved lamb live weight gains from grazing tetraploid perennial ryegrass cultivars, due to consistent improvements in digestibility have been demonstrated over standard diploid perennial ryegrass cultivars (Westwood & Norriss 1999). Tetraploids however require more careful management during stressful conditions. Increased palatability can lead to over grazing in dry periods. During wet periods the reduced tiller density increases the risk of stock treading and pugging damage (Stewart *et al.* 2014). Tetraploids are more suited to intensive, high production systems where grazing is more controlled. In these systems tetraploid perennial ryegrass can be used to effectively increase productivity. Diploids in contrast are well adapted to more extensive systems where there is less control of grazing management.

Tetraploid perennial ryegrass should be used in intensive finishing properties where its high palatability can be fully utilized. Pasture mixtures with white clover and tetraploid perennial ryegrass can be

effective finishing pastures. Swift *et al.* (1993) reported clover build up to around 20% of sward DM in continuously stocked plots of tetraploid perennial ryegrass and a small-leaved cultivar of white clover (Swift *et al.* 1993). This was probably due to the relatively open growth habit of tetraploid perennial ryegrass which allows dense stolon growth from white clover. Sowing rates for tetraploid perennial ryegrass are higher than diploid perennial ryegrass rate due to larger seed size. Sowing rates of 25-30 kg/ha should be used, but this can be reduced if conditions are ideal (Stewart *et al.* 2014).

2.4 White clover

White clover is the most important forage legume in New Zealand. It is sown widely in New Zealand and is a vital component in many pasture mixtures. White clover is a perennial and has a prostate growth habit with stolons. Stolons root from the nodes and produce plants that become independent of the original plant (White & Hodgson 2000). Leaves are trifoliate with oval leaflets. White clover performs well on moderate to high fertility soils. White clover can be less productive in summer in dry periods due to its limited root system, for this reason persistence can be poor when rainfall is under 600-700 mm (White & Hodgson 2000). Moot *et al.* (2000) estimated an average base temperature for vegetative development of white clover from a number of cultivars to be $0.8 \pm 0.28\text{ }^{\circ}\text{C}$ (Table 2.1). Thermal time required for germination was estimated at $41 \pm 1.6\text{ }^{\circ}\text{Cd}$. White clover stops growing at approximately 8-9 $^{\circ}\text{C}$ and reaches maximum growth rates at 25 $^{\circ}\text{C}$, which is why the winter growth of white clover is poor (White & Hodgson 2000).

Table 2.1 Estimates of base temperature (T_b) and thermal time (T_t) required for germination of white and red clover. Adapted from Moot *et al.* (2000).

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White clover can biologically fix N via rhizobia in root nodules. Biological N fixation from white clover in grazed pastures has been assessed at between 55 and 296 kg N/ha/yr (Ledgard & Steele 1992). The amount of N fixed per year is mainly dependent on the amount of clover in the pasture and the

environment (climate) it grows in. Hoglund *et al.* (1979) reported N fixation per year from white cover ranging from 34 – 342 kg N/ha/yr (Table 2.2). N fixation was higher in irrigated and wetter areas of New Zealand and was also influenced by temperature and soil fertility. N fixation was higher when clover proportions in the pasture were increased. Clover yields ranged from 150 – 5040 kg DM/ha/yr in pastures 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) in mixed pastures.

Table 2.2 White clover yield, percentage pf total pasture yield and N fixation per year for a range of sites in New Zealand. Adapted from Hoglund *et al.* (1979).

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White clover mixes well with most pasture species but does best when mixed with a pasture with a relatively open sward, which allows white clover to thrive. White clover is usually sown in pasture mixtures at a rate of 2-5 kg/ha (Stewart *et al.* 2014; White & Hodgson 2000). Ryegrass and white clover have been the most popular seed mixture, but there is increasing popularity in growing white clover with forage herbs such as plantain and chicory.

White clover has high levels of crude protein and a higher ratio of soluble to structural carbohydrate in comparison to ryegrass and many other legumes (White & Hodgson 2000). This makes white clover a high quality feed to grazing livestock. Rattray *et al.* (2007) reported white clover had a superior feeding value compared with other common pasture species (Table 2.3). When sown in a pure sward, white clover has twice the feeding value relative to a pure sward of perennial ryegrass. Increasing lamb live weight gains have therefore been reported with increases in the clover proportion in pasture mixtures (Figure 2.1).

Table 2.3 Feeding value of forages based on live weight gain when fed *ad libitum* to growing lambs. The ranking is relative to white clover (100). Adapted from Rattray *et al.* (2007).

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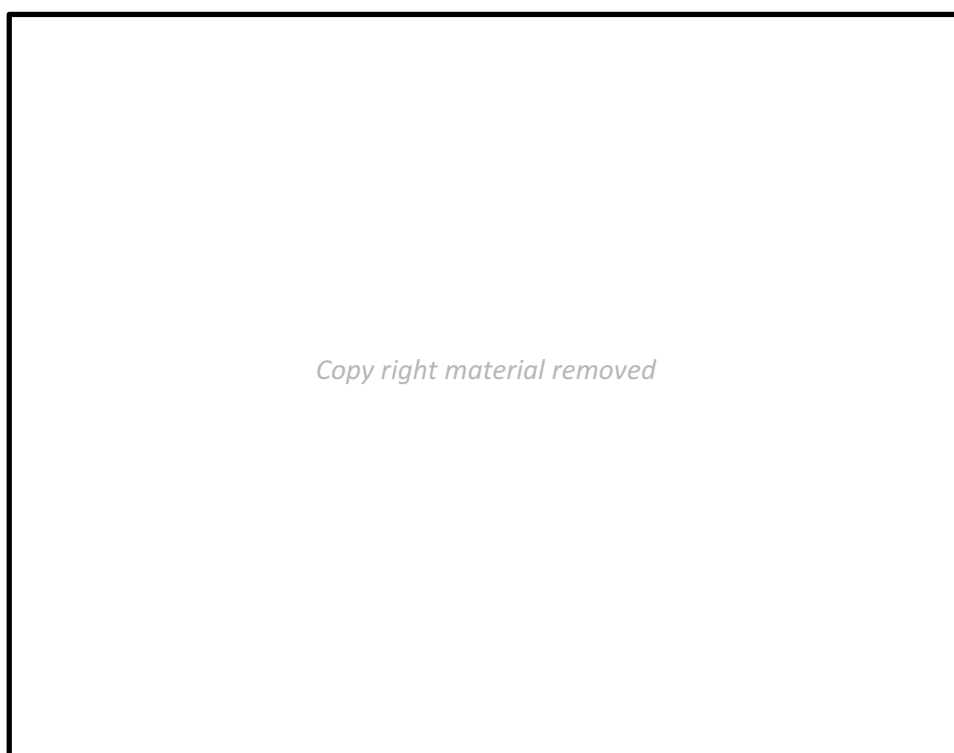


Figure 2.1 Relationship between percentage white clover in the diet on offer and live weight gain in young sheep grazing either tall fescue and white clover (—●—) or ryegrass and white clover (---●---) swards. From Hyslop *et al.* (2000).

2.5 Plantain

Plantain is a perennial herb that is highly palatable to grazing animals and is a mineral rich forage (Stewart 1996). Plantain has a rosette of broad leaves that are produced from a crown. Plantain also contains biologically active compounds such as condensed tannins which have been linked to have antimicrobial and anthelmintic effects in grazing livestock. A tap root allows plantain to access water during dry periods which allows quality to be maintained over summer. Improved performance from lambs and sheep grazed on plantain in spring have been reported (Judson *et al.* 2009; Sinhadipathige *et al.* 2012). Plantain is relatively short lived in pastures, lasting 2-4 years depending on grazing

management (Stewart *et al.* 2014). Plantain is not tolerant of set stocking and should be rotationally grazed to maintain persistence. It is important to graze plantain in summer to reduce reproductive stems that have low palatability.

Narrow leaf plantain occurs naturally in many pastures as a weed. The readily acceptance of narrow leaf plantain by livestock and adaption to grasslands has led to the selection of productive cultivars for agricultural production in New Zealand. 'Grasslands Lancelot' was the first cultivar to be released which has since been followed by 'Ceres Tonic'. The use of herb species such as plantain and also chicory has been increasing in New Zealand as farmers recognize their potential forage quality. The two cultivars differ quite a bit with 'Grasslands Lancelot' being semi-erect, having medium size leaves, high tiller numbers, low winter growth and high summer growth. 'Ceres Tonic' has very erect, very large leaves, with medium tiller numbers and a high summer and winter growth. Plantain is usually sown as a component of pasture mixtures to increase seasonal yields, especially in drier climates and in low fertility areas. Sowing plantain in mixtures with clovers as a specialist finishing pasture has been increasing in popularity as a means of increasing lamb live weight gains (Golding *et al.* 2011). Typical sowing rates for plantain in pasture mixtures are 3-5 kg/ha (White & Hodgson 2000). When sown in a pure sward plantain should be sown at a rate of 7-10 kg/ha (Stewart *et al.* 2014). Establishment for plantain is very rapid and comes close to that of perennial ryegrass (Blom 1978).

Annual production of plantain is 10-15% lower than that of perennial ryegrass, with lower winter growth rates. DM yields can be greatly influenced by seasonal rainfall with production in wet season yields of 11-15 t DM/ha possible, however in dry seasons the yields of 6-9 t DM/ha are likely (Stewart *et al.* 2014). Stewart (1996) reported the yield of 'Lancelot' and 'Tonic' plantain in comparison to perennial ryegrass and other pure sown pasture species under dryland conditions (Table 2.4). Plantain yield over establishment was approaching that of perennial ryegrass and higher than most alternative grasses. The more recently released 'Tonic' plantain obtained higher yields over all periods, compared with 'Lancelot' plantain. Summer yields of both plantain cultivar were superior to perennial ryegrass.

Table 2.4 Yields of pure dryland species plots relative to perennial ryegrass (100) over 4 years. From Stewart (1996).

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2.6 Red clover

Red clover is a perennial legume which is short lived (lasts 3-4 years). Red clover has an upright growth habit and grows from a crown. Inflorescences are large and range in colour from pink to purple (White & Hodgson 2000). The young shoots of red clover grow from the crown which is above ground and can be susceptible to damage during grazing. Red clover performs well on soils with moderate to high fertility. A deep tap root allows red clover to have greater survival and DM production in summer and in dry periods. This allows red clover to provide high quality feed over summer when water is more limiting to other pasture species. Estimated average base temperature for development of red clover cultivars has been reported as 0.6 ± 0.15 °C, with average thermal time to germination estimated as 66 ± 2.1 °Cd (Table 2.1) (Moot *et al.* 2000).

Red clover can have superior summer growth than white clover in dry summers, however winter growth is very poor. White and Hodgson (2000) reported the seasonal distribution of red clover production for “Grasslands Pawera” as 30% of the total annual production in spring, 50% in summer and the remaining 20% in autumn and winter. Due to its low winter growth and high summer growth red clover is commonly used in mixtures with perennial ryegrass or Italian ryegrass (*Lolium multiflorum*) for feed conservation as hay or silage (Black *et al.* 2009). Red clover can yield up to 17 t DM/ha/yr when sown in a pure sward and under irrigation (Brown & Moot 2004). Typically DM yields are much lower than this due to competition with other pastures species and also water limitations in dry summer periods.

The use of red clover in grazed pasture mixtures is also common along with the use of red clover in specialist finishing pastures. Sowing rates for red clover are dependent on seed size which is larger for

tetraploids and lower for diploid cultivars. Usually a rate of 4-8 kg/ha is used in pasture mixtures (White & Hodgson 2000). Red clover is less able to withstand grazing in comparison to white clover, due to its growth point being above ground and vulnerable to damage during grazing. With increasing defoliation frequency of red clover, yield and persistence decrease (Black *et al.* 2009). Because of this red clover is best suited to lax grazing or cutting to ensure persistence of red clover in the sward. Grazing management is key to the persistence and performance of red clover.

Like white clover, red clover is a high quality forage that is widely accepted by grazing livestock. Rattray *et al.* (2007) reported red clover had a feed value that was 30% lower than the feeding value of white clover (Table 2.3). Red clover can provide high levels of both milk production and lamb live weight gain over standard white clover/ryegrass pastures (Keogh & Thomson 1996).

2.7 Plant competition in mixed pastures

Plant competition is the competition between plants for limited resources (e.g. light, water, nutrients and space). Plant competition has a major influence on species composition and longevity in mixed pastures. In pure monocultures there is only intraspecific competition, which is the competition between plants of the same species. In mixed pastures there is interspecific competition, which is the competition of different species of plants with each other (e.g. perennial ryegrass and white clover). It is generally accepted that intraspecific competition is more intense than interspecific competition, due to the two plants requiring exactly the same resources (Haynes 1980). Intraspecific competition is more dependent of plant density with more competition as plant density increases. Initially when seedlings are young there is very little to no competition as there is plenty of space, water, nutrients and light for each plant. As plants develop and begin to expand plants begin to be shaded by other plants which is the beginning of competition. The most competitive plants out compete the less competitive plants that do not persist. Competition plays a vital role in succession of ecosystems in which species succeed one another until a steady state is reached.

Interspecific completion is very important in understanding how pasture mixtures establish and then consequent the persistence of certain sown species. Generally in pasture mixtures clovers will have a faster germination and emergence than grasses (Table 2.5). However grasses such as perennial ryegrass have rapid germination, emergence and canopy development and will out compete clover for light and space. This results in pasture mixtures that are dominated by ryegrass with low clover proportions (often less than 20%). Tall and erect growing ryegrass shades out white clover that has small and prostrate growth. For this reason it is important to consider the compatibility of pasture species before sowing them in a mix. For example sowing either white or red clover, which have relatively low thermal time requirements for emergence with another slow emerging pasture species like timothy (Table 2.5) can provide a more appropriate option for a companion grass than a fast

establishing ryegrass. Plantain is likely to have similar properties to perennial ryegrass with its rapid rate of establishment (Blom 1978). Although plantain, being a broad leaf species may cause further shading on clovers than ryegrass.

Table 2.5 Summary of thermal time to 75% germination and 50% emergence of herbage species. Analysis assumes a base temperature of 0°C. Adapted from Moot *et al.* (2000).

| |
|------------------------------------|
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In regards to this dissertation, it is likely that the pasture mixtures containing perennial ryegrass will be dominant in perennial ryegrass. White and red clover will likely make up a relatively low proportion when sown with either plantain or perennial ryegrass, due to their slow establishment especially when sown in autumn.

2.8 Spatial separation of species in pastures

The inter-specific competition between perennial ryegrass and clover when they are sown in a mixture is one of the primary causes of low clover content. White clover often constitutes less than 20% of the pasture. This is a result of the aggressive and competitive nature of perennial ryegrass. Decreasing competition between clovers and grasses is one potential option to increase clover proportions in pasture. One method of achieving this is through spatially separating species within a field at sowing. Spatial separation may reduce the complex competitive interactions between clover and grass (Chapman *et al.* 1996). This has the potential to increase clover proportions in grazed pasture systems, through allowing the clover to establish without the aggressive competition of ryegrass. This would also allow grazing livestock a better balanced diet selection compared with a grass dominated pasture. The benefits to animal performance through increasing clover proportions have already been stated in this review. The application of species separation can be taken beyond white clover and perennial ryegrass to other slow establishing species. Studies into the practice of sowing individual pasture species in alternative drill rows is currently lacking. However, the benefits gained from mixing certain species together in pasture, compared to sowing them alone (e.g. grass and clover mixes can yield more than either species sown alone) may be different when species are sown in alternate rows. This system has not been tested for pastures with species sown in alternate rows.

2.9 Leaf area index

Interception of solar radiation by plant leaves is a main driver of photosynthesis and plant growth. Plants require green leaves to absorb photosynthetically active radiation (PAR) and use it for photosynthesis and growth. As plants develop they branch out and spread to increase green leaf area and increase light interception. Canopy leaf area is quantified as the leaf area index (LAI) which is the green leaf area per unit area of ground (Ratnayake *et al.* 2007). As plants develop the LAI increases as the plant spreads out and covers more area with green leaves. However even a high leaf area index there are gaps in the canopy, therefore 100% light interception is usually unobtainable. For this reason 95% light interception is defined as the critical leaf area index (LAI_{crit}). Brougham (1957) investigated the relationship between leaf area and light interception in regrowth of pure and mixture swards of pasture species (Figure 2.2). For a mixed sward of perennial ryegrass and white clover the critical LAI occurs at a LAI of 3.5-4.5 (Joggi *et al.* 1983; Ratnayake *et al.* 2007). In comparison a pure perennial ryegrass sward will have a critical LAI of around 6-7. White clover has a critical LAI of 3.5. Critical LAI for red clover has been reported at 3.3 (Joggi *et al.* 1983). No literature was found for critical leaf area index of plantain. Differences in LAI_{crit} are due to the orientation of leaves towards the sun. Ryegrass for example has long and slender and usually erect leaves. White and red clover leaves are more horizontal, which makes them more effective at intercepting light than ryegrass.

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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).

2.10 Conclusions

- Sowing species in pasture mixtures provides advantages over sowing pasture species in pure swards.
- White clover is a valuable pasture species due to its high feed quality to grazing animals and also the ability to biologically fix N.
- Red clover is a valuable addition to pasture mixture as it provides high quality feed through summer and can be another source of biological N fixation.
- The addition of plantain has the potential to increase palatability and seasonal growth in pasture mixtures especially in dry summers.
- Tetraploid ryegrass provides direct advantages over diploid ryegrass cultivars through increased palatability to livestock which allows increases in intake.
- Competition between pasture species can be a large determinant of the persistence and proportions of sown pasture species.
- There is a lack of literature on the effects of separating species at sowing.

- Leaf area index is a critical measurement in determining the development of plant canopies and explaining yield differences in pasture mixtures.

3 Materials and methods

3.1 Experimental design

Nineteen seed mixtures of four species were created based on a simplex centroid design (Cornell 2011). The four species were 'Base' tetraploid perennial ryegrass, 'Tonic' plantain, 'Apex' white clover (with prill seed coating) and 'Sensation' red clover.

There were four monocultures, six two-species mixtures sown evenly, four three-species mixtures sown evenly, one mixture with all four species in equal parts and four four-species mixtures with one of the four species dominate in the mixture in turn and the other three species all equal. In addition, there were four mixtures of perennial ryegrass, white clover and plantain, repeated with each species sown in alternate drill rows. There were three two-species mixtures and one three-species mix. The 23 blends were randomly assigned to plots within each of four replicates according to a randomized complete block design. The plot size was 2.1 m x 6 m.

3.2 Experimental site

The experiment was conducted in the Horticulture Research Area (H8) at Lincoln University, Canterbury, New Zealand (Plate 3.1). The soil was a Templeton silt loam (Cox 1978).

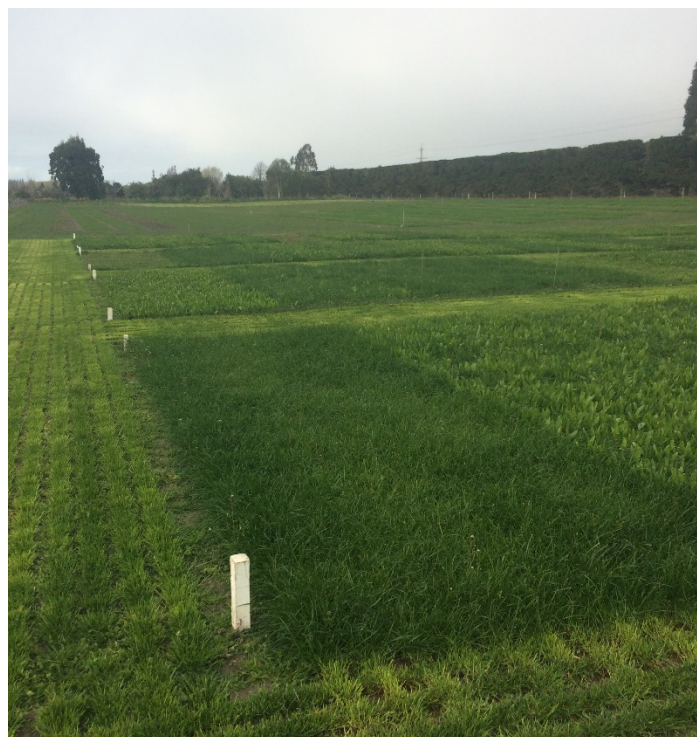


Plate 3.1 Experiment area at Lincoln University (paddock H8) on the 21st of September 2015 prior to the September harvest.

3.3 Paddock history

The paddock was sown in lucerne in 2011-2012 and was then put into oil-seed rape (*Brassica napus*) in 2013. In 2014 the paddock was put into oats (*Avena sativa*) and in summer was cultivated and left in a fallow.

A soil test for the entire paddock was taken in early March 2015. The soil pH was 5.4, Olsen P of 14 mg/L, Calcium of 7.4 me/100g, Magnesium at 0.9 me/100g and the Sulphur sulphate of 12 mg/kg. Another soil test was taken on the 4th of May from the trial area. The soil test results showed the pH to be slightly less acidic than entire paddock soil test at 5.7 pH (Table 3.1). Olsen P was well below optimum levels at 13 mg/L, which is similar to the previous Olsen P of 14 mg/L. Magnesium and Sodium were both slightly below optimum at 0.84 and 0.17 me/100g each respectively. Sulphate sulphur was similar to previous soil test.

Table 3.1 Soil test results (0-10cm depth) on the 4th of May 2015, for experimental site in H8, Lincoln University.

| | pH | Olsen P (mg/L) | SO ₄ -S (mg/kg) | K ⁺ | Ca ²⁺ | Mg ²⁺ (me/100g) | Na ⁺ |
|---------------|-----------|-------------------|-------------------------------|----------------|------------------|-------------------------------|-----------------|
| Test Results | 5.7 | 13 | 13 | 0.32 | 7.3 | 0.84 | 0.17 |
| Optimum Range | 5.8 - 6.3 | 20 - 30 | 10 - 12 | 0.30 - 0.50 | 3.0 - 9.0 | 1.00 - 1.50 | 0.20 - 0.40 |

3.4 Seed bed preparation

Prior to cultivation the paddock was sprayed with 3 litres of Roundup (570 g/L glyphosate) to kill off weeds. The paddock was then rotary hoed and rolled with a Cambridge roller. The paddock received 60 mm of irrigation as well as a further 20 mm in rainfall at the start of March, 2015 to ensure sufficient moisture after a dry summer. The paddock was rolled again in March prior to drilling. The plots were drilled on the 26th of March 2015 with a Flexi-seeder drill. The drill had 15cm drill spacing's and sown at a depth of 15-20mm.

3.5 Thousand seed weights

Three replicates of a 100 seeds were counted to determine the thousand seed weight (TWS) of each species (Table 3.2). The counts of the white clover were repeated but with washed clover seed to account for the weight of the seed coating around the seeds.

Table 3.2 Thousand Seed weight (TSW).

| | 'Base' Perennial ryegrass | 'Apex' White clover (Coated seed) | 'Apex' White clover (washed seed) | 'Tonic' Plantain | 'Sensation' Red clover |
|---------|---------------------------------|---|---|---------------------|---------------------------|
| TSW (g) | 3.64 | 0.883 | 0.822 | 2.73 | 2.14 |

3.6 Germination test

Germination tests were run on all four species, on tissue paper with 50 grids on each. Three replicates of each species with 50 seeds placed in each grid of the paper. The germination paper was placed in plastic containers with lids. The paper with the seeds was watered and then stored in an incubator at 15°C. The seeds were checked daily to ensure they were still moist. After 7 days the germinated seeds were counted and removed and the remaining seeds left and placed back in the incubator for another 7 days. After 7 days the seeds that germinated were counted and the remaining seeds tested for poor vigour or hard seed. Pressure was applied to each seed by pressing down on the seed with the tip of the finger. Seeds that were crushed easily were classed as having poor vigour and seeds that were still hard were classed as having hard seediness (Table 3.3).

Table 3.3 Average percentage of hard seed, poor seed vigour and seed germination of 'Base' tetraploid perennial ryegrass, 'Apex' white clover, 'Tonic' plantain and 'Sensation' red clover after 14 days in an incubator at 15°C.

| | Perennial ryegrass | White clover | Plantain | Red clover |
|-------------|--------------------|--------------|----------|------------|
| Hard seed | 6.0 | 3.3 | 0.0 | 8.7 |
| Poor vigour | 1.3 | 5.3 | 0.7 | 3.3 |
| Germination | 92.7 | 91.3 | 99.3 | 88.0 |

3.7 Seed rates

The 23 different blends (the proportions of each species in the mix) were created using the mixture design in the statistical software package Minitab 17 (Table 3.4). The seed rates were based on a plant population basis. A total seed population of 820 seeds per m² was used to acquire seed rates for all seed mixtures. To achieve this seed population a sowing rate of 30, 7.5, 23 and 18 kg/ha were used for perennial ryegrass, white clover, plantain and red clover respectively. Enough seed was weighed out to drill seven meters of every plot. This ensured that the seed did not run out too early and the 2 meter gaps between blocks allowed a buffer for the seed to be all used before the next plot was drilled. The thousand seed weights were used to calculate the seed rates for each species. The four plots sown in alternate drill rows had the same species proportions and seed rates as mixtures 5, 6, 8 and 11 (Table

3.4 & 3.5). The sowing rates were not adjusted for any differences in germination between species (Table 3.3).

The Flexiseeder drill that was used to drill the plots was specially adapted to allow alternate drill row planting. The drill had a second cell wheel attached that allowed species to be drilled separately. When drilling the two-species alternate drill row plots the pipes carrying the seed from the cell wheel to the tines were set up to allow the alternate drill row sowings of the two species. With the three-species alternate drill row sowing the fertiliser hopper was used to sow the third species (ryegrass). Because the three species could not be evenly split into the 14 drill rows, ryegrass and white clover were sown in five of the drill rows and plantain in four. The drill was set up to allow the species to be sown in order of ryegrass, white clover, plantain and then repeating this sequence going from the left most tine to the right. From each cell wheel and the fertiliser hopper there were several pipes from each that were not attached to a tine and went to waste. The waste seed was caught by attached containers and later weighed to ensure accurate drilling.

Table 3.4 Proportion of perennial ryegrass (RG), white clover (WC), plantain (P) and red clover (RC) sown in each of the 19 pasture mixtures. Mixture 5, 6, 8 and 11 were repeated with species sown in alternate drill rows.

| Mixture ID | RG | WC | P | RC |
|------------|-------|-------|-------|-------|
| 1 | 1 | 0 | 0 | 0 |
| 2 | 0 | 1 | 0 | 0 |
| 3 | 0 | 0 | 1 | 0 |
| 4 | 0 | 0 | 0 | 1 |
| 5 | 0.5 | 0.5 | 0 | 0 |
| 6 | 0.5 | 0 | 0.5 | 0 |
| 7 | 0.5 | 0 | 0 | 0.5 |
| 8 | 0 | 0.5 | 0.5 | 0 |
| 9 | 0 | 0.5 | 0 | 0.5 |
| 10 | 0 | 0 | 0.5 | 0.5 |
| 11 | 0.333 | 0.333 | 0.333 | 0 |
| 12 | 0.333 | 0.333 | 0 | 0.333 |
| 13 | 0.333 | 0 | 0.333 | 0.333 |
| 14 | 0 | 0.333 | 0.333 | 0.333 |
| 15 | 0.25 | 0.25 | 0.25 | 0.25 |
| 16 | 0.625 | 0.125 | 0.125 | 0.125 |
| 17 | 0.125 | 0.625 | 0.125 | 0.125 |
| 18 | 0.125 | 0.125 | 0.625 | 0.125 |
| 19 | 0.125 | 0.125 | 0.125 | 0.625 |

Table 3.5 Sowing rates of perennial ryegrass (RG), white clover (WC), plantain (P) and red clover (RC) sown in each of the 19 pasture mixtures (kg/ha) (dom = dominate in the mix). Mixtures 5, 6, 8 and 11 were repeated with species sown in alternate drill rows.

| Mixture ID | Species in mix | RG | WC | P | RC | Total |
|------------|------------------|------|-----|------|------|-------|
| 1 | RG | 30.0 | 0.0 | 0.0 | 0.0 | 30.0 |
| 2 | WC | 0.0 | 7.5 | 0.0 | 0.0 | 7.5 |
| 3 | P | 0.0 | 0.0 | 23.0 | 0.0 | 23.0 |
| 4 | RC | 0.0 | 0.0 | 0.0 | 18.0 | 18.0 |
| 5 | RG,WC | 15.0 | 3.8 | 0.0 | 0.0 | 18.8 |
| 6 | RG,P | 15.0 | 0.0 | 11.5 | 0.0 | 26.5 |
| 7 | RG,RC | 15.0 | 0.0 | 0.0 | 9.0 | 24.0 |
| 8 | WC,P | 0.0 | 3.8 | 11.5 | 0.0 | 15.3 |
| 9 | WC,RC | 0.0 | 3.8 | 0.0 | 9.0 | 12.8 |
| 10 | P,RC | 0.0 | 0.0 | 11.5 | 9.0 | 20.5 |
| 11 | RG,WC,P | 10.0 | 2.5 | 7.7 | 0.0 | 20.2 |
| 12 | RG,WC,RC | 10.0 | 2.5 | 0.0 | 6.0 | 18.5 |
| 13 | RG,P,RC | 10.0 | 0.0 | 7.7 | 6.0 | 23.7 |
| 14 | WC,P,RC | 0.0 | 2.5 | 7.7 | 6.0 | 16.2 |
| 15 | 4 species | 7.5 | 1.9 | 5.8 | 4.5 | 19.6 |
| 16 | RG dom 4-species | 18.8 | 0.9 | 2.9 | 2.3 | 24.8 |
| 17 | WC dom 4-species | 3.8 | 4.7 | 2.9 | 2.3 | 13.6 |
| 18 | P dom 4-species | 3.8 | 0.9 | 14.4 | 2.3 | 21.3 |
| 19 | RC dom 4-species | 3.8 | 0.9 | 2.9 | 11.3 | 18.8 |

3.8 Climate data

Climate data was taken from the Broadfields Meteorological Station (Lincoln, Broadfield Electric weather station, Network No. 17603) located at latitude -43.6262, longitude 172.4704 and 18 m above sea level (approximately 1.9 km from experiment site). Mean monthly rainfall, air temperature and soil temperature are given in Figures 3.1, 3.2 and 3.3 respectively.

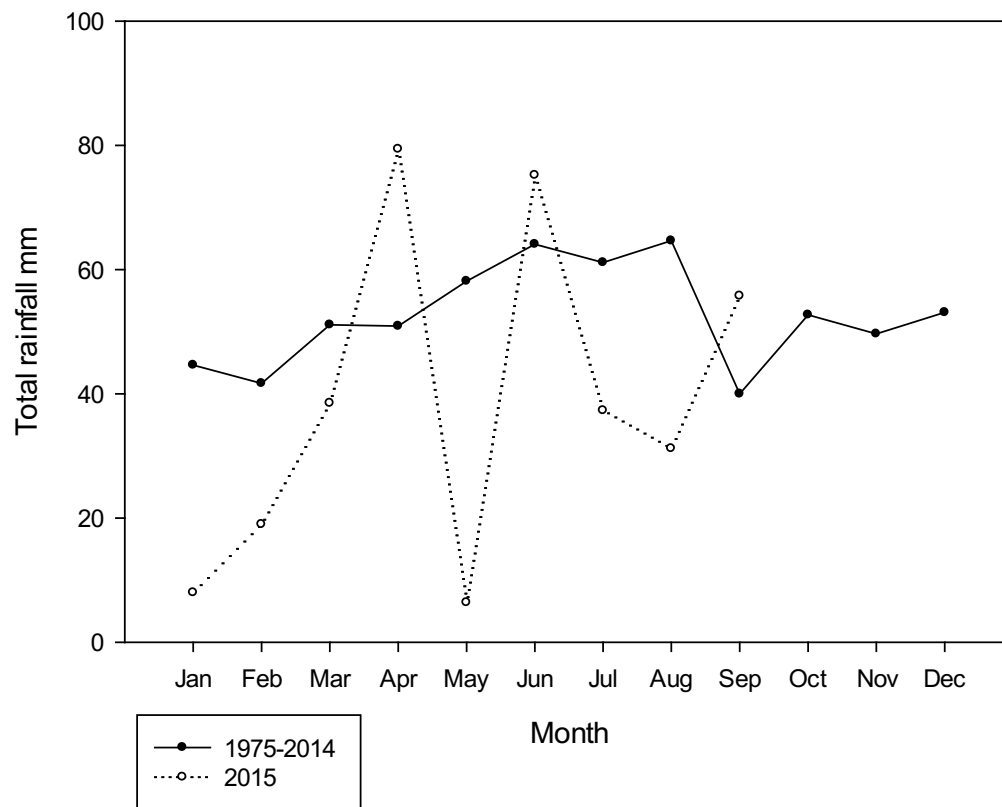


Figure 3.1 Average monthly rainfall from 1975-2014 and 2015 for Lincoln, New Zealand (NIWA 2015).

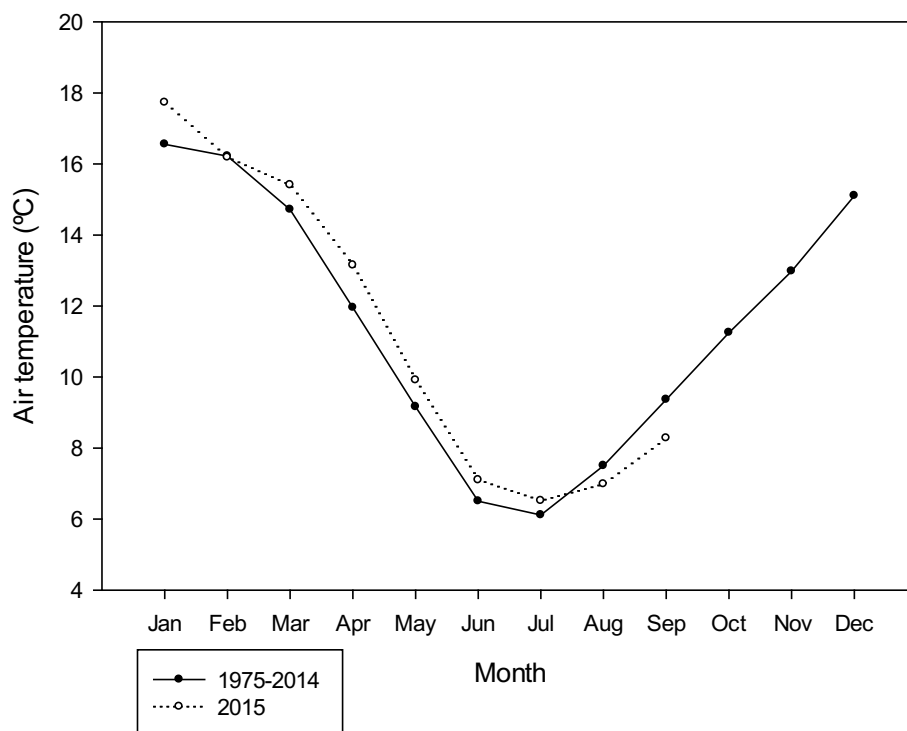


Figure 3.2 Average monthly mean air temperature from 1975-2014 and 2015 for Lincoln, New Zealand (NIWA 2015).

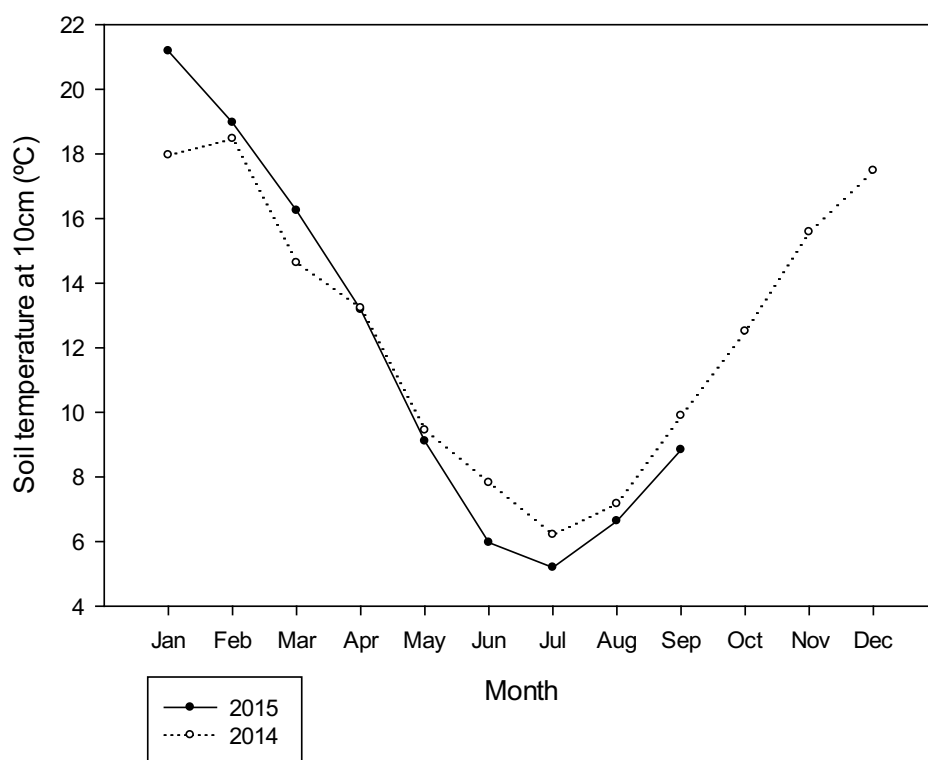


Figure 3.3 Average monthly soil temperature (°C) for 2014 and 2015 at 10cm depth, for Lincoln, New Zealand (NIWA 2015).

3.9 Management

Plots were mown on the 15th of August following the completion of the August harvest to simulate first grazing. Plots were cut to a height of 5 cm with a standard lawn mower, with all cut herbage being removed from the plots. To rectify the P deficiency identified through the soil test in May, 500 kg/ha of superphosphate (9% P, 11% S) was applied in September to the experiment. Following the completion of the September harvest (22 September) the plots were lightly grazed by hoggets for 3-4 days and the residual mown to 4-5 cm.

3.10 Plant population measurement on the 5th of May 2015

Population counts were done on each plot on the 5th of May, 2015 to quantify the plant populations present. Plant counts from one meter along two adjacent drill rows for 22 of the treatments and three adjacent drill rows for the three-species sown in alternate drill rows. The population count was taken for the individual species of ryegrass, white clover, plantain and red clover within each drill row.

3.11 May harvest

The first physical harvest was on the 5th of May and was done by cutting five individual plants of each sown species from each plot. The plants were cut about 1 cm from the ground using scissors. The plant material was then separated into leaf and stem of each species with counts of the number of leaves and stems also recorded. The plant material was then dried in an oven at 60°C for at least 48 hours. The material was then weighed with a fine scale to determine leaf and stem dry weight. The total dry weight could then be determined and then with the according population count data, an estimate of DM production was made.

3.12 August harvest

The second harvest was taken on the 4th of August, 2015. The harvest involved cutting the plant material from 1 m of two adjacent drill rows for 22 of the treatments and of three adjacent drill rows for the three-species alternate drill row sowing treatment. Plant material was cut 1 cm from the ground using scissors. All plant material from every plot was then separated into each sown species as well as weeds and dried in an oven at 60°C for a least 48 hours. After drying the samples were weighed.

To measure plant populations from the harvest at this date, five plant of each sown species were harvested from each plot on the 4th of August. The plants were cut underground to ensure entire plant collection. Soil was removed and the plants were cut to the same height as the previous harvest. The tiller numbers per plant were recorded for each sown species. The leave and stem material was then separated and bagged. The plant material was then dried in an oven for at least 48 hours at 60°C. The

material was then weighed and the average plant weight used to calculate plant populations for each plot.

3.13 September harvest

The third and final harvest was taken on the 21st of September, 2015. The harvest protocol followed the previous harvest date in August with the exception of harvests of plants for population measurements and the use of electric hand piece rather than scissors. All plant material from every plot was then separated into botanical composition of sown species as well as weeds and dried in an oven at 60°C for at least 48 hours. After drying the samples were weighed. Weighed samples were then re-bagged and kept with the exception of weeds which were discarded. Sub samples from the four monocultures and the four equal species mixture were taken for each sown species to determine leaf and stem percentages. These samples were also kept for pasture quality.

For pasture quality analysis plant material from rep 1 and 2 were used. Firstly each individual sample was mixed together by hand to ensure species were well mixed together after being weighed separately. After mixing samples were placed in the oven at 60°C for 1 hour to remove any moisture. A small representative sub sample was then taken from each individual sample and ground one at a time through a grinder to a fine powder. Ground samples were collected in small plastic containers. Between each sample being ground the grinder was cleaned with an air compressor to remove any plant residual from the previous sample. Samples were then analysed using Near Infrared Spectroscopy with calibration to dry green leafy material. Metabolisable energy (ME) was calculated by $ME = (DMD + 3) \times OM/100 \times 0.16$.

3.14 Leaf area measurement

Leaf area was measured on the 5th of May, 3rd of June and the 11th of August, 2015 for ryegrass, white clover, plantain and red clover. 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 four-species mixture with all species equal, Mixture 15. This was to reduce the amount 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 stay together. The plants were cut under the ground with scissors to ensure plants stayed together 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. The number of leaves, petioles and tillers were counted and the stems bagged. The leaves between the plastic were then

scanned at high resolution and in colour. The plant images were then measured for total leaf area of each plant using the computer programme imageJ. Plant leaves of the five individual plants of each species from each plot were dried for a minimum of 48 hours in an oven at 60°C. The stems/bases from the five plants of each species were also dried as previous. The plant material was then weighed in grams to three decimal places. The weight of each individual plant leaves were labelled so they could be matched to the according plant leaf area. Specific leaf area was then calculated by dividing the leaf area of the plant by the dry weight of the leaves of the plant. Specific leaf area was then used to calculate leaf area index (LAI) through the following calculation:

$LAI = \text{Specific leaf area} \times \text{Leaf \%} \times \text{the dry matter yield per unit area (i.e. kg DM/ha)}$

3.15 Statistical analysis

The effects of species proportions in the seed mixture on accumulated sown yield were analysed using the mixture regression method in Minitab 17. A quadratic mixture model was fit to the data as follows:
 $SY \text{ (kg DM/ha)} = b_1*RG + b_2*WC + b_3*P + b_4*RC + b_5*RG*WC + b_6*RG*RC + b_7*WC*P + b_8*P*RC$
 SY is the sown yield response from a mixture; RG, WC, P and RC are the sown proportions of tetraploid perennial ryegrass, white clover, plantain and red clover respectively, b1 is an estimate of the response of the perennial ryegrass monoculture, while b2, b3 and b4 represent the estimated response of white clover, plantain and red clover monocultures. b5 to b8 represent the interaction effects for the combination of two species, with non-significant species interactions excluded from the quadratic equation. Another mixture analysis was used on the alternate drill row sown pasture mixtures to access treatment effects.

Significance of mixtures were tested using one way and two way ANOVA. The stats package GenStat 16 was used to obtain P values and standard error of difference (SED). Sigma plot 13 was used to present results.

4 Results

4.1 May harvest

Average plant population in May ranged from 555 to 335 plants/m² with an average of 435.5 plants/m² (Figure 4.1). Based on the sowing population of 820 seeds/m² the actual emergence was just over 50% of the seeds that were sown. There were differences in the plant population between the pasture mixtures ($P=0.015$). The perennial ryegrass monoculture (1) had the highest plant population at 555 plants/m² which was also evident throughout the mixtures that contained perennial ryegrass. Evidence of increased perennial ryegrass population was evident in the two species mixtures (5, 6 & 7). Where perennial ryegrass was sown with either white clover, red clover or plantain, in a 50/50 mix the plant population was composed of 56-58% perennial ryegrass with the other 41-43% composed of the other sown species. White clover and red clover monocultures had lower populations compared with both perennial ryegrass and plantain which lead to slightly lower contribution to total plant population. Over all the pasture mixtures the proportion of sown species composing the total plant population was very similar to the proportions that they were sown at. The four species mixtures sown with one species dominate (16, 17, 18 & 19), provides evidence that species abundance was similar to the proportion of seeds of each species sown in the mixture, with each mixture showing a clear dominance for the species that was sown dominantly.

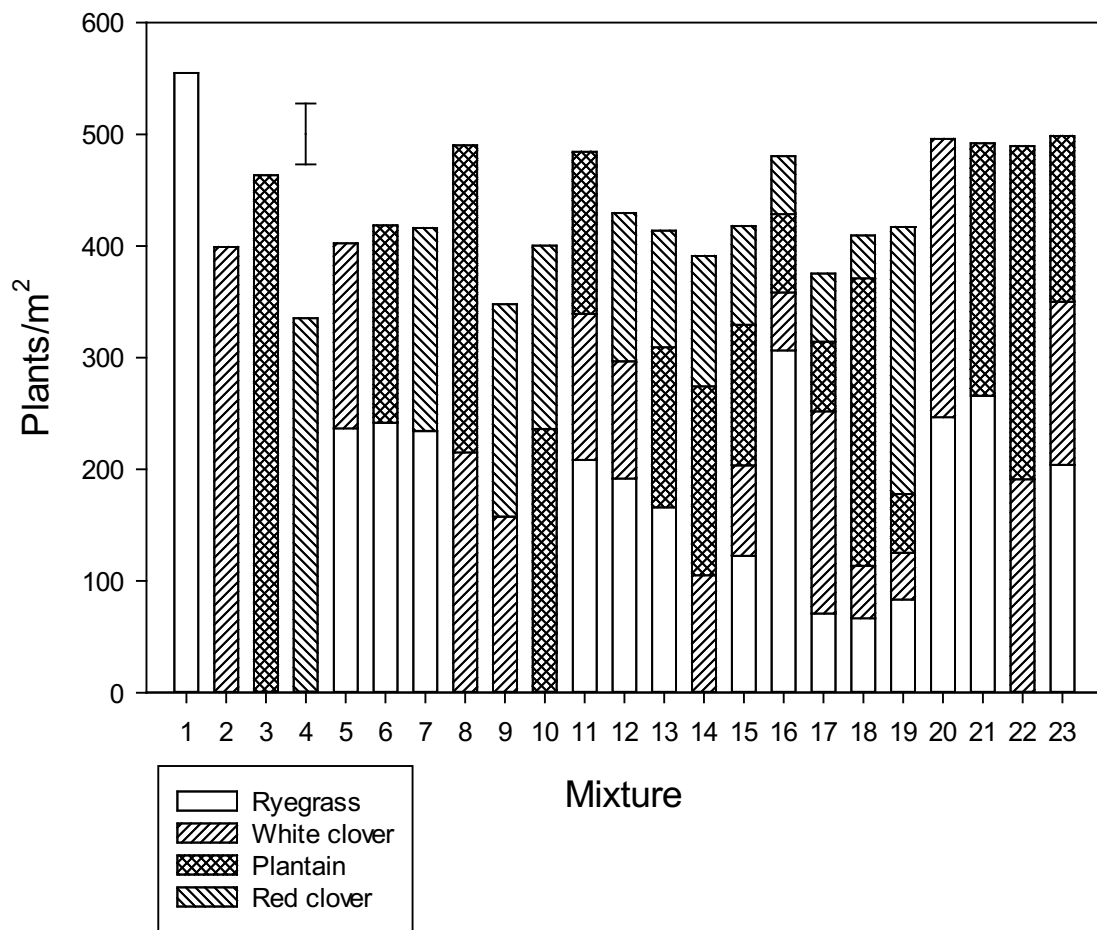


Figure 4.1 Average plant population per square meter of perennial ryegrass, white clover, plantain and red clover and their mixtures on the 5th of May 2015 at Lincoln University. P value of 0.015. Error bar represents SED.

The average shoot DW of perennial ryegrass and plantain per plant, were much greater than white and red clover in May (Figure 4.2). Perennial ryegrass ranged in weight from 22 to 36 mg in comparison to plantain which had lower shoot weights that ranged in weight from 17-28 mg. Perennial ryegrass and plantain shoot weights showed a range of variation between pasture mixtures, but were relatively constant in shoot weight over all the pasture mixtures. White clover ranged in shoot DW from 1.2-2.6 mg while red clover ranged from 4.2-6.9 mg. The shoot DW of both white and red clover was relatively constant over all the pasture mixtures. All sown species had relatively similar shoot DW over all the pasture mixtures in May.

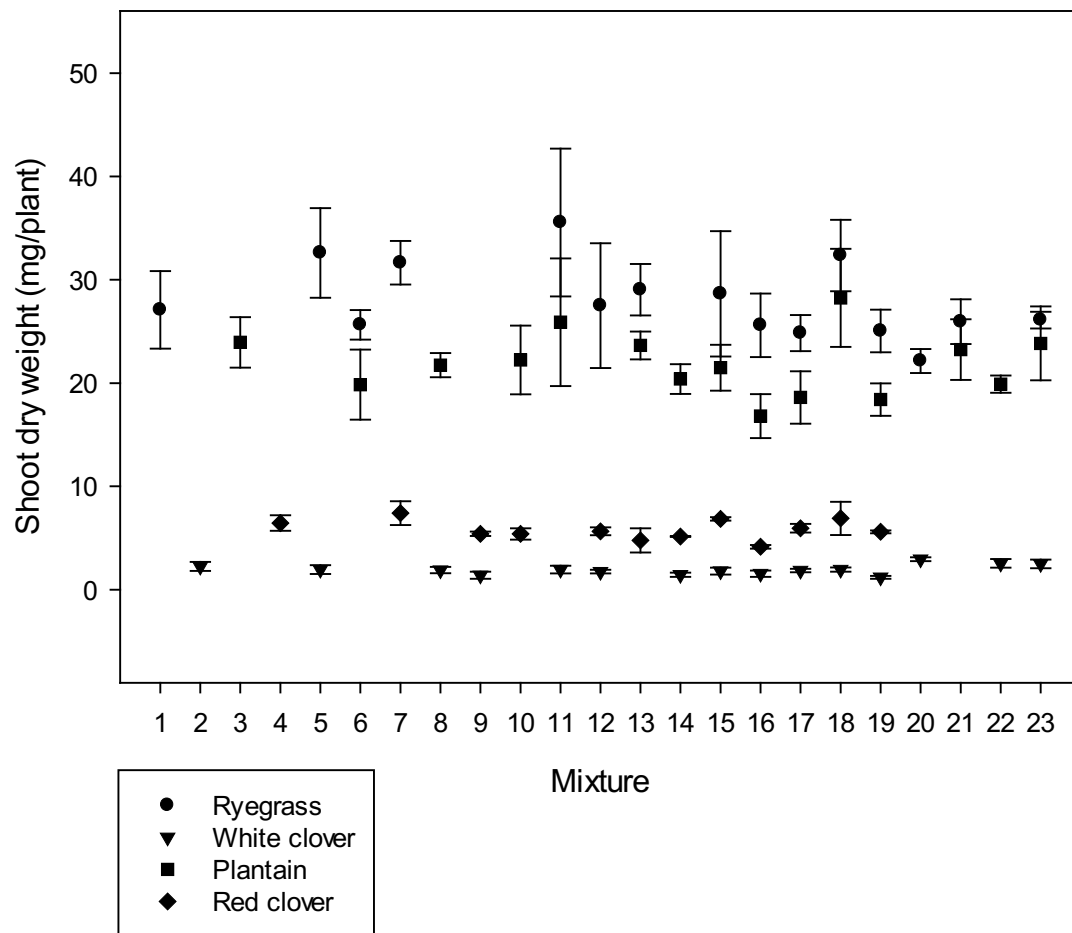


Figure 4.2 Average shoot dry weight of perennial ryegrass, white clover, plantain and red clover on the 5th of May 2015 at Lincoln University. Error bar represent standard error of mean.

Total average leaves per plant of perennial ryegrass, white clover, plantain and red clover showed no differences ($P>0.05$) over the different mixtures in May (Figure 4.3). On average perennial ryegrass had 6.5 leaves, white clover 1.7, plantain 1.9 and red clover 2.3. White clover had notably lower total leaves (1.2) in mixture 19, where red clover was sown dominate with perennial ryegrass, white clover and plantain. Plantain leaves were also notably lower in mixture 10 when sown with red clover.

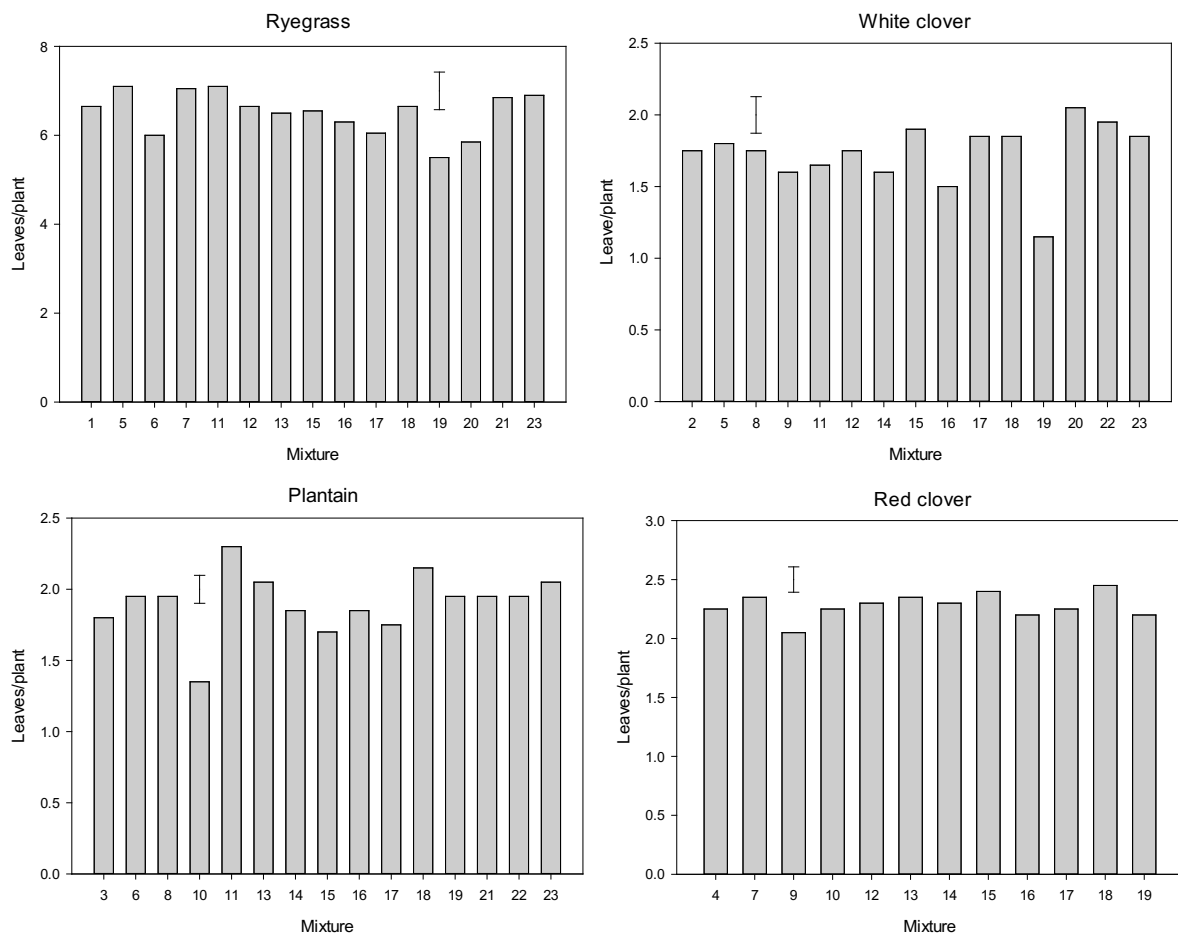


Figure 4.3 Average total leaves per plant for perennial ryegrass, white clover, plantain and red clover in all mixtures on the 5th of May 2015 at Lincoln University. P values for perennial ryegrass = 0.802, white clover = 0.168, plantain = 0.248, red clover = 0.903. Error bars represent SED.

Average DM yield for perennial ryegrass, white clover, plantain and red clover over all pasture mixtures was different ($P < 0.001$) in May (Figure 4.4). The perennial ryegrass monoculture (1) had the greatest DM yield at 148 kg DM/ha. High perennial ryegrass yield was also evident in the pasture mixtures containing perennial ryegrass in the mix. The plantain monoculture produced 111 kg DM/ha and was well represented in the pasture mixtures. The white and red clover monoculture in comparison to perennial ryegrass and plantain had very low yields of 9.3 and 21.6 kg DM/ha respectively. The white and red clover mixture also produced low DM yields of 12.2 Kg DM/ha. Both clovers contributed very little DM yield when sown in a mixture with plantain and/or perennial ryegrass. Pasture mixtures that contained high proportions of perennial ryegrass and plantain resulted in higher yields than mixtures with low proportion or no perennial ryegrass and/or plantain in the mix. Alternative drill row sown mixtures was higher in mixture 21 and lower in mixture 23 in comparison with the equivalent mixtures sown together (6 & 11).

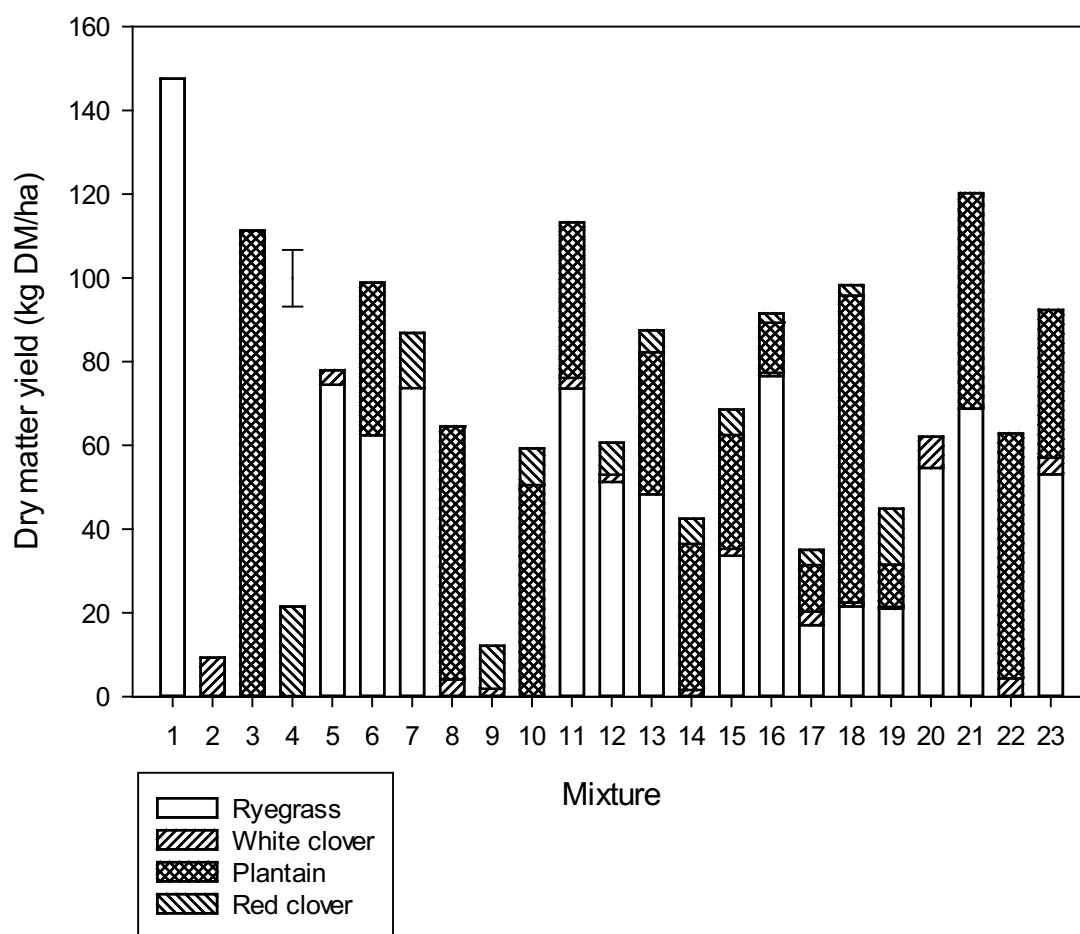


Figure 4.4 Average dry matter yield of perennial ryegrass, white clover, plantain and red clover and their mixtures on the 5th of May 2015 at Lincoln University. Yields based on the average shoot weights of these species and plant population from pasture mixtures on the 5th of May 2015. Error bar represent SED. P value was <0.001.

Average leaf area index from pasture mixtures was different ($P < 0.001$), ranging from 0.16 to 2.43 LAI (Figure 4.5). The plantain monoculture (3) produced the highest LAI at 2.43. The perennial ryegrass monoculture (1) was similar to plantain with a LAI of 2.17. The white clover and red clover monoculture (2 & 4) in comparison had very low LAI of 0.16 and 0.40 respectively. The species trends in the monocultures were carried through to the pasture mixtures with white and red clover contributing very little to total LAI. Perennial ryegrass and plantain when in a pasture mixture provide the majority of contribution to total LAI. Pasture mixture containing perennial ryegrass and/or plantain in adequate proportions (above 25%) provided the pasture mixtures with greater overall LAI than pasture mixtures contain no or low proportions of perennial ryegrass/plantain.

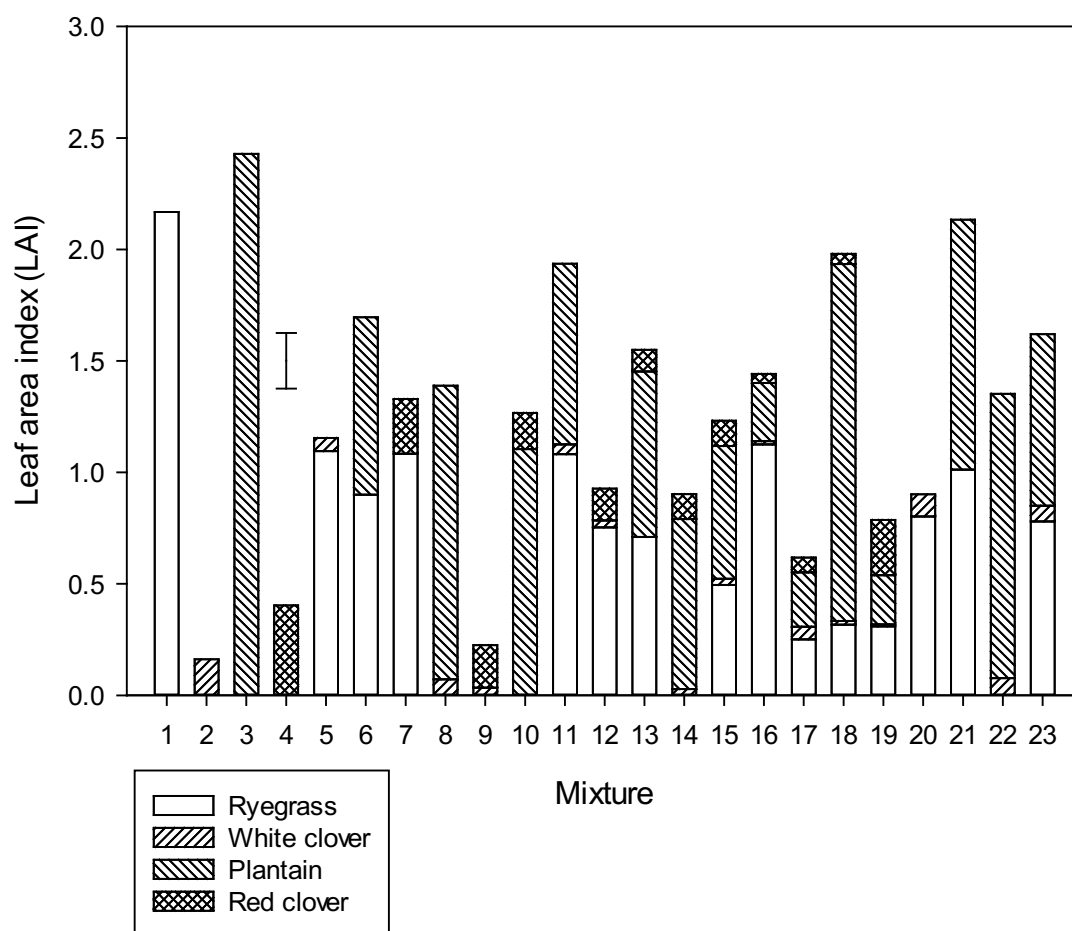


Figure 4.5 Average leaf area index of pasture mixtures with contribution of perennial ryegrass, white clover, plantain and red clover to the total LAI on the 5th of May 2015 at Lincoln University. Error bars represent SED. P value was <0.001.

4.2 August harvest

Average DM yield for pasture mixtures was different between pastures ($P < 0.001$) in August (Figure 4.6). DM yield ranged from 252 to 1891 kg DM/ha. The white clover and the red clover monoculture and two species mixture had the lowest yields which were all below 450 kg DM/ha. These pure clover pasture mixtures had over 50% of the yield composed of weeds. The plantain monoculture had the highest yield of the four monocultures at 1839 kg DM/ha. The perennial ryegrass monoculture yielded 300 kg DM/ha lower than the plantain monoculture. Mixture 22 that contained plantain and white clover, sown in alternate drill rows yielded the highest (1891 kg DM/ha), with the majority of the yield coming from the plantain. Mixtures yielded higher when they contained high proportions of plantain and/or perennial ryegrass. When perennial ryegrass and plantain were sown together in a two or three species mixture (6, 11, 13, 21 & 23) they had superior yield over mixture with either perennial ryegrass or plantain with clovers. This was evident in mixture 15 when the four species were sown equally and in mixture 16 and 18 when perennial ryegrass and plantain were sown dominate respectively, in a four

species mixture. These mixtures yielded around 1400-1600 kg DM/ha compared with the four species mixtures that contained less than 0.125 proportions of perennial ryegrass and plantain in the seed mix (17 & 19), that yielded 790 and 894 kg DM/ha (17 and 19 respectively). When sown in alternate drill rows mixture 22 had higher and mixture 23 had lower yields than their equivalent mixtures (8 & 11) sown together.

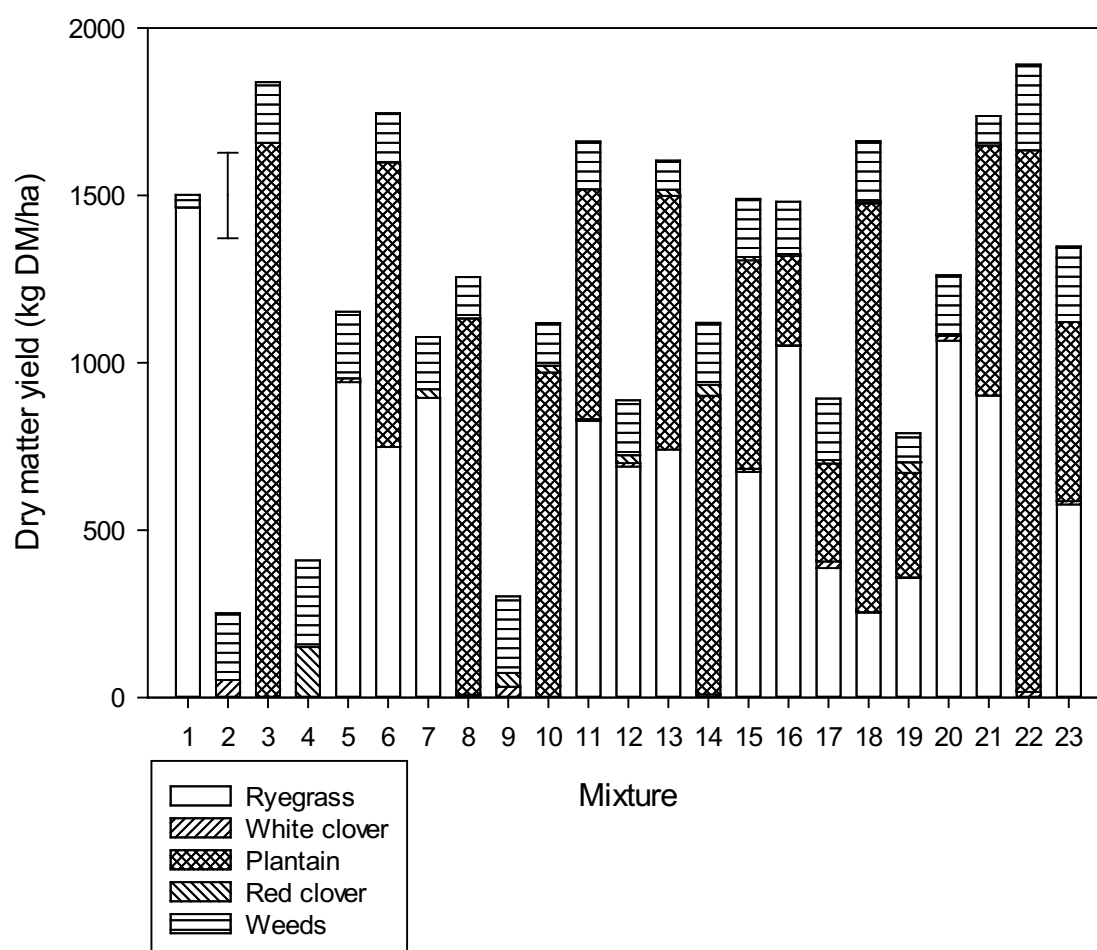


Figure 4.6 Average dry matter yield of perennial ryegrass, white clover, plantain, red clover and weeds of pasture mixtures on the 4th of August 2015 at Lincoln University. Error bars represent SED. P value was <0.001.

Average weed proportion ranged from 0.03 to 0.80 and was different over pasture mixtures ($P < 0.001$). Weed proportion was highest on the plots that contained only clovers in the mix (2, 4 & 9). The white clover and red clover monocultures contained 0.80 and 0.64 proportion of weeds of the total yield. The white and red clover mixture had a weed proportion intermediate of the two monocultures at 0.76. In comparison all the other pasture mixtures contained 0.2 or less 0.2 proportion of weeds. The perennial ryegrass monoculture had the greatest suppression of weeds (0.03). Some of the plots that contained a high proportion of clover had increased weed proportion such as mixtures 5, 12 and 17.

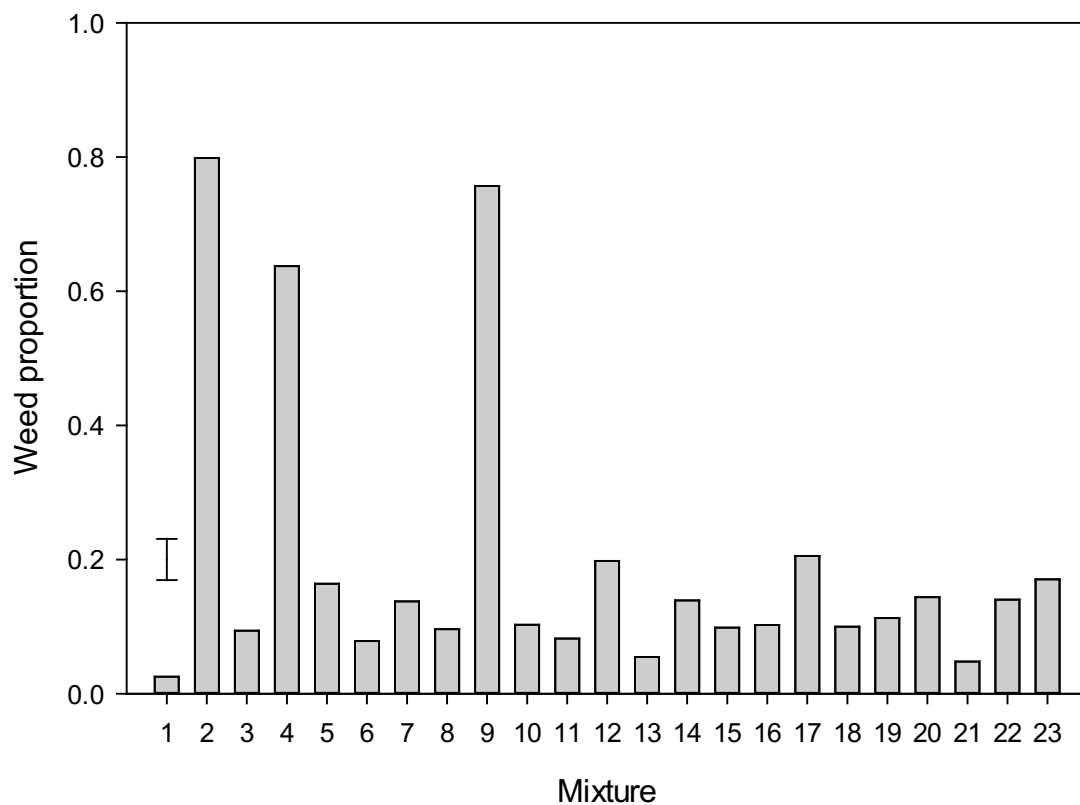


Figure 4.7 Average weed proportion of perennial ryegrass, white clover, plantain and red clover and their mixtures on the 4th of August 2015 at Lincoln University. Error bars represent SED. P value was <0.001.

Mean estimated plant population in August ranged from 168 – 460 plants/m² and was on average the plant population was 298 plants/m². There was no differences ($P = 0.434$) between plant population over all the pasture mixtures. The red clover monoculture had the highest plant population at 460 plants/m². The plantain monoculture also had high plant population. Over all the mixtures red clover and white clover plant populations were not very representative of the actual proportions they were sown at. Perennial ryegrass and plantain in comparison were well represented in each pasture mixture and tended to compose the majority of the total plant population.

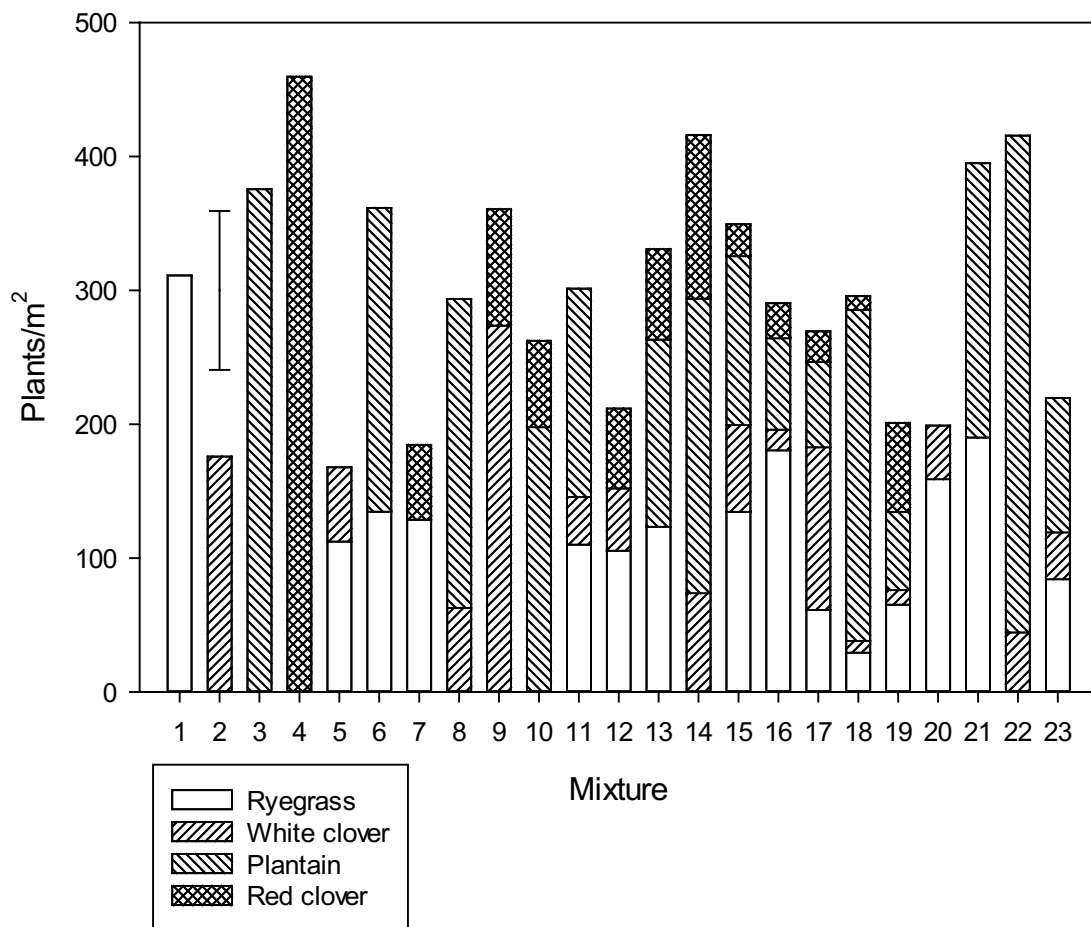


Figure 4.8 Average plant population per square meter for perennial ryegrass, white clover, plantain and red clover and their mixtures on the 4th of August 2015 at Lincoln University. Estimate based on average plant dry weight and DM yield of sown species per square meter. Error bars represent SED. P value was 0.434.

Average tillers per plant for perennial ryegrass were different ($P=0.007$) over pasture mixtures containing perennial ryegrass (Figure 4.9). The number of tillers per plant ranged from 12.0-18.5 and on average over all the mixtures was 15.6. The number of tillers per perennial ryegrass plant were noticeably lower in mixtures 1, 6, 13 and 21, where perennial ryegrass was either sown alone (mixture 1), with plantain (6), with plantain and red clover and sown in alternate drill rows with plantain. In contrast tiller numbers per perennial ryegrass plant were higher when perennial ryegrass was mixed with white clover and/or red clover (mixture 5, 7 & 12) and also in the four species mixtures (15, 17 & 18).

Average white clover tillers (stems) per plant ranged from 1.20-2.05 with an average of 1.55 and there was difference ($P<0.001$) between pasture mixtures. The number of tillers per plant was higher when sown in a monoculture (2), with perennial ryegrass and red clover in a three species mixture (12) and

when sown in alternate drill rows (20, 22 & 23). The rest of the mixtures had similar tiller numbers per plant which ranged from 1.2-1.5.

The average number of plantain tillers per plant ranged from 1.75-3.25 and on average was 2.49 over all the mixtures. There was differences between pasture mixtures ($P < 0.001$). The number of tillers per plant was lower on mixtures that were sown with perennial ryegrass and when sown in alternate drill rows (6, 11, 21, 22 & 23). Tillers per plant were highest at 3.15-3.25 in the four species equal mix (15) and the two four species mixtures with white and red clover dominate (17 & 19).

Average red clover tillers per plant ranged from 1.25-2.05 and was on average 1.63 over all the pasture mixtures. There was differences ($P = 0.004$) between pasture mixtures. Tiller numbers per plant were reduced in pasture mixtures the three species mixture with perennial ryegrass and plantain (13) and in the four species mixture where perennial ryegrass (16) and plantain (18) were sown dominate. Tiller numbers were highest from mixtures that contained high proportions of red clover or were sown with low proportions of perennial ryegrass and plantain (mixtures 4, 9, 12, 17, 19).

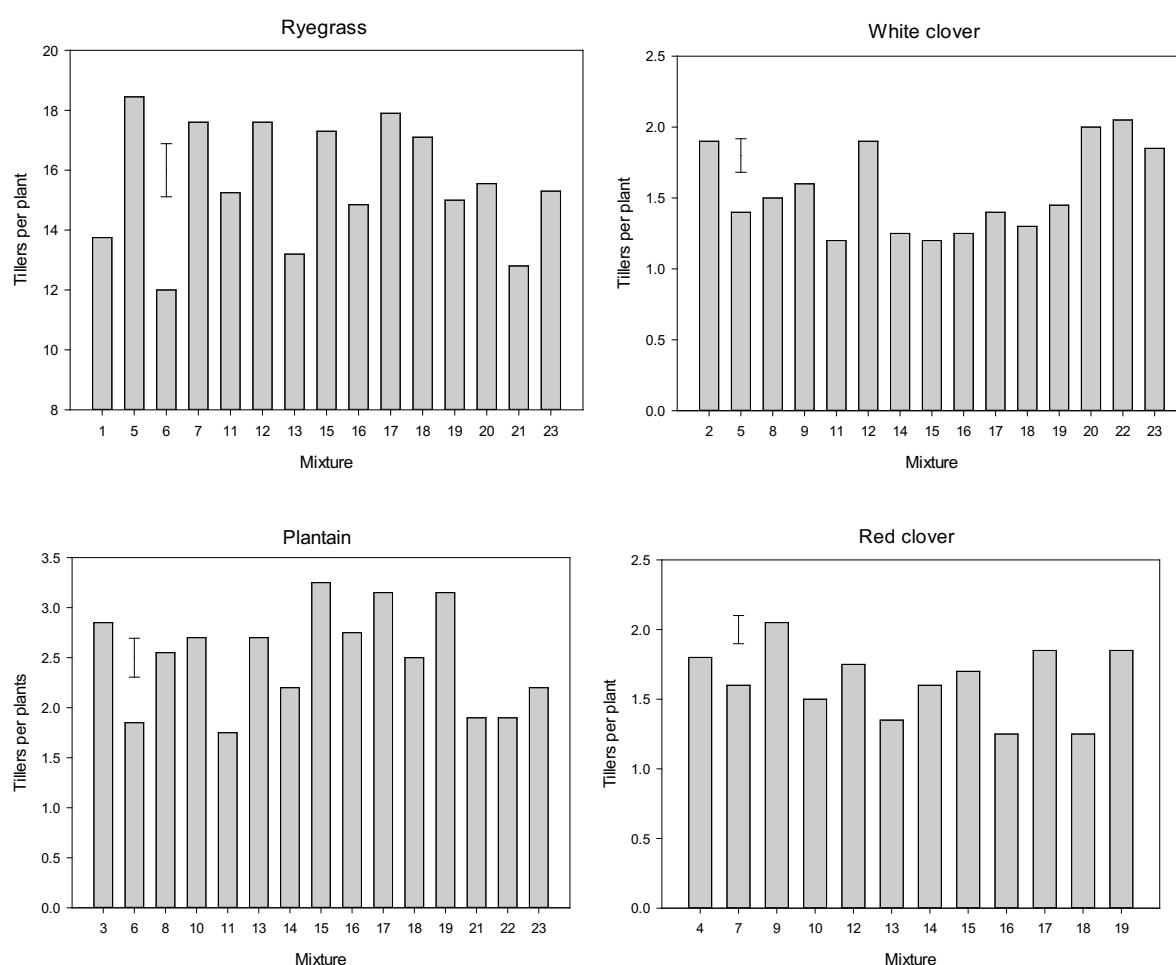


Figure 4.9 Average total tillers for ryegrass, white clover, plantain and red clover and their mixtures on the 4th of August 2015 at Lincoln University. P value were ryegrass = 0.007, white clover = <0.001, plantain = <0.001, red clover = 0.004. Error bars represent SED.

4.3 September harvest

Average DM yield over September was on average 1923 kg DM/ha and ranged from 710 to 2321 kg DM/ha. There were difference ($P<0.001$) between pasture mixtures. The monoculture of perennial ryegrass and plantain both yielded similarly and produced over 900 kg DM/ha more than the white and red clover monocultures. The clover monoculture had the lowest yield at 710 and 1016 kg DM/ha for white and red clover respectively. The two species mixture of white and red clover also yielded poorly at 1511 kg DM/ha. The yields of these pure clover mixtures was composed of more weeds than clover. The majority of the mixtures performed well above 1900 kg DM/ha with high perennial ryegrass and plantain content, with the exception of mixtures 14, 17, 19, 22 and 23. Contribution of white and red clover to total yield of all pasture mixtures was very low even when sown in alternate rows. The alternate drill row treatment of pasture mixtures (20-23) produced lower yields compared to the equivalent mixtures sown together (5, 6, 8 & 11).

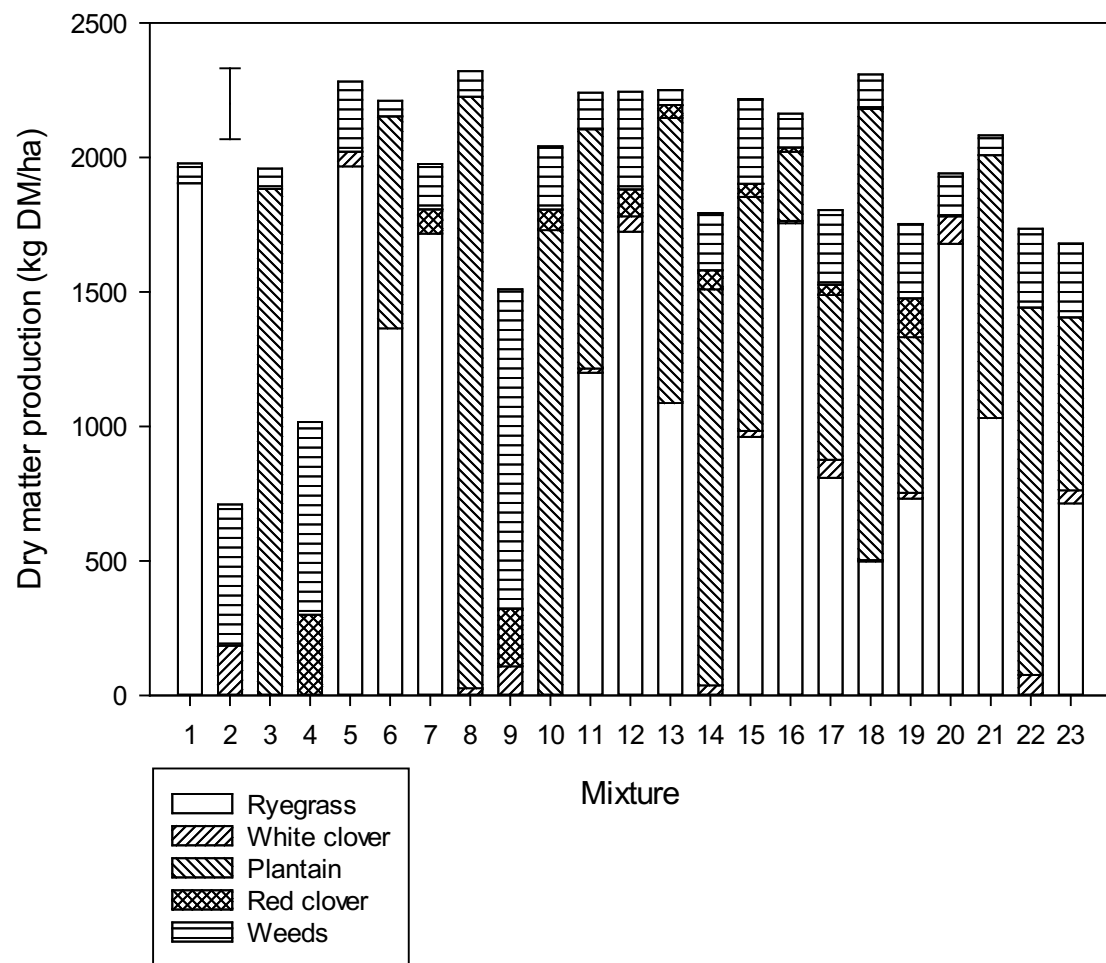


Figure 4.10 Average DM yield of perennial ryegrass, white clover, plantain, red clover and weeds of pasture mixtures on the 21st of September 2015 at Lincoln University. Error bar represents SED. P value was <0.001.

The average weed proportion was different (<0.001) over the pasture mixtures. The proportion of weeds ranged from 0.02 to 0.72. The proportion of weeds was highest in mixtures 2, 4 and 9 which were the white and red clover monoculture and two species mixture. The weeds in these mixtures were all above 60% of the total yield. When these three mixtures were excluded the average weed proportion was 0.09. The alternate drill row sown mixtures 22 and 23 contained a higher proportion

of weeds than the equivalent mixtures sown together (0.17 and 0.16 for mixture 22 and 23 and 0.04 and 0.06 for mixture 8 and 11).

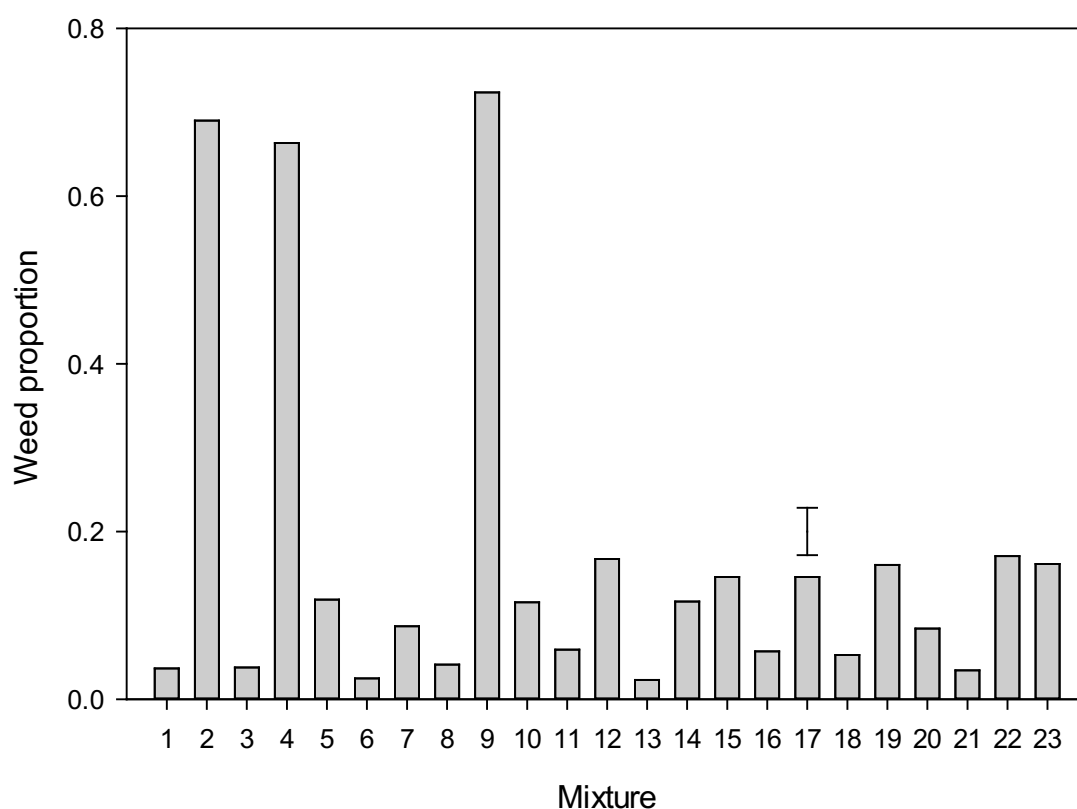


Figure 4.11 Average weed proportion of perennial ryegrass, white clover, plantain and red clover and their mixtures on the 21st of September 2015 at Lincoln University. Error bar represents SED. P value was <0.001.

Average leaf area index for September was different ($P < 0.001$) over the pasture mixtures. LAI ranged from 0.34 to 3.97 over all the pasture mixtures and was on average 2.82. Mixtures 2, 4 and 9 had the lowest LAI at 0.34, 0.58 and 0.61 respectively. All other pasture mixtures had LAI between 2 and 4. Perennial ryegrass and plantain monoculture both had LAI above 3 (3.06 and 3.36). The majority LAI of pasture mixtures was made up by perennial ryegrass and plantain with very little contribution from white and red clover to total LAI.

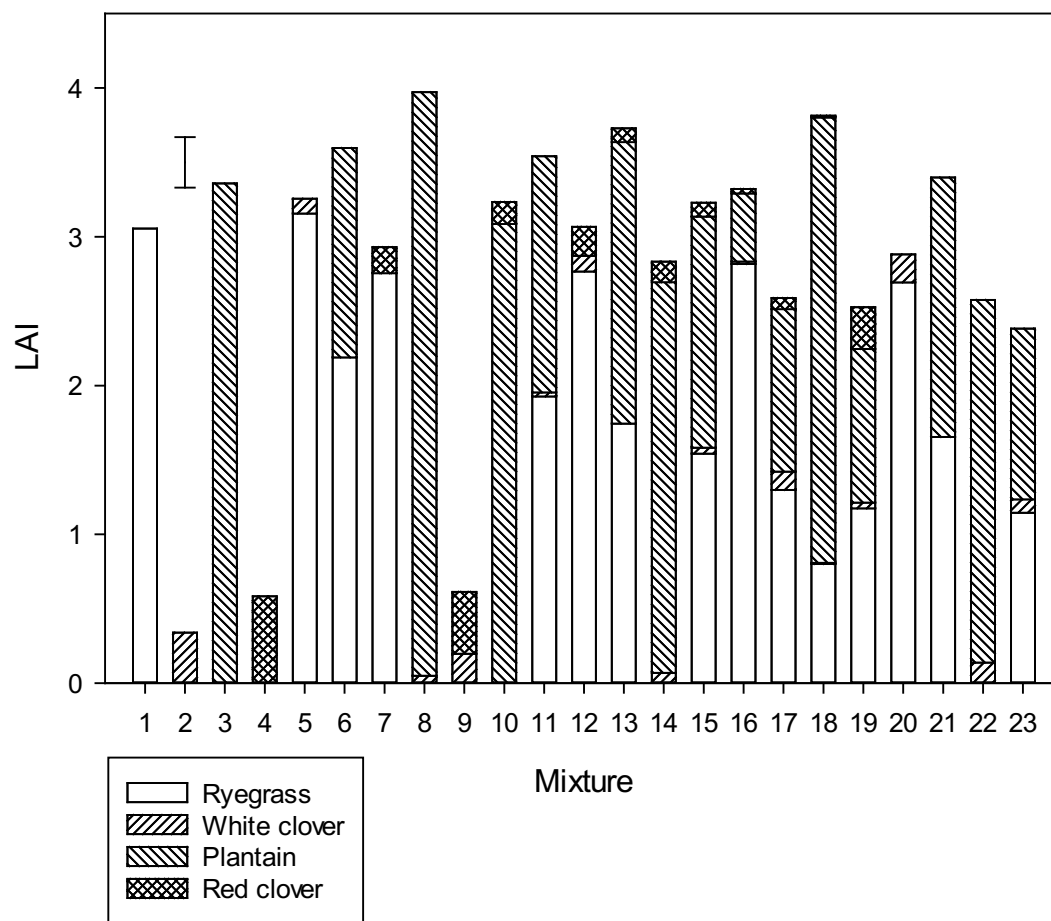


Figure 4.12 Average leaf area index (LAI) for perennial ryegrass, white clover, plantain and red clover and their mixtures on the 21st of September 2015, at Lincoln University. (Specific leaf area from August harvest used to calculate LAI) Error bars represent SED. P value of <0.001.

Average metabolisable energy (ME) content of pasture mixtures were different ($P = 0.004$) in September 2015 (Figure 4.13). ME ranged from 10.92 to 12.74 MJ ME over all the pasture mixtures. The perennial ryegrass monoculture contained the highest ME compared with the other three monoculture at 12.73 MJ ME. White clover had then next highest ME at 12.09 MJ ME for the monocultures, followed by plantain and red clover which had similar ME (11.21 and 11.16 MJ ME respectively). Mixtures 5, 6, 12, and 20 had a high proportion of perennial ryegrass and white clover which caused higher ME. In comparison, mixtures with no perennial ryegrass in the mixture (8, 9, 10 & 14) had much lower ME.

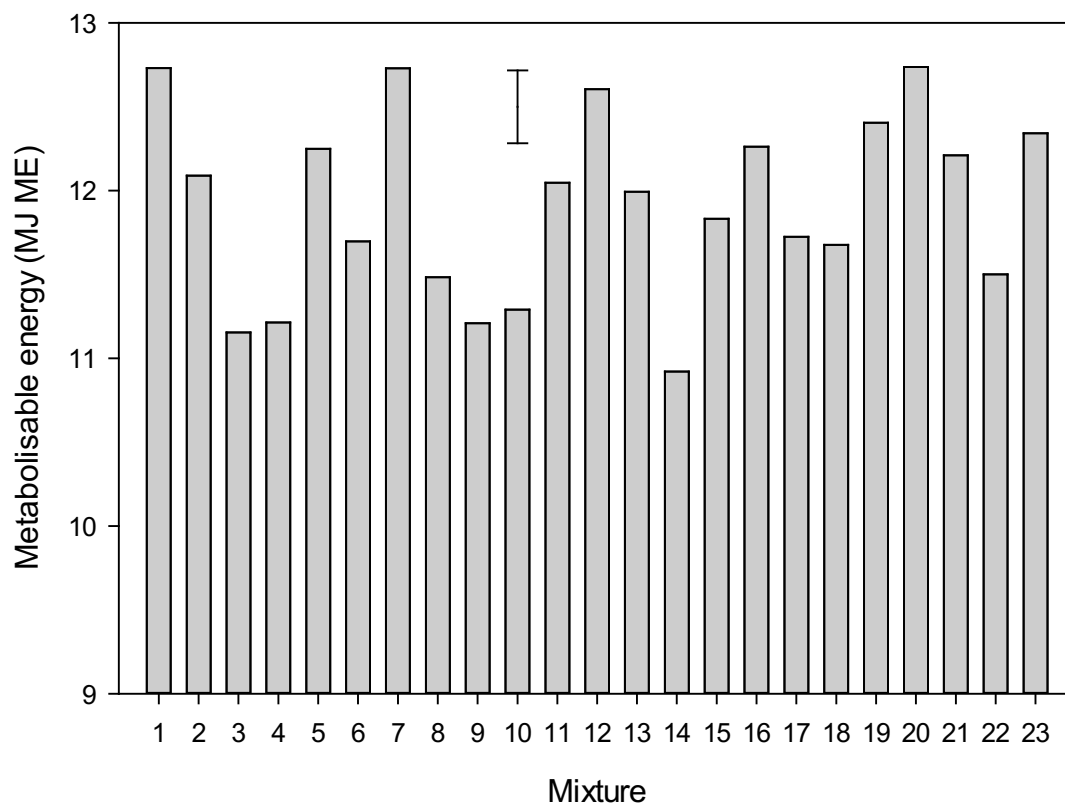


Figure 4.13 Average metabolisable energy of perennial ryegrass, white clover, plantain and red clover and their mixtures on the 21st of September 2015, at Lincoln University. Error bar represents SED. P value was 0.004.

Average nitrogen percentage of the pasture mixtures was different ($P = 0.001$) in September 2015 (Figure 4.4). All the pasture mixtures had similar N% (average 3.14% N), except the three mixtures that contained pure clovers (2, 4 & 9). The white clover and red clover monocultures and two species mixture had 4.51, 4.16 and 4.48 N% respectively.

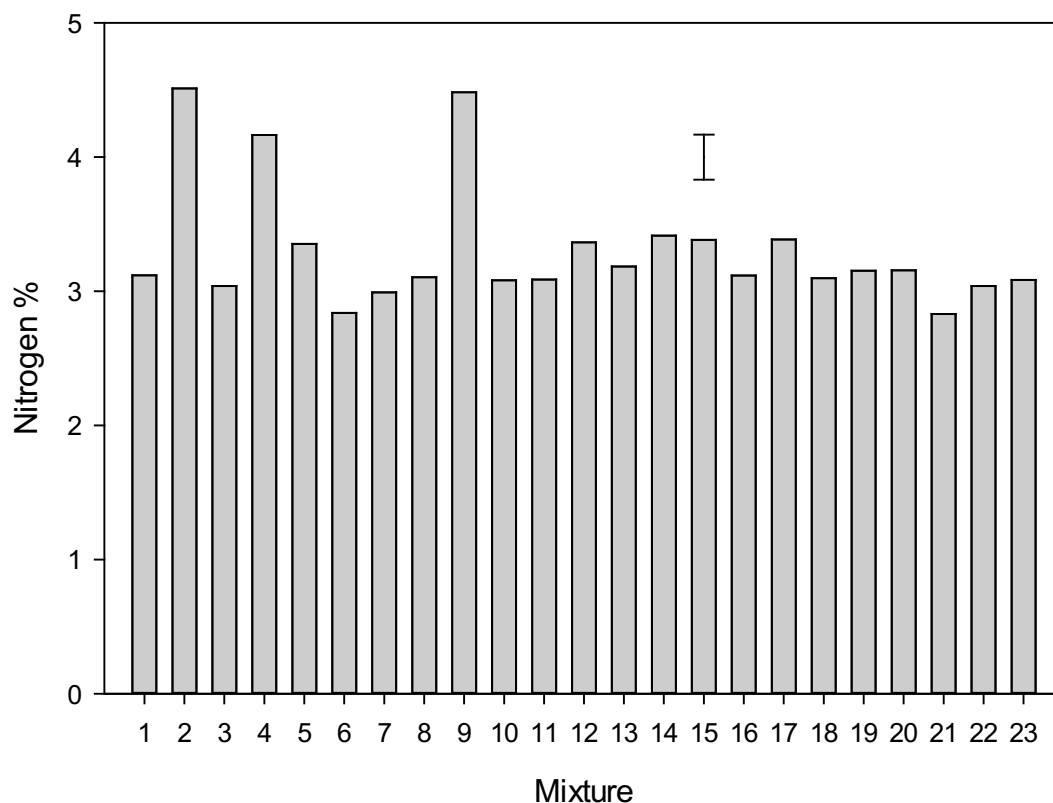


Figure 4.14 Nitrogen percentage of perennial ryegrass, white clover, plantain and red clover and their mixtures on the 21st of September 2015 at Lincoln University. Error bars represent SED. P value was 0.001.

Images of the pasture mixtures (Plate 4.1 A-D) on September, 2015 show that the monocultures of perennial ryegrass (A) and plantain (C) have established well and covered all available area. The white and red clover monocultures (B & D) in comparison did not establish as well and have much higher bare ground and also visual weed content. The two species mixtures (Plate 4.2 A-F) showed perennial ryegrass and plantain establishing very well in all mixtures. The white clover and red clover mixture (E) had more bare ground and higher weed content than the other two species mixtures. Clover content was noticeable in the mixtures sown with ryegrass or plantain but was in relatively low proportions. The three species mixtures showed (Plate 4.3 A-D) perennial ryegrass and plantain dominating the pasture mixtures with relatively low clover proportions present. The four species equally sown mixture (15) again showed (Plate 4.4) dominance of perennial ryegrass and plantain, with clover in relatively small proportions. The four species mixtures with one species in turn dominate (Plate 4.5 A-D), were relatively well representative of the proportion they are sown at. Clovers when sown dominate caused a more open sward and still resulted in relatively low clover percentages (visual). The alternate drill row sown plots showed (plate 4.6 A-D) perennial ryegrass and plantain established well and had dense populations in each drill row they were sown in. Alternate drill row sowing improved white clover establishment, although weeds were still present (Plate 4.6 A, C & D).

A.



B.



C.



D.



Plate 4.1 Monocultures of perennial ryegrass, white clover, plantain and red clover (Mixtures 1-4).
A (RG), B (WC), C (P) and D (RC).

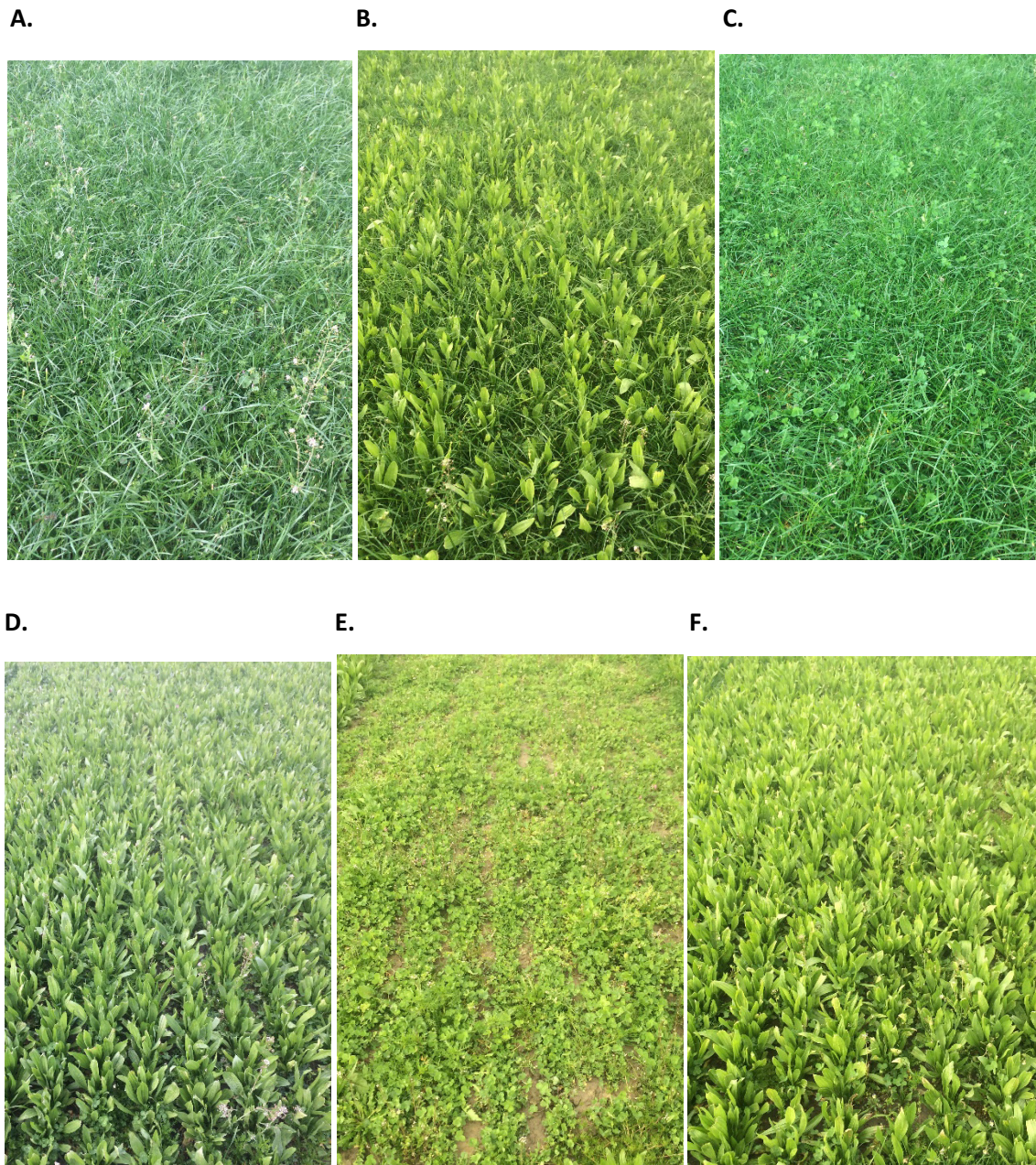


Plate 4.2 Two species mixtures of perennial ryegrass, white clover, plantain and red clover (Mixtures 5-10). A (RG + WC), B (RG + P), C (RG + RC), D (WC + P), E (WC + RC) and F (P + RC).

A.



B.



C.



D.



Plate 4.3 Three species mixtures of perennial ryegrass, white clover, plantain and red clover. A (RG, WC, P), B (RG, WC, RC), C (RG, P, RC) and D (WC, P, RC).



Plate 4.4 Four species mixture with all components equal (15). RG, WC, P, RC.

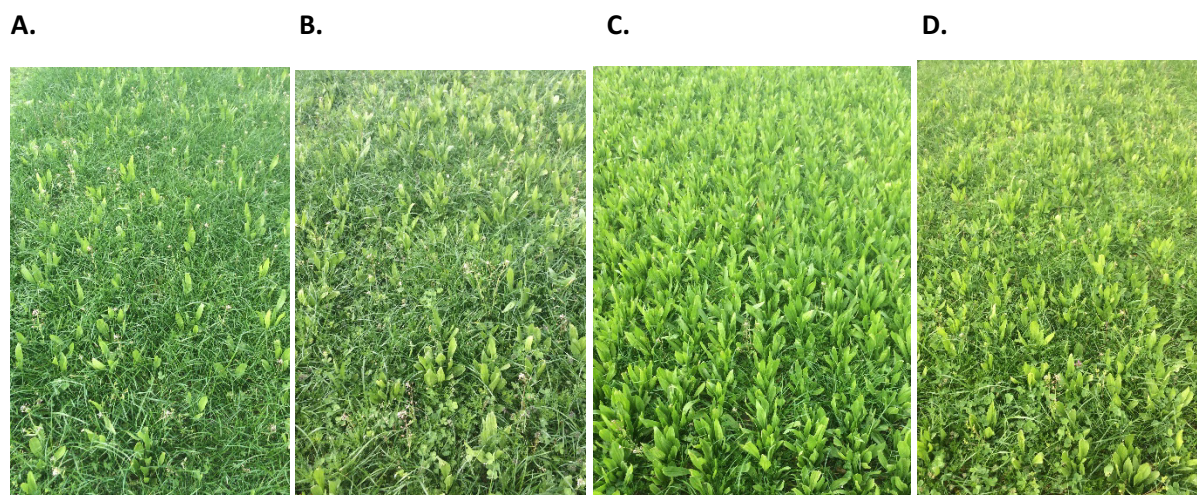


Plate 4.5 Four species mixture with one species sown in the mix dominate of perennial ryegrass, white clover, plantain and red clover (16-19). A (RG dominate), B (WC dominate), C (P dominate) and D (RC dominate).

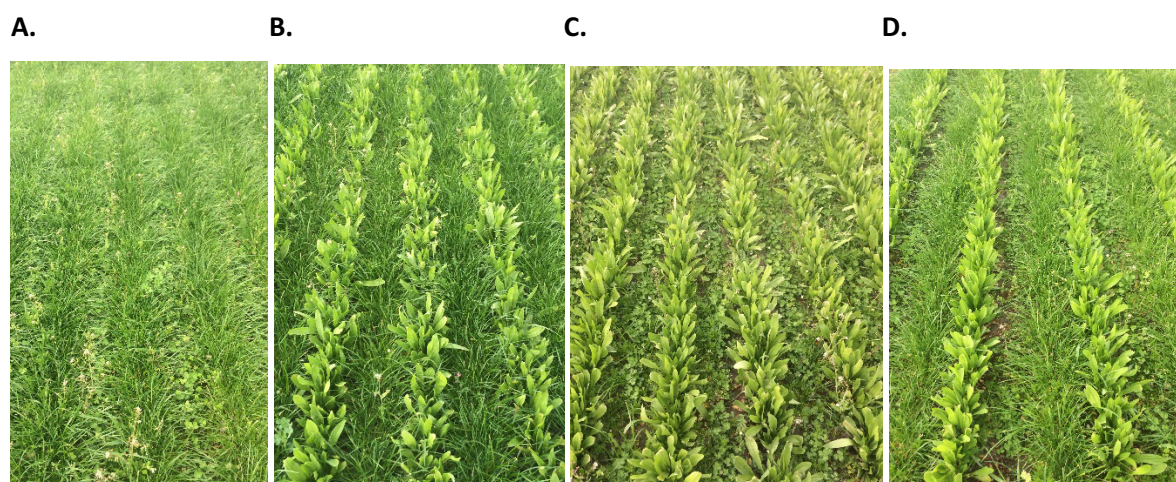


Plate 4.6 Alternative drill row sown mixtures of perennial ryegrass, white clover and plantain (Mixtures 20-23). A (RG, WC), B (RG, P), C (WC, P) and D (RG, WC, P).

4.4 Leaf counts from leaf area harvest

The average number of perennial ryegrass leaves and stems per plant were different ($P < 0.001$) over each harvest date as a result of plant development (Table 4.1). The amount of leaves increased from 7.15-8.80 leaves per plant at the initial harvest in May to 43.7-48.8 at the final harvest in August. The numbers of tillers followed the same pattern and increased from 2.85-3.65 to 14.3-17.3 by the final harvest. The number of perennial ryegrass leaves per plant was different ($P = 0.039$) between the monoculture and the mixture. Mixing the species caused higher amounts of perennial ryegrass leaves to be present compared with the monoculture of perennial ryegrass. The number of tillers was also different ($P = 0.009$) between the mixture and monoculture for ryegrass. The amount of tillers increased with the mixing of perennial ryegrass with the other pasture species.

Table 4.1 Average number of total ryegrass leaves and tillers per plant from the monocultures and the four species equal mixtures (mixture 15), on the 5th of May, 3rd of July and 11th of August 2015, at Lincoln University. P value <0.001 for date, with P value of 0.039 and 0.009 for leaf and tiller respectively for mixture effect.

| | | Date | | |
|---------|-------------|-----------|-----------|------------|
| | | 5/05/2015 | 3/07/2015 | 11/08/2015 |
| Leaves | Monoculture | 7.15 | 26.1 | 43.7 |
| | Mixture | 8.80 | 31.0 | 48.8 |
| Tillers | Monoculture | 2.85 | 9.05 | 14.3 |
| | Mixture | 3.65 | 10.2 | 17.3 |

The average number of total white clover leaves was different ($P < 0.001$) over harvest date. Total leaves per plant increased from 5.70-5.85 initially in May to 11.4-22.4 in August. The number of leaves was different ($P < 0.001$) between the monoculture and mixture for white clover. Differences were less pronounced in May with 0.15 of a leaf difference between the monoculture and mixture. Over time the difference in total leaves per plant increased and by the August harvest the monoculture had double the leaves that the mixture did (22.4 and 11.4 respectively). Mixing white clover with the other pasture species had negative impact on the amount of leaves present.

Table 4.2 Average number of total white clover leaves per plant from monocultures and the four species equal mixture (mixture 15) on the 5th of May, 3rd of July and 11th of August 2015, at Lincoln University. P value for date <0.001 and <0.001 for mixture effect.

| | | Date | | |
|-------------|--|-----------|-----------|------------|
| | | 5/05/2015 | 3/07/2015 | 11/08/2015 |
| Monoculture | | 5.85 | 14.1 | 22.4 |
| Mixture | | 5.70 | 11.8 | 11.4 |

The average number of plantain leaves per plant was different ($P < 0.001$) over three harvest dates. The total number of plantain leaves per plant increased from 3.4-3.7 in May to 12-14.3 leaves in August. There was differences ($P = 0.044$) in the total number of leaves per plant between the monoculture and mixture for plantain. When mixed with the other pasture species, plantain had more total leaves per plant than in the monoculture over all harvest dates.

Table 4.3 Average number of total plantain leaves per plant from monocultures and the four species equal mixture (mixture 15) on the 5th of May, 3rd of July and 11th of August 2015, at Lincoln University. P value for date <0.001 and 0.044 for mixture effect.

| | | Date | | |
|-------------|--|-----------|-----------|------------|
| | | 5/05/2015 | 3/07/2015 | 11/08/2015 |
| Monoculture | | 3.4 | 7.95 | 12.0 |
| Mixture | | 3.7 | 9.85 | 14.3 |

The total number of leaves per plant of red clover was different ($P < 0.001$) over the three harvest dates. The number of leaves per red clover plant increased from 8.2-8.5 in May to 17.6-18.0 in August. There was no difference ($P = 0.532$) in the total number of red clover leaves between the monoculture and mixture.

Table 4.4 Average number of total red clover leaves per plant from monoculture and the four species equal mixture (mixture 15) on the 5th of May, 3rd of July and 11th of August 2015, at Lincoln University. P value for date < 0.001 and 0.532 for mixture effect.

| | Date | | |
|-------------|-----------|-----------|------------|
| | 5/05/2015 | 3/07/2015 | 11/08/2015 |
| Monoculture | 8.20 | 14.9 | 17.6 |
| Mixture | 8.50 | 15.8 | 18.0 |

4.5 Accumulated yield

Average accumulated yield was different ($P < 0.001$) over pasture mixtures (Figure 4.15). Accumulated yield from sowing in May (26th) to the 22nd of September 2015, ranged from 962-3972 kg DM/ha and on average over the pasture mixtures was 3166 kg DM/ha. Mixtures 2, 4 and 9 had the lowest yield (962, 1426 and 1812 kg DM/ha for each respectively) and highest weed yield. These were the monocultures and two species mixture of white clover and red clover. The perennial ryegrass and plantain monocultures had similar yield with the plantain yielding 318 kg DM/ha more. The highest yielding mixtures were approaching 4000 kg DM/ha and were from mixtures 3, 6, 11, 13, 18 and 21 which all contained high proportions of plantain and/or perennial ryegrass in their seed mixtures. Mixtures 17, 19 and 14 had noticeably lower yields than some of the other mixtures that contained either perennial ryegrass and/or plantain. These mixtures did contain high proportions of clover in the seed mixtures (0.6-0.625). Alternate drill row sowing produced similar yields compared with the equivalent mixtures sown together, except for the three species alternate drill row sowing (23) that had lower yields than the three species sown together (11) (3029 and 3902 kg DM/ha respectively).

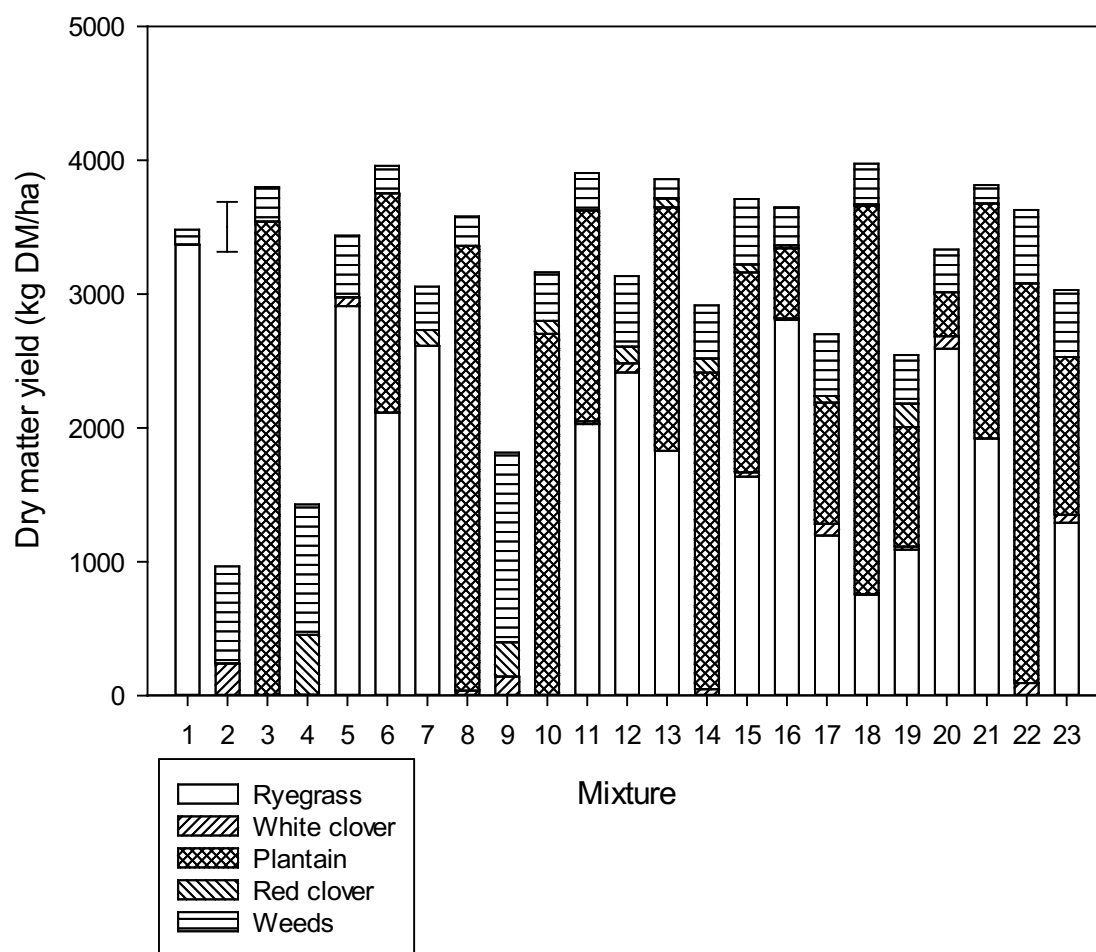


Figure 4.15 Average sum of total yield for perennial ryegrass, white clover, plantain, red clover and weeds of pasture mixtures from March 26th to September 21st 2015, at Lincoln University. Error bar represents SED. P value was <0.001.

Average accumulated weed yields ranged from 0.03-0.72 and was on average 0.18 over all pasture mixtures. There was differences ($P < 0.001$) between pasture mixtures. Mixture 2, 4 and 9 had the highest weed proportion at 0.72, 0.65 and 0.72 each respectively. Excluding these mixtures the rest had weed proportions below 0.2. The perennial ryegrass monoculture, three species mixture of ryegrass, plantain and red clover and the alternate drill row sown perennial ryegrass and plantain had the lowest weed proportions (0.03-0.04). Mixture 22 and 23 which were sown in alternate drill rows had higher weed proportions than there equivalent mixtures sown together (8 & 11) (0.15 and 0.16 vs 0.06 and 0.07 for alternate sowing and sown together respectively).

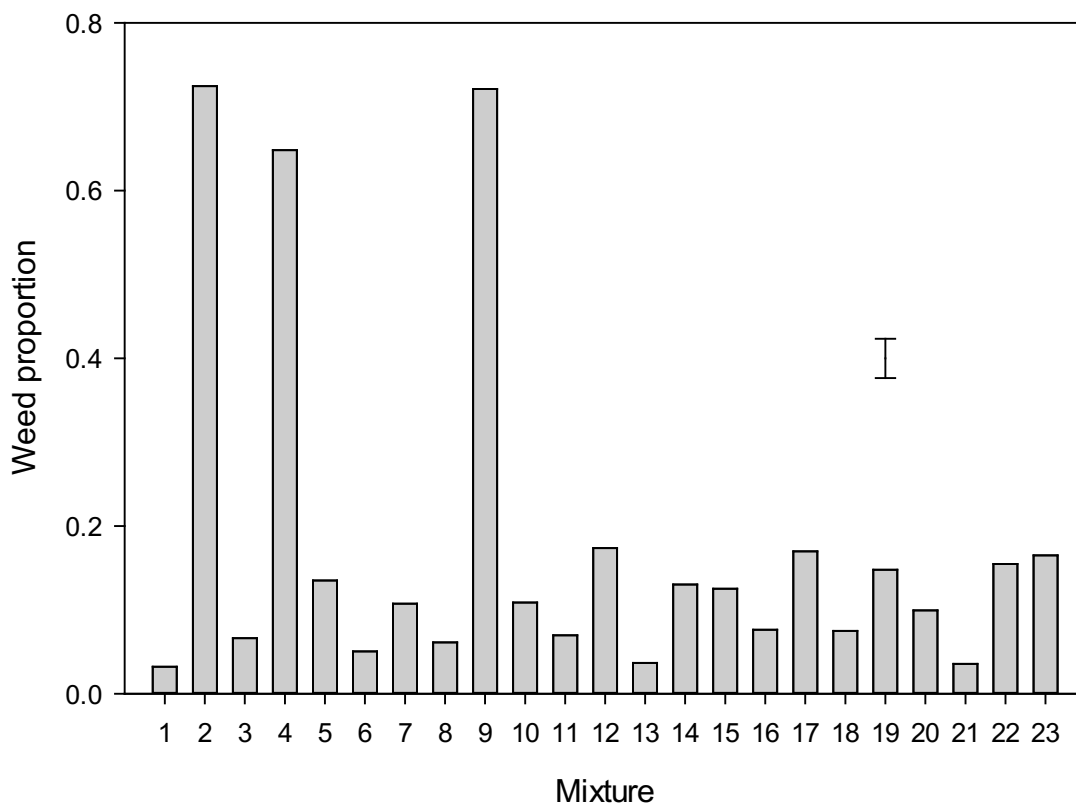
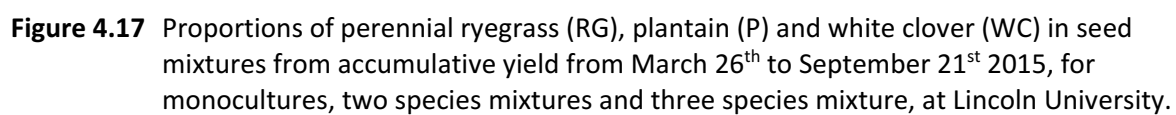


Figure 4.16 Average sum of weed proportion of perennial ryegrass, white clover, plantain and red clover and their mixtures from March 26th to September 21st 2015, at Lincoln University. Error bars represent SED. P value was >0.001.

Proportions of perennial ryegrass, white clover and plantain in monocultures, two species mixtures and three species mixture were dominated by perennial ryegrass and plantain (Figure 4.17). White clover proportions were on average 0.013 when sown in a mixture with perennial ryegrass and/or plantain. The proportion of perennial ryegrass was on average 0.70 when sown in a mixture. The proportion of plantain was on average 0.59 when sown in a mixture. When species were sown in alternate drill rows the proportion of white clover was increased to an average of 0.032 (Figure 4.18) when sown with perennial ryegrass and/or plantain. Average perennial ryegrass proportion was 0.67 and plantain was 0.64 when sown in a two or three species in alternate rows.



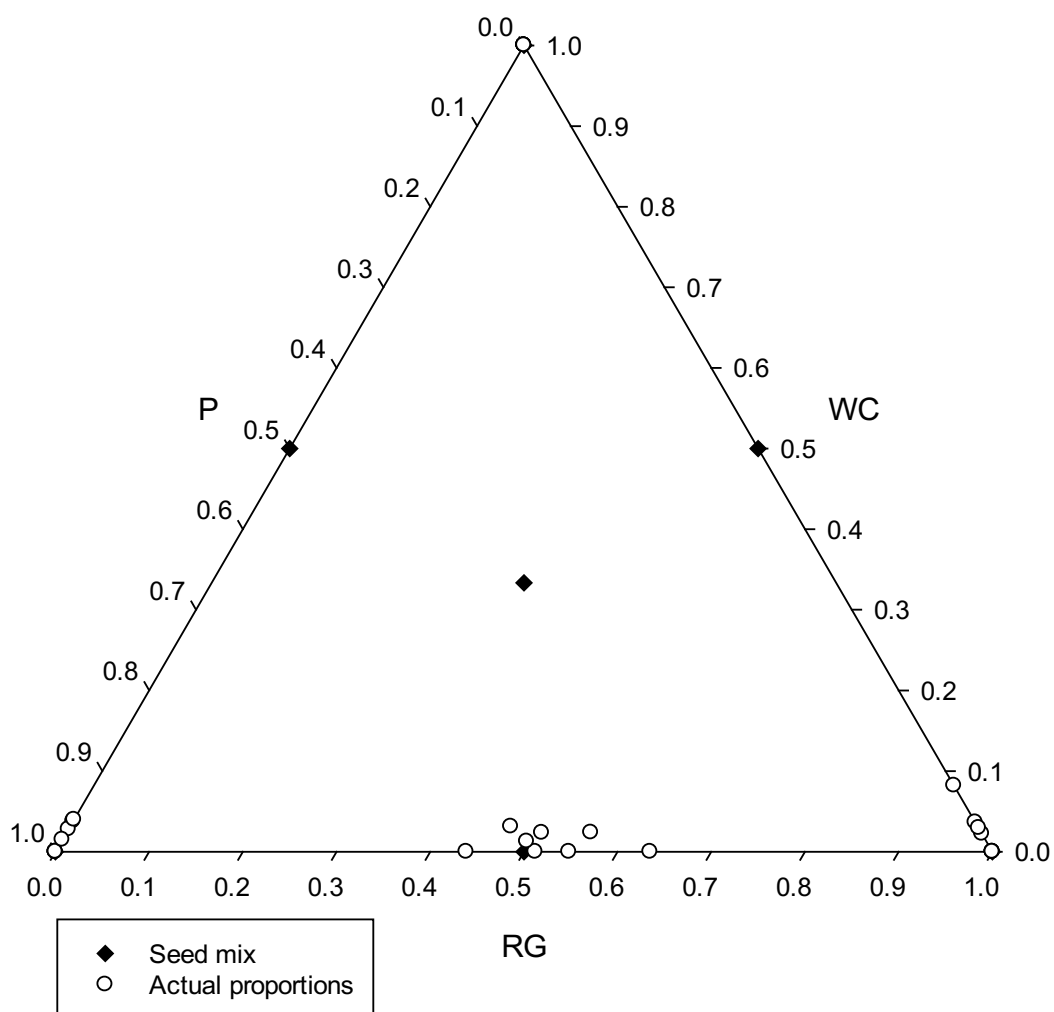


Figure 4.18 Proportions of ryegrass (RG), plantain (P) and white clover (WC) in seed mixtures and from accumulative yield for monocultures, two species and three species sown in alternative drill rows, at Lincoln University.

Results from mixture analyse using a quadratic equation found differences ($P < 0.001$) in sown yield (SY) between perennial ryegrass, white clover, plantain and red clover (Table 4.5). Plantain had the highest yield (coefficient) at 3509.1 kg DM/ha followed by perennial ryegrass at 3273.6 kg DM/ha. When the species were mixed there were significant interactions ($P < 0.001$) between species for perennial ryegrass and white clover, perennial ryegrass and red clover, white clover and plantain and plantain and red clover. Using this quadratic equation sown yield (kg DM/ha) can be modelled for pasture mixtures (only significant interactions included. Model:

$$\text{SY (kg DM/ha)} = 3273.6 \cdot \text{RG} + 256.4 \cdot \text{WC} + 3509.1 \cdot \text{P} + 438.4 \cdot \text{RC} + 4961.8 \cdot \text{RG} \cdot \text{WC} + 4224.4 \cdot \text{RG} \cdot \text{RC} + 5803.5 \cdot \text{WC} \cdot \text{P} + 3807.5 \cdot \text{P} \cdot \text{RC}$$

Where RG, WC, P and RC represent the proportion of perennial ryegrass, white clover, plantain and red clover respectively sown in the pasture mix.

Table 4.5 Mixture analyse for the sum of the sown yield of perennial ryegrass, white clover, plantain and red clover and their two species mixtures from March 26th to September 21st 2015, at Lincoln University. *** represents P value <0.001.

| Term | Coefficient | P value | Significance |
|-------|-------------|---------|--------------|
| RG | 3273.6 | 0.000 | *** |
| WC | 256.4 | 0.000 | *** |
| P | 3509.1 | 0.000 | *** |
| RC | 438.4 | 0.000 | *** |
| RG*WC | 4961.8 | 0.000 | *** |
| RG*P | 1511.6 | 0.061 | NS |
| RG*RC | 4224.4 | 0.000 | *** |
| WC*P | 5803.5 | 0.000 | *** |
| WC*RC | 751.7 | 0.347 | NS |
| P*RC | 3807.5 | 0.000 | *** |

Mixture analyse found there was no effect on the sown yield of perennial ryegrass, white clover and plantain when sown in alternate drill rows ($P = >0.05$) (Table 4.6).

Table 4.6 Mixture analyse for sum of sown yield from March 26th to September 21st 2015, for ryegrass, white clover, plantain and red clover and their two species mixtures sown in a mixture and in alternate drill rows, at Lincoln University. *** represents P value <0.001.

| Term | Coefficient | P value | Significance |
|-----------|-------------|---------|--------------|
| RG | 3403 | 0.000 | *** |
| WC | 272 | 0.000 | *** |
| P | 3575 | 0.000 | *** |
| RG*WC | 3777 | 0.000 | *** |
| RG*P | 315 | 0.663 | NS |
| WC*P | 4630 | 0.000 | *** |
| RG*Trt | 30 | 0.849 | NS |
| WC*Trt | 30 | 0.849 | NS |
| P*Trt | 30 | 0.849 | NS |
| RG*WC*Trt | -824 | 0.257 | NS |
| RG*P*Trt | -788 | 0.278 | NS |
| WC*P*Trt | -1163 | 0.112 | NS |

5 Discussion

5.1 Species effects

Accumulated DM yields of perennial ryegrass, white clover, plantain and red clover and their mixtures over the 7 month establishment period, from sowing on the 26th of March to final harvest on the 21st of September varied with yields ranging from 962 - 3972 kg DM/ha. The monoculture yields (1-4) of each species provided means of explaining yields in the mixtures based on sown proportions (5-23). The ryegrass had the highest composition of the plant population of all the mixtures which was also evident with the perennial ryegrass monoculture having the highest plant population. All other sown species were proportions in similar plant populations relative to the proportion of seed that was sown in each mixture.

Both perennial ryegrass and plantain shoot dry weights per plant were on average 28.0 and 21.9 mg respectively, and were far above the shoot dry weights of white and red clover which on average were 1.9 and 5.8 mg on the 5th of May (40 days after sowing) (Figure 4.2). This result was similar to what Moot *et al.* (2000) reported with white clover plants averaging 3.6 mg shoot dry weight compared with 24.8 mg for perennial ryegrass, 43 days after sowing (4 April 1996). Red clover in this same experiment had a shoot dry weight of 15.6 mg which much heavier than the shoot weights at a similar time in the experiment (5.8 mg). This is likely due to the red clover cultivar 'Pawera' which was used by Moot *et al.* (2000), being a tetraploid and having larger leaves and plant size, compared with diploid 'Sensation' red clover used in this experiment (Stewart *et al.* 2014). These differences in shoot weight were due to the rapid germination, emergence and seedling growth of perennial ryegrass, which give it a competitive advantage over species like white clover and red clover over establishment (Brougham 1953). Plantain also has fast establishment which comes close to that of perennial ryegrass, which could explain why plantain had heavier shoot weights than the clovers (Blom 1978). Although white and red clover have rapid germination, slow seedling growth limited the shoot weight of the clovers. The average shoot weight of perennial ryegrass, white clover, plantain and red clover did not vary substantially in May which likely due to little or no interspecific or intraspecific competition. Further evidence of this was shown with the number of total leaves per perennial ryegrass, white clover, plantain and red clover plant which was not significantly different over the pasture mixtures (Figure 4.3). This directly relates to ability of plants to intercept light for photosynthesis, which is measure by the LAI of the mixtures. LAI for the monocultures was 2.17 and 2.43 for perennial ryegrass and plantain and was below 0.40 for white and red clover. The LAI of mixtures was well represented by the monoculture LAI, with perennial ryegrass and plantain providing the majority of the LAI of the mixtures. The LAI over all the pasture mixtures (and monocultures) were well below critical LAI

reported in the literature (Joggi *et al.* 1983; Rattray *et al.* 2007), which suggests that at this stage in development the plant population were not limited by light. During May the plants were still in early development and had not started to be shaded by other individual plants. At this stage the plants were most likely limited by soil temperature which in May was on average 9 °C (Figure 3.3) which is getting to the point where white clover stops growing (8-9 °C) (White & Hodgson 2000).

The rapid establishment and seedling growth resulted in the ryegrass monoculture having the highest yield of the monocultures and the mixtures (148 kg DM/ha) (Figure 4.4). This was a result of the high shoot dry weight of perennial ryegrass and the high plant population which were the factors used to estimate yield in May. The plantain monoculture was also high in comparison with the two clover monocultures and the majority of the other mixtures. This again related to the high plant population and shoot weights of plantain. White clover and red clover in contrast produced the lowest yields which again related to the slow seedling growth of white clover and also red clover. The trends in the monocultures were also present when the pasture species were mixed, with perennial ryegrass and plantain providing the majority of DM yield. Mixtures with high proportions of perennial ryegrass and plantain in the mix provided the greatest yields (e.g. mixtures 6, 11, 16, 18, 21 & 23). White clover had very low contribution to total yield as a result of low seedling growth in the mixtures. Red clover yields in the mixtures was above white clover, but was still limited due to lower shoot weight compared with perennial ryegrass and plantain.

In August the plantain monoculture yield higher than the perennial ryegrass monoculture. When ryegrass was sown with plantain, with a combined proportion of at least 50% of the seed mixture, there was clear yield advantages relative to mixtures with lower proportions of perennial ryegrass and plantain or no plantain (Mixtures 6, 11, 13, 15, 16, 18, 21 & 23) (Figure 4.6). In comparison the white and red clover monocultures had poor yields which relates back to the slow seedling development of white clover and the winter dormancy of red clover. Subsequently pasture mixtures sown with high proportions (0.5-0.625) had lower yields relative to the other mixtures. The proportion of weeds in the pasture mixtures in August was large in mixtures sown in pure clovers, reaching up to 80% of total yield (2, 4 & 9) (Figure 4.7). Mixtures sown with high proportion of white and/or red clover had higher weed proportions compared with mixture with low clover proportions in the mix. Higher weed content in clover dominated mixtures is due to the slow seedling establishment and poor seedling vigour of clovers that offers little competition to the more aggressive weed species that establish quickly and shade clovers severely (Lee 1985). Perennial ryegrass in contrast had the greatest suppression of weeds when sown as a monoculture, because of its rapid emergence, seedling growth and aggressive competitive nature.

Plant population in August (Figure 4.8) was poorly estimated with larger error bars. This was due to the estimates being made by average shoot weight and species yield per m². This estimate showed that species mixtures with perennial ryegrass and/or plantain with red clover and or white clover had low clover populations. This is likely due to the small size of the clovers at the time of the harvest in these pasture mixtures, which made accurate harvesting difficult. This then cause an under estimation of the clover populations. Tiller numbers per plant in August provided strong evidence of the performance of species in pasture mixtures (Figure 4.9). Perennial ryegrass had lower tiller numbers per plant sown in a monoculture and when in mixtures with high proportion of plantain. Plantain followed the same pattern for tiller numbers as perennial ryegrass. The number of tillers for both perennial ryegrass and plantain were highest when sown in with high proportions of clover (30 - 62.5% clover). This was due to the open swards that occurred from sowing clovers at 30-62.5% of the sown species. This allowed perennial ryegrass and plantain plenty of space to branch out (produce more tillers) to increase photosynthetic area. White and red clover in contrast to perennial ryegrass and plantain the total number of tillers per plant was negatively affected by mixing with high proportions of perennial ryegrass and plantain. Tiller numbers were highest when sown in the monocultures and the two species clover mixture for both red and white clover. These trends hold for the amount of leaves per plant when comparing species sown in a monoculture or a four species (equal) mixture (Tables 4.1 - 4.4). Leave counts from three dates (5th of May, 3rd of July and 11th of August) showed both perennial ryegrass and plantain more leaves per plant when sown in a mixture compared with a monoculture. This relates to the level of competition present in monoculture and mixed swards. It is generally accepted there is more intense competition in pure swards as the plants are competing for the same resources (Haynes 1980). This hold true for perennial ryegrass and plantain in this instance. However, the number of white clover leaves are negatively affected by mixing with perennial ryegrass, plantain and red clover. This most likely due to the more erect and taller perennial ryegrass and plantain plants shading white clover. Unlike white clover, red clover showed no difference in total leaves between the monoculture and mixture. This was likely due to red clover having a more erect growth habit which was not affected by shading from perennial ryegrass and plantain (White & Hodgson 2000).

Early spring yield (September) showed the majority of the pasture mixtures yielding above 1600 kg DM/ha, with the exception being the red and white clover monocultures and mixture (Figure 4.10). Yields of perennial ryegrass and plantain monocultures were not different and again provided the majority of total DM yield of pasture mixture, with little contribution from clover. As in August weed content in the pure clover mixtures was above 60% of total yield (0.66-0.72) (Figure 4.11). Weed proportions were also still high in many of the mixtures with 50% or greater clover in the seed mix. LAI of the pasture mixtures in September provided further evidence for yield differences with higher yields coming from pasture mixture with a LAI around 3. Based on critical LAI from the literature the majority

of the pasture mixtures were approaching critical LAI (3.5-4.5) (Ratnayake *et al.* 2007). The monocultures of white and red clover were well below reported critical LAI of 3.3-3.5 at 0.34 and 0.58 respectively (Jaggi *et al.* 1983). This explains why the pure clover monocultures had lower yields, because they had lower leaf area for light interception and consequently lower growth and yield. The clovers in the mixtures composed very little of total LAI which was again due to the poor growth of white and red clover seedlings from establishment. Metabolisable energy from pasture mixtures ranged from 10.9 to 12.7 MJ ME and was on average 11.9 MJ ME (Figure 4.13). The tetraploid perennial ryegrass and white clover monocultures had superior feed value (12 MJ ME) compared to the plantain and red clover monocultures (11 MJ ME). Consequently the pasture mixtures with high sown proportions of perennial ryegrass and white clover had the highest ME. In relation to N% all pasture mixtures had similar N content of around 3% except for the three mixtures which contained pure clover (Figure 4.14). Nitrogen leaves were higher due to the clovers having a readily available source of N, via biological N fixation which allows high N content in leaves to be maintained.

The accumulated DM yield showed when perennial ryegrass and/or plantain composed 50% or more of the seed mixture the pasture mixtures yield between 3000 – 3900 kg, regardless of the white and red clover sowing proportions (Figure 4.15). The dominance of perennial ryegrass and plantain was visually evident in September with very little clover content in most pasture mixtures (Plate 4.1 – 4.6). Sowing clovers alone in pasture mixtures resulted in low yields and high weed proportions due to the slow seedling growth and low competitiveness of clover (Figure 4.16). There were significant differences between pasture species when sown in monocultures (Table 4.5). With plantain yielding the highest followed by perennial ryegrass and then red and white clover (accumulated yield). Sowing non legumes (perennial ryegrass and plantain) with legumes had a significant interaction. Høgh-Jensen and Schjoerring (1997) reported positive interaction between perennial ryegrass and white clover due to high N up take because of biological N fixation from clover. There was no clover to clover or perennial ryegrass to plantain interactions as they were competing for similar resources. Using a quadratic equation based on the output from the mixture analyse, accumulated sown yield could be predicted based on different proportions of species sown in the mixture.

5.2 Species richness

Based on plant population in May (Figure 4.1) the proportions of each species sown in the mixtures was well represented of the proportion of each species present in the plant population. There was a trend towards increased perennial ryegrass population and decrease in the plantain, white clover and red clover. Accumulated yield showed that species richness (amount of species in the mix) had no impact on the yield of pasture mixtures (Figure 4.15). This was evident by the similar yields obtained from certain monocultures, two species mixtures, three species mixtures and four species mixtures.

The yields were instead related to the pasture species that were sown in the pasture mixtures and the relative proportions of each species in the seed mixture. This was likely due to the experiment being over establishment which favoured the fast establishing species (perennial ryegrass and plantain). The low contribution of clover to DM yields was a consequence of slow seedling growth due to low temperatures after sowing and in winter. Yield advantages for mixing perennial ryegrass and/or plantain with the clovers may have been more apparent if the clover establishment was improved. The nitrogen availability in the soil was also likely a contributor to the lack of differences in yield between mixtures. The cultivation of the soil prior to sowing, likely resulted in mineralisation of N which was then readily available for plants to take up, so plants were likely not severely nitrogen deficient. This as well as the young age of the plants may have been the cause of no differences in sowing a monoculture of perennial ryegrass and a mixture of perennial ryegrass and white or red clover.

5.3 Relative abundance

Relative abundance of seed in the seed mixture was well represented in the plant populations of pasture mixtures in May (Figure 4.1). However, the yield at this date was dominated by perennial ryegrass and plantain. Four species mixtures sown equally or with each component sown dominate in-turn, all resulted in the mixtures being dominated by plantain and perennial ryegrass. The dominate sowing of perennial ryegrass or plantain did result in higher yields relative to the four species equal mixture and the clover dominate mixtures. Perennial ryegrass dominance was evident in the two and three species mixtures as well. This trend was continued in the August and September harvest (Figure 4.6 & 4.10). Accumulated yield from the four species mixtures showed clover percentage was still low (>10%) even when clover is sown dominate in the seed mixture (62.5%). Dominate sowing of clover did however, result in 1000 kg DM/ha lower accumulated yields compared with the four species mixtures sown with 0.5 or lower clover proportions. Visual observation of the four species mixtures in September show clear differences between mixtures with different sowing rates of species (Plate 4.4 & 4.5 A, B, C & D). The relative abundance of tetraploid perennial ryegrass, white clover, plantain and red clover in the seed mixture was poorly represented in the botanical composition of pasture mixtures. White and red clover botanical contribution was poor even when sown at high proportions of the seed mixture. Perennial ryegrass and plantain in comparison with the clovers contributed the majority (78 – 92%) of the total pasture yield.

5.4 Species separation

The practice of sowing tetraploid perennial ryegrass, white clover and plantain in alternate drill rows was used to test the effects of spatial separation on the establishment of these pasture species when sown in three two-species mixtures and one three-species mixture (20, 21, 22 & 23). These were compared with the three equivalent pasture mixtures sown together (5, 6, 8 & 11). Plant population

from the alternate drill row sown mixtures was very even with a range of 489 – 498 plants/m² in May (Figure 4.1). The mixtures sown together had similar plant population in comparison with the alternate row sowing, except for mixtures 5 and 6 which were lower. The higher plant population in the two alternate drill row mixtures was likely due to the separation of species allowing higher establishment. Yield in May was similar between sowing treatments for mixture sown with plantain and white clover (8 & 22). There were differences between mixtures 5, 6 and 11 and 20, 21 and 23 (Figure 4.4). Where ryegrass and plantain sown in alternative drill rows (21) had higher yield than when species were mixed (6). This was a result of the higher white clover population when sown in alternate rows, which was likely due to the separation of species. Perennial ryegrass and white clover and the perennial ryegrass, white clover and plantain mixtures produced higher yields than when the same mixtures were sown in alternate drill rows. This was due to higher shoot weights when the species were mixed which was likely due to the lower perennial ryegrass plants per row. LAI in May was directly related to DM yields in May (Figure 4.5).

In August yields were higher for plantain and white clover sown in alternate drill rows (22) compared with the same species sown together (8). This could have been a result of increased establishment from the plantain. Perennial ryegrass, white clover and plantain sown in alternate drill rows (11) had lower yields than the same species sown together. This was a result of lower perennial ryegrass and plantain yield, which been a result of increased intraspecific competition within the rows of ryegrass and plantain relative to the same species sown in a mixture. Weed proportions from this harvest were increased for the three species mixture when sown in alternate drill rows. This was likely due to the separation of white clover into separate drill rows which increased the potential for weed competition, which white clover is vulnerable too (Lee 1985). Alternate drill row sowing caused decreases in total tiller number for plantain and perennial ryegrass when both were sown with white clover in alternative drill rows (Figure 4.9). This relates back to competition between plants of the same species, which is higher than competition between different species (inter vs intra specific competition) (Haynes 1980). White clover in contrast had higher tiller number per plant when sown in alternate drill rows. Here interspecific competition in mixed pastures is the factor that limits the number of tillers and growth of clover as perennial ryegrass and also plantain shade white clover causing reduced growth. When sown separately clover has more space and can branch out (tiller).

In September the yields from pasture mixtures sown together was higher than the alternate drill row sown mixtures, with the exception of the mixtures sown with perennial ryegrass and plantain (Figure 4.10). This was likely due to the limited expansion of perennial ryegrass and plantain within each drill row they were sown in because of intraspecific competition. White clover yields were however, notably higher when sown in alternate drill rows. High weed proportion were also evident in mixtures 22 and 23 for the September harvest, similar to August (Figure 4.11). The weed content in these

mixtures could also have been linked to the amount of bare ground (available space) in these mixture which was visually observed (plate 4.2 D & 4.3 A). There were small difference in the ME in September, with alternate drill row sown mixtures having a ME of 12.2 MJ ME and the same mixtures sown together an average ME of 11.9 MJ ME.

The average accumulated yield of the two sowing treatments were not different, with small differences between mixture 11 and 23 (Figure 4.15). Weed proportions were increased through the sowing of the two species mixture of plantain and white clover and for the three species mixture in alternate drill rows compared with sowing the species together in a mixture. This was a result of white clover being sown in separate drill rows which allowed weeds to take advantage of the slow seedling growth of white clover and establish. In relation to species proportions, species separation caused a more even 50:50 split between perennial ryegrass and plantain when sown in the same mixture (Figure 4.17 & 4.18). White clover was increased on average by 0.032 when sown in alternate drill rows. This increase was due to white clover having no competition from perennial ryegrass and plantain, however the white clover proportions were still low. The low white clover proportions relative to total yield were due to the slow seedling growth and poor competition with weeds. The late March sowing date also influenced the clover as the temperature limited growth over late autumn and winter (Figure 3.2 & 3.3) (Moot *et al.* 2000). Mixture analyse for the two sowing treatments found no significant interactions from the alternate drill row sown mixtures.

5.5 Practical implications

The results of this study show that sowing tetraploid perennial ryegrass, white clover, plantain and red clover in various pasture mixtures, in early autumn (March) will result in high proportions of perennial ryegrass and plantain and low proportions of white clover and red clover in relation to total yield. Sowing clover in proportions, 50% or above resulted in lower DM yields. Autumn was not favourable for the establishment of clovers, which will likely perform better from a spring sowing. When selecting pasture seed mixtures perennial ryegrass and plantain should consist of at least 50% of the seed mixture to achieve high yields. Even when white or red clover was sown as the dominate pasture species the yield was still dominated by perennial ryegrass and plantain. Increasing species richness (amount of species in the mix) in the pasture mixtures did not result in increased yields. Pasture quality is highest in pastures with tetraploid perennial ryegrass and white clover.

Sowing pasture species in alternate drill rows had no yield advantages and only very small increases in clover content. Sowing pastures using this method would not be recommended based on no yield advantages over establishment and the difficulty in setting up drilling equipment for this sowing practice. However, yields and pasture composition following establishment may provide better assessment of this practice.

When farmers are selecting pasture seed mixtures for livestock production tetraploid perennial ryegrass and plantain should make up the majority of the seed mixture. White clover and red clover should be included in the mixture to provide added pasture quality and biological N fixation but are not essential to obtain high DM yields.

5.6 Future Research

The continuation of the experiment will be valuable to gain long term data on the performance of the pasture mixtures. This will be especially important to measure how the botanical composition changes with continued grazing/harvesting and with increasing age of the pasture. The comparison of the alternate drill row sown mixtures and their equivalent mixtures sown together, over the next year or so will provide a better assessment of the alternate sowing practice than could be achieved over the establishment phase in this experiment. Late spring and summer growth for the trial will provide a better estimate of clover contribution as the clovers become more active and develop more. The yield over this period will also be of interest as plantain and red clover are both highly productive in summer. The quality of the pasture will be another important measure over late spring and summer to measure. The long term persistence of perennial ryegrass, white clover, plantain and red clover in the pasture mixtures is another key aspect, especially for red clover and plantain which have been reported to be relatively short lived in pastures (3-4 years) (Stewart *et al.* 2014).

Repeating this experiment with a spring sowing date may be another potential research area as clover proportion would likely be higher due to higher temperatures associated with spring. Future trials are required on the lamb performance and finishing capability from these pasture mixtures. Ewe and lamb performance from these pasture mixtures may also be another important performance attribute to quantify from these pasture mixtures.

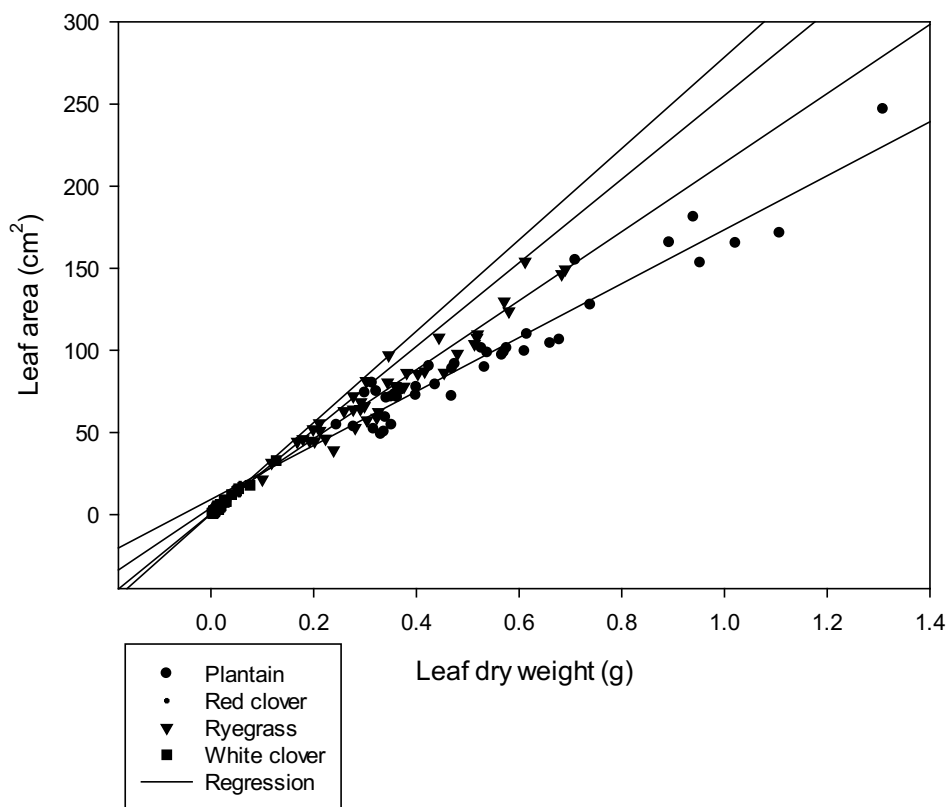
6 Conclusions

Over establishment from an autumn sowing, pasture mixtures with proportions, of 50% or more of tetraploid perennial ryegrass and/or plantain results in high accumulated yields between 3000 – 3900 kg DM/ha. When white and red clover were sown as monocultures or as a two species clover mixture, yields were poor with high weed proportions (above 0.6) relative to total yield. Alternate drill row sowing resulted in small increases in white clover proportions, but there were no advantages in yield when compared with the equivalent mixtures sown together. Pasture quality was highest for pasture mixtures containing tetraploid perennial ryegrass and white clover. Farmers selecting a pasture mixture for spring production from an autumn sowing should select mixtures with at least 50% of the seed mixture made up of plantain and/or perennial ryegrass. The addition of white and red clover will assist in the increasing feed quality and have little impact on yield when sown at low proportions. Further research is required into late spring and summer performance of these mixtures following establishment.

Appendix

A.1 Leaf area

Figure A 1 Linear regression for leaf area and leaf dry weight for ryegrass, white clover, plantain and red clover for monocultures and four species equal mixtures on the 3rd July 2015, at Lincoln University.



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