Dry matter production and botanical composition of multi-species pasture mixtures during the second year after establishment

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

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A pasture mixture experiment was conducted, growing four species; perennial ryegrass, plantain, white clover and red clover under irrigation in Canterbury, New Zealand. The experiment set out to identifty an optimal pasture seed mixture that maximised annual dry matter (DM) yield by quantifying species identity and diversity effects from pasture mixtures. The plots were sown containing one- to four-species, based on a simplex mixture design containing all possible combinations. The annual and seasonal DM production were statistically analysed with a special cubic model created from the simplex mixture design, enabling predictions to be made on the mixture that would produce maximum yield for the second year of growth (May 2016 to May 2017). The DM production and botanical composition of each monoculture and mixture were measured, compared and analysed. To explain differences in yield between mixtures, N content, leaf area index and light interception were also measured. The modelled analysis showed that five two-species mixtures and one three-species mixture showed significant species interactions and subsequent yield increases over the mean of their component monocultures. The seed mix that was predicted to maximise yield consisted of 0.29 ryegrass, 0.20 plantain and 0.51 red clover in terms of seeds/unit area, predicted to yield 16.44 t DM/ha for the second year of growth. This mix produced 5.19 t DM/ha more than the rygrass and white clover (RG*WC) mixture, which yielded 11.25 t DM/ha. The greatest diversity effects were shown by the two species mixtures that included red clover, and produced 4.93 t DM/ha (RG*RC) and 5.31 t DM/ha (P*RC) above the mean yield of the monocultures.

Species interactions and botanical compositions within the mixtures were not consisitent over time; the optimal mixture (ryegrass, plantain and red clover) displayed transgressive overyielding over six of the eight sampling dates. There were no significant species interactions across any of the mixtures at the early spring harvest (August 2016) and the late autumn harvest (May 2017). The botanical composition of the highest yielding mixture RG*P*RC vaired in botanical composition over the season. It deviated from its sown proportions of 0.33 RG -0.33 P - 0.33 RC to 0.24-0.54-0.22 of RG, P, RC respectively at the time of first harvest (2/08/2016). By the second harvest (22/09/2016) the botanical composition of the mixture had changed to 0.10-0.26-0.64; halving the proportion of ryegrass and plantain and tripling the proportion of red clover. There were significant differences in N content between the four monocultures, ranging from 2.30% (ryegrass) to 3.75% (red clover). The mixture N% was lower when white clover was included in the mixture (2.71% for RG*WC, 2.82% for P*WC) than when red clover was included (3.22% for RG*RC, 3.49% for P*RC). The N content of the mixtures can be used to explain yield as the average N concentration of the mixtures increased by 1%, the annual yield increased by 6.60 t DM/ha. Leaf area index and light interception analysis within the seventh and eighth harvests showed that significantly higher yielding mixtures such as RG*RC, P*RC and RG*P*RC reached critical light interceptance and had a significantly higher leaf area index than their component monocultures and mixtures that produced lower yields, such as RG*P, P*WC and WC*RC.

Keywords: Ryegrass, plantain, white clover, red clover, seasonal yield, seed mixtures, simplex design

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Table of Contents

List of Tables

List of Figures

Table of Plates

Chapter 1

Introduction

Traditionally New Zealand pastoral agriculture has been based on pasture mixtures of perennial ryegrass (*Lolium perenne* L.) and white clover (*Trifolium repens* L.) pastures. Ryegrass-white clover pasture mixtures are generally regarded as sustainable systems of livestock production and have been extremely successful as a production base in pastoral agriculture (Goh & Bruce, 2005). This combination has provided reliable, quality feed for most of the year, with good yield and persistence qualities (Daly *et al.*, 1996; Sanderson & Webster, 2009). The downside to this pasture is that it can lack quantity and quality during the summer period. Reduced quality in ryegrass is predominantly due to reproductive stem development, with metabolisable energy (ME) content shown to decline from 11 mega joules (MJ) /kg DM in spring to between 7.6 and 9 MJ/kg DM (Brown *et al.,* 2005). Pasture-based farming has become more intensive over the last 40 years (MacLeod & Moller, 2006) and as a result, more options of pasture species and cultivars have become available for farmers to utilise. Other species options include but are not limited to; red clover (*Trifolium pratense*), plantain (*Plantago lanceolata*), chicory (*Cichorium intybus*), lucerne (*Medicago sativa*), birdsfoot trefoil (*Lotus corniculatus*), lotus major (*Lotus pedunculatus*) and timothy (*Phleum pratense*). These species produce high quality forage that is capable of supporting high animal growth rates through the dry summer period (Brown *et al.,* 2005).

Sward mixtures based on herbs and legumes can have superior herbage quality (Daly *et al.,* 1996; Goh & Bruce, 2005) and greater tolerance of dry summers (Goh & Bruce, 2005) than a mixed perennial ryegrass and white clover pasture. Young stock performance increases substantially when grazed on a herb-clover sward. For example, Li and Kemp (2005) and Cranston *et al.* (2015) showed average increase in lamb liveweight gain (LWG) grazing a herb instead of perennial ryegrass was 51% over four trials, while LWG of Friesian bull calves increased by 29.3% when groups were fed on herb-clover pastures compared to ryegrass. These data show the difference that mixing herb species into a whole, or part of, a farm system can increase the potential of stock finishing on farm.

Any differences in stock performance may be linked to the greater quality and yield of mixed pastures. Combining two or more species into a pasture can create species and diversity effects within the sward, forming from the different physiological traits and genetic differentiation of each of the contained species, e.g. the way white clover can facilitate the growth of grasses and herbs in mixtures (Harris, 1977). These effects may explain the differences in stock performance seen from previous studies (Li and Kemp, 2005; Cranston *et al.,* 2015). These interactions between species within a sward can cause ‘transgressive overyielding’, where the functional response of a pasture mixture (such as dry matter yield, nutritive value, or animal performance) is more than the yield of the best performing species when grown as a monoculture.

Adding a legume species to a pasture mix not only creates a benefit of added DM growth, but the other species in the mix also gain access to plant available N ’fixed’ from the atmosphere by root nodules on the legume root structure (Harris, 1968; Sanderson *et al*., 2005). There are N benefits with growing more legumes in a mixture, more biological N fixation can occur and a reduced amount of N fertiliser may be required (Black *et al.,* 2009).

A study of plants growing in mixture, no matter how detailed, cannot separate the direct effects of treatment and environment from those due to the presence of the companion species. Only by comparing mixture and monoculture can this be achieved (de Wit & van den Bergh, 1965). Despite the potential agricultural importance of mixed grass/clover swards, few studies have made the comparison for this association (Davidson & Robson, 1986). The objectives of this research were to quantify species identity and diversity effects from pasture mixtures; being the sum of the positive and negative interactions between two or more pasture species in the same sward, in terms of increased yield compared to the species grown in monocultures (Frankow-Lindberg *et al.,* 2009). The quantification of this effect also helps identify the optimal seed mixture that maximised dry matter (DM) yield (Sanderson *et al.,* 2005; Connolly *et al.,* 2009; Black *et al*., 2017).

This information will hopefully provide some basis to support or argue the make-up of commercial seed mixtures by seed companies, which recommend a mix of seeds with little known scientific backing behind their formation. Sheep and beef seed mixes for breeding and finishing from major seed companies include multiple species and cultivars of predominantly ryegrass and white clover; with some herb options starting to emerge (Table 1.1). Commercially available cultivars are predominantly ryegrass based, with a small legume proportion. The mixtures are also very rich in terms of the number of species and cultivars that are included in the mixture, despite literature suggesting that the use of simpler mixtures results in high yields. The mixtures do not have a set proportion of legume or non-legume species, and can range from 14% to 29% legume in terms of kg seed/ha.

Table 1.1. Commercially available seed mixes currently offered to farmers.

Company	Species 'Cultivar'	Rate (kg/ha)
Agriseeds – Sheep/Beef	Tetraploid Perennial Ryegrass 'Viscount'	15
	Diploid Perennial ryegrass 'Trojan'	10
	White Clover 'Kotare'	2
	White Clover 'Weka'	2
Total		29
Agriseeds - Finishing	Tetraploid Perennial Ryegrass 'Viscount'	30
	White Clover 'Weka'	2
	White Clover 'Apex'	2
	Red Clover 'Tuscan' (coated)	6
Total		40
Agricom -Sheep/Beef	Diploid Perennial Ryegrass 'Samson'	12
	Diploid Long Rotation Ryegrass 'Supreme'	8
	White Clover 'Tribute'	4
	Red Clover 'Sensation'	4
Total		28
Agricom- Finishing	Tetraploid Perennial Ryegrass 'Halo'	26
	White Clover 'Emerald'	2
	White Clover 'Tribute'	2
	Red Clover 'Sensation'	4
	Chicory 'Choice'	2
	Plantain 'Tonic'	1
Total		37

This dissertation conducted a mixture experiment as a field trial at Lincoln University where four species were sown in all possible combinations from one to four species swards. The chosen species and cultivars were 'Base' perennial ryegrass, 'Tonic' plantain, 'Apex' white clover and 'Sensation' red clover. Measurements analysing DM production were taken and tests performed on the corresponding data to quantify the effects of growing the species as monocultures or as a multi-species pasture in terms of annual and seasonal DM yield. From this information an optimal seed mixture was calculated from the modelled data that would produce the highest DM yield over the second year of growth. The dissertation includes a literature review of previously published studies involving the species within pasture mixes, the formation of mixtures, and how species interactions have been previously quantified.

Further analysis of the swards was undertaken by measuring specific leaf area, formulated from measuring the area of green leaf and per gram of dry leaf matter. This calculation is then converted using the mass of green leaf harvested into leaf area index (LAI), being the ratio of leaf area to ground area occupied by the sward. The aim of this was to try and explain any differences in yield between pasture mixtures by quantification of light interception and leaf area.

Another way of explaining difference in DM yield between pasture mixtures is the concentration of N within the pasture, calculated from the concentration (%) of crude protein. N is the major determinant of plant growth in the presence of sufficient moisture and temperature, and can be supplied to non-legumes in the presence of legume species, such as white and red clover. Near-Infrared Spectroscopy (NIR) analysis of the mixture samples was undertaken to generate data of N concentration and enables a comparison between average N concentration in the sward, and sward yield.

Chapter 2

Literature review

2.1 Introduction

Perennial ryegrass and white clover pasture mixtures are often perceived as permanent or only needing replacing every 10 years, but the agronomic research on their productivity suggests it declines after 3 to 5 years (Sanderson & Webster, 2009). Pasture renewal is a major development increasing the productivity of all farm systems in New Zealand. Exploring other species options to compliment perennial ryegrass and white clover for pasture renewal has the potential to lift farm productivity. In addition, multi-species mixtures could provide greater and more stable DM production through temporal, spatial and resource niche complementarities (Sanderson *et al.,* 2004).

Large inputs of artificial N fertiliser of 200-500 kg N/ha/year are required to maximize the productivity of non-legume monocultures (pure grass and pure herb pastures) (Reid, 1970). This is increasingly regarded by policy makers and public opinion as being environmentally unacceptable (Frankow-Lindberg *et al.,* 2009). Mixing legume and non-legume species carries the benefit of potentially reduced artificial fertiliser applications through the supply of fixed N from legumes. The use of mixtures could therefore be recommended as a way of reducing N inputs, while also increasing production benefiting from genetically different species in the same sward.

The purpose of this review was to study previous literature pertaining to the principles behind pasture mix formulation; how species functional groups interact and contribute to yield. This leads to the selection of species involved in our own mixture formulation trial, being perennial ryegrass, plantain, white clover, and red clover, so as to understand the effects of having each of these species as part of a multi-species sward. The species richness and evenness relating to botanical composition, competition and succession, and LAI of pastoral swards are also covered within the basis of this review.

2.2 Pasture mix formation

The idea of combining different pasture species into a mix is not a new one, with the proposal mooted in international production agriculture as early as 1908 (Elliot, 1908). The benefits of using different species to increase sward quantity and quality have been well realised in many studies globally throughout the 20th and 21st centuries. In forming a pasture mix, Frankow-Lindberg *et al.* (2009) hypothesized that: (1) positive interactions between species would result in larger yields from mixtures than expected from the performance of the individual species sown as monocultures; (2)

these benefits would persist over several years; (3) a broad genetic-base composite of species that have different physical and physiological traits would provide a platform for further and sustained positive effects of mixing species; and (4) increased species diversity and the use of broad genetic-base composites would help to prevent invasion of unsown species into the swards. The study found that species diversity had a strong, persistent and positive effect on yield and on the ability of the sward to resist invasion by unsown species. In the experiment, all hypotheses were confirmed apart from the third hypothesis, as growing different genotypes of the same species proved to be of significant value in terms of increased yield. Proposed benefits of mixing pasture species in a sward include a longer grazing season benefited by the differing growing seasons of multiple species; better soil occupation and interaction as deep-and-shallow rooted species occupy more of the soil column; and greater persistence as a richer species presence suppresses weed infiltration (Alexander, 1933).

Species diversity effects are the culmination of all the positive and negative effects of interspecific interactions between plant species. These interactions can be synergistic or antagonistic in their effect on pasture responses (Figure 2.1). Synergistic interactions are normally the result of differences in resource use among species, and facilitation, such as the way clovers can favour a companion grass or herb. This synergism usually results in an increase in yield, such as between a grass species and a clover species. Species may also interact antagonistically to have a negative effect on pasture responses, such as when two similar species compete for the same resources and niche within the sward, and as a result yield can decrease (Figure 2.1). Interactions may involve two or more species, and several positive and negative interactions may occur at the same time. The diversity effect is the net result of all of these interactions.

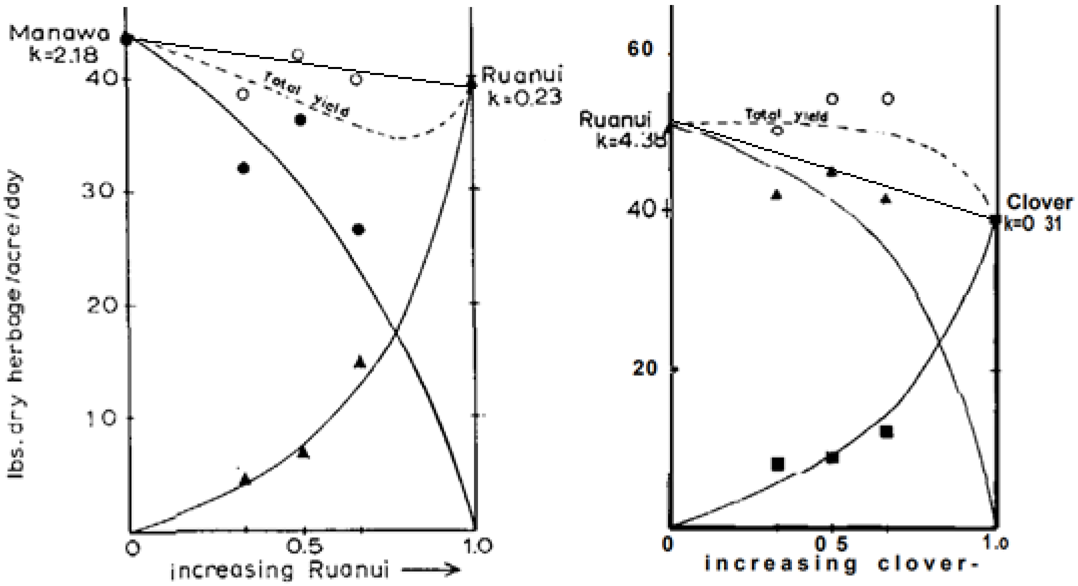

Figure 2.1. Replacement diagrams based on yields of Manawa ryegrass, Ruanui ryegrass and white clover displaying antagonistic and synergistic interactions respectively. From Harris (1968).

The thinking towards creating a mixed pasture with species showing complimentary characteristics was carried on by Harris (2001), who discussed three principles towards a successful seed mixture. The first principle was in agreement with Alexander (1908) and that was matching the species to be included in the mixture with the environment they are to be grown in. In an irrigated, high fertility soil situation, this principle does not discount many species, although the Canterbury area experiences cool winters, so cold-sensitive species would not be an option. The second principle was to include fast establishing species, to outcompete weed species for canopy space and nutrients. For example, slow establishing species such as Caucasian clover (*Trifolium ambiguum* M.) would be less suitable as part of a mixed species pasture for this characteristic. The third principle is to select species with differing characteristics, providing versatility that allows for both the heterogeneity of environmental microsite or ecological niches within the area being sown. 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.

In contrast to ryegrass–white clover based pastures, which are usually ryegrass dominant, the mix generally includes species chosen for compatibility and diversity within the sward (Daly *et al.,* 1996). Genetic diversity might be expected to provide additional benefits through improved resistance to biotic and abiotic environmental stresses, and enhanced niche complementarity (Frankow-Lindberg *et al.,* 2009). Combining a range of species, some with different growth periodicity, can improve yield and seasonal growth patterns (Harris & Hoglund, 1977). As species decline or fail to persist with changing conditions, the opportunity for substitution by a lower-order species is more likely within a diverse mixture, allowing continuity of production and quality (Daly *et al.*, 1996). Frankow-Lindberg *et al.* (2009) showed annual sown yield over 3 years showed a considerable and significant decline in the yield of monocultures for all species. This highlighted the role that lower-order species play within a sward to extend the persistence of a mixed species sward.

The number of species to include in a pasture mix, described as species richness, has developed from pioneering mixtures in New Zealand, where up to 20 species were sown in a seed mixture in the hope that a few would establish (Harris, 1968). Levy (1936), suggested the simplification of mixtures, to create swards with less species richness but where each species is expected to contribute to the productivity of the sward. Sanderson *et al.* (2005) showed that a three-species grass-herb-clover mixture containing cocksfoot, white clover, and chicory yielded 54% more herbage in one season than a two-species cocksfoot-white clover mix. Despite this increase of one added species, there was no further benefit in adding more species in the six- and nine-species mixes that were trialled alongside the two- and three-species mixtures. Dalgety (2016), found in the 2015/16 year of the mixture trial, that the grouped average of three-species mixtures yielded significantly more than the grouped average of one- and two-species mixtures, but there was no added benefit of adding a fourth species in to the mix.

The species involved in the experiment grow very differently as monocultures in terms of functional type and growing point and rooting type. These differences lead to potential annual yield differences that may introduce a species effect into the pasture mixtures, i.e. the species involved in the mixture may have an effect on the yield of the mixture.

Table 2.1. Pasture species included in the experiment; ryegrass, plantain, white clover and red clover functional group, traits and typical yield when grown as monocultures.

Species	Type	Functional Group	Growing Point	Rooting Type	Annual Yield (t DM/ha)	Study
Perennial Ryegrass	Grass	Non-legume	Tiller	Fibrous	10.0	Stewart, (1996)
Plantain	Herb	Non-legume	Tiller	Deep fibrous	7.5-8.5	Stewart, (1996)
White Clover	Clover	Legume	Stolon	Fibrous	6.0-7.0	Hyslop, (1999)
Red Clover	Clover	Legume	Crown	Tap-rooted	11.0-15.0	Hyslop, (1999)

The special cubic simplex design of the experiment design of the mixture experiment carried out in this honours study included every possible combination of these four species from one- to four-species mixtures. This incorporation means that conclusions can be drawn on species diversity effects that wouldn't otherwise be able to be made if some mixtures were excluded, as is the case in other mixture experiments (Nibilly *et al.,* 2013; Sanderson *et al.,* 2005). These diversity effects can be quantified and directly related to the species grown as monocultures, binary mixtures, ternary mixtures, and quaternary mixtures under the same conditions.

The plant population of the pasture has the potential of alter the diversity effect of pasture species, as species respond differently to higher levels of competition within the sward. For example, Italian ryegrass (*Lolium multiflorum* L.), red clover and balansa clover (*Trifolium michelanium* S.) were sown as a mixture at two differing sowing rates (20 kg/ha and 30 kg/ha) at Lincoln University (Ryan-Salter & Black, 2012). The seasonal yield of each mixture was measured to discern differences in species diversity effect influenced by sowing rate. There were no mixture-by-sowing rate interactions, indicating the mixture effects were consistent over sowing rates. The mixtures sown at 30 kg/ha produced on average 0.16 t DM/ha more yield than those sown at 20 kg/ha in May, July and March ($P < 0.001$). However, accumulated yield after 12 months was not affected by overall sowing rate (Table 2.2).

Table 2.2. Average yield (t DM/ha) from seed mixtures of Italian ryegrass, red clover and balansa clover repeated at two overall sowing rates. From Ryan-Salter and Black, (2012).

Sowing Rate	Harvest Yield (t DM/ha)						
	11 May	19 July	18 Oct	13 Dec	2 Feb	30 Mar	Total
20 kg/ha	0.50	0.68	4.24	3.26	2.09	1.27	12.03
30 kg/ha	0.66	0.84	4.20	3.20	2.10	1.44	12.45
Significance	< 0.001	< 0.001	NS	NS	NS	< 0.05	NS

Therefore, mixture experiments that test species effects and interactions in mixtures at a single overall abundance (based on seed count per unit), which is within the range used in the industry, would be a sufficient first step in the identification of optimal seed mixtures that maximise DM yield.

2.3 Species attributes

Each of the species carried out in this honours project had differing physical and biophysical traits and the most important traits concerned were; growing season, their individual species yield and their ability to persist in a sward over a number of seasons. In agronomic grasslands, grass species have an excellent ability to acquire and convert available resources (including N transferred from legumes) into high quality forage. Legumes also provide high quality forage and, due to their ability to fix atmospheric N, can contribute significantly to synergistic interactions through interspecific transfer of fixed N (Finn *et al.,* 2013).

Many trials undertaken researching pasture performance only run for 1-2 years (e.g. Powell *et al.,* 2007; Goh & Bruce, 2005), and persistence of species in a mixed sward is often overlooked in the aim of generating short term performance. The persistence of a pasture is increasingly important to famer's as the cost of establishing pastures increases and more efficient techniques are investigated.

The persistence as fully productive pastures of legume and herb species ranges from 2 to 5 years depending on the weather, management, pest and disease presence, and species (Li & Kemp, 2005).

2.3.1 Perennial ryegrass

Perennial ryegrass is the base species of pasture production in New Zealand. It is a highly versatile species able to establish successfully in a range of environments, though it prefers high fertility soils and mild temperatures. Rapid germination, emergence and seedling growth give it a competitive advantage over species like white clover and red clover during the establishment period (Brougham 1953). Perennial ryegrass is well adapted to grazing with strong tillering ability and recovers well from both frequent and intensive grazing techniques.

Annual production of ryegrass pastures can be consistent up to 6 years, yielding 12-12.6 t DM/ha (Li & Kemp, 2005; Moloney, 1991) under an irrigated environment, and 10 t DM/ha under a fertilised dryland environment (Stewart, 1996). This persistence is important to farmers as a sward that holds a high proportion of its sown species or a longer amount of time results in a lower proportion of weed content and less reseeding costs over a long-term period. Ryegrass pastures, although tolerant of a wide range of grazing and environmental conditions, are disadvantaged by low rainfall, high soil temperatures and susceptibility to attacks by grass grub (*Costelytra zealandia*), black beetle (*Heteronychus arator*), pasture mealy bug (*Planococcus poae*), porina (*Wiseana* spp.) and Argentine stem weevil (*Listronotus bonariensis*) in New Zealand (McFarlane, 1990).

New cultivars of perennial ryegrass have been developed and modified into a tetraploidy genetic makeup. This genetic change means that cells have twice as much genetic material contained within them. One specific commercial tetraploid perennial ryegrass cultivar was chosen for use in this trial; namely 'Base' supplied by PGG Wrightson seeds. 'Base' has high density tillering and strong cool season growth (Stewart *et al*., 2014). The extra material contained in tetraploid cells affects seed size, the size of individual cells within the plant, and also its physical traits. The increased cell size of tetraploid perennial ryegrass increases the amount of water soluble carbohydrate (WSC) and reduces the amount of neutral detergent fibre (NDF) (Smith *et al.,* 2001); leading to an increase in nutritive value of the pasture. 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). For these reasons, tetraploid perennial ryegrass is used for this trial for its ability to produce a large quantity of high-quality feed.

2.3.2 Plantain

Plantain is quickly becoming a top pasture option for farmers to finish stock quickly. It is rapid to establish, grows on a wide range of agricultural soils and is tolerant of drought and of many common diseases and pests. New Zealand plantain cultivars have been chosen for their erect growth habit and production of large leaves. 'Ceres Tonic' was the cultivar used in this trial, and is the most common cultivar in New Zealand, showing greater winter growth compared to other plantain cultivars, such as 'Grasslands Lancelot' (Stewart, 1996).

The seasonal growth pattern of plantain begins its growth earlier in spring and continues later into autumn than white clover, red clover and ryegrass species (Kemp *et al.,* 2010) but is dormant during the winter period. Nevertheless, from spring to autumn a combined herb and legume pasture was shown to produce as much dry matter, or more, than the annual production of a traditional perennial ryegrass and white clover pasture (Goh & Bruce, 2005).

Plantain is able to establish quickly and produce over 10 t DM/ha in a short growing period. Powell *et al.* (2007) showed plantain is capable of first-year production of 17 t DM/ha as an irrigated monoculture. This strong period of growth is not prolonged, with average yields of only 8.36 t DM/ha shown over 4 years under a fertilised dryland environment (Stewart, 1996).

Plantain has been reported to be relatively short lived in pastures, with the useful life of the species being around 3-4 years (Stewart *et al.,* 2014). This persistence is highly dependent on how the sward has been managed. Grazing with long intervals rather than continuous grazing, under dryland conditions, and in swards containing "less aggressive" and slower-establishing companion species give plantain an edge of other species competing for resources. Grazing plantain during winter severely reduces the ability of the species to persist over a number of years (Cranston *et al.,* 2015).

2.3.3 White clover

White clover is New Zealand's most common legume, spread through all environments across both the North and South islands. The abundance of white clover originated from Grasslands 'Huia', which has been used during the past 50 years to oversow dry hill country pastures in New Zealand (Charlton, 1984). Since this time extensive research and development has been undertaken on low-land white clover cultivars, and as a result, many cultivars are now available for providing a high-quality legume forage. Limitations of white clover production are due to difficulties in establishment, maintenance and persistence, especially in drought prone regions (Brock & Caradus, 1996; Knowles *et al.,* 2003). This difficulty in establishing white clover was shown by Moot *et al.* (2000), where white clover germinated quickly in terms of growing degree days (°Cd), but did not have correspondingly rapid field emergence. In this situation the seedlings would be out-competed for light and resources in a mixed sward.

The growing season of white clover begins later than other pasture species such as perennial ryegrass, when temperatures exceed 8-9°C, and grows at maximum rates around 25°C (White &

Hodgson, 2000). The temperate nature of the species means winter growth is low, and its yield traits limit white clover's ability to perform as a monoculture for grazing.

Typically, white clover yields range from 6-7 t DM/ha (Hyslop, 1999). Yields commonly decrease when mixed with another species, ranging from 150-5040 kg DM/ha (Hoglund *et al.,* 1979) as the competition for light usually restricts white clover growth. This competition means that white clover may only make up 2-40% of the total DM for the sward. White clover is best utilised when mixed with species that allows light penetration through the sward down to where white clover can thrive. Traditionally, this species has been perennial ryegrass, but work has also been done with other pasture species such as tall fescue (*Festuca arundinacea*), cocksfoot (*Dactylis glomerata*), meadow fescue (*Festuca pratensis*) and plantain (Moloney, 1991).

White clover's ability to persist in a sward is dependent on the environment and management of the pasture. In drought prone regions, the stoloniferous nature of white clover growth makes the growing points on the soil surface susceptible to overheating when not sufficiently shaded by a companion species. In a mixed sward, a companion grass can protect white clover from high temperature damage. Loss of leaves *per se* does not kill clover (Brock *et al.,* 2003) but the temperature of the soil surface can. The critical temperature at the soil surface for stolon death in environments with low soil moisture is 30°C (Watson *et al.,* 1996).

The purpose of using white clover as a companion species in a pasture is its ability to fix atmospheric N into a plant available form for the use of non-legume species within the sward. Ledgard & Steele (1992) quantified biological N fixation from white clover in grazed pastures at between 55 and 296 kg N/ha/yr. These findings were supported by Hoglund *et al.* (1979), who reported N fixation per year from white cover ranging from 34 – 342 kg N/ha/yr. The amount of N fixed per year is mainly dependent on the amount of clover in the pasture and the N-level of the soil in environment that it is grown in, as soil N also regulates legume N fixation (Høgh-Jensen & Schjørring, 1997).

White clover has been shown to be a superior facilitator of ryegrass growth, compared to red clover. This is hypothesised that perhaps it can transfer N to grass better or because it is less competitive for light (Ergon *et al.,* 2016). Gierus *et al.* (2012) supported this statement, finding that at the University of Kiel in Germany, perennial ryegrass yields were higher when paired with white clover in a mixture (441 g/m^2) than in a perennial-ryegrass-red clover mixture (318 g/m^2).

In terms of managing clover content by manipulating sward grazing severity and frequency, grazing frequency is the more important aspect of defoliation controlling white clover growth (Brock *et al.,* 2003). This is due to the leaf placement of white clover high in the sward canopy in order to be able to intercept light. Defoliation is therefore usually severe, as the fully expanded, elevated leaves are removed relatively early in the grazing process. Therefore, it is important the clover plant receives sufficient time between grazing events to regrow their leaves and restore nutrient reserves before further defoliation caused by the next grazing.

2.3.4 Red clover

Red clover is commonly used as a high DM producing companion legume in crops predominantly used for finishing young stock. It is genetically different from white clover and as a result has different physical traits. Red clover's growing point is from a crown above ground and has a more upright growth habit. It has larger trifoliate leaves than white clover and possess a taproot; as opposed to the fibrous rooting system of white clover.

Red clover is more productive than plantain and white clover during summer in areas that experience drought stress. This is due to the presence of a taproot, allowing greater access to water when dry conditions bring water stress to swards (Kemp *et al.,* 2002). 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.

Red clover has a strong yield in the establishment season, producing 12 t DM/ha in one example under irrigation (Brown *et al.,* 2005), and 11-15 t DM/ha in a summer safe environment (Hyslop, 1999). In corresponding seasons productivity of red clover declines and monocultures are easily succeeded by weed growth, as the open ground between crowns promotes growth of unsown species. After four growing seasons, Brown *et al.* (2005) showed red clover had been completely succeeded by weeds (mostly voluntary white clover) under an irrigated environment.

As a result of the ease of establishment of unsown species, the persistence of red clover within pasture is low. Red clover proportions decreased by 80% when grown with chicory (*Chicorium intybus*) after 2 years (Sanderson *et al.*, 2005), and populations in monocultures decreased by 40-60% by the third year of growth. However, there was a significantly greater percentage of parent plants surviving in the lax than the hard-grazing intensity (Hyslop *et al.,* 1999).

Though not quite as effective as white clover at producing plant available N (Gierus *et al.,* 2012), red clover is still a useful legume for N fixation. Goh & Bruce (2005) showed a red clover mixed species pasture (MSP) produced 26 kg/t legume DM in a dryland situation, which was a 4.7 kg N/ha/year contribution as a proportion of total DM yield, when clover was 11.3% of seed by weight (4 kg seed/ha) in the 35.2 kg/ha mix.

2.4 Botanical composition and evenness

Plant species diversity refers to the number of species and their relative abundance in a defined area. Diversity measurements incorporate both species richness (the number of plant species in a community) and species relative abundance (an estimate of species distribution within a community). A community is perfectly “even” if all the species in the community have an equal number of individuals and are all the same size (Sanderson *et al.*, 2004). The strength of an interaction between species may depend on the relative abundances of the species involved.

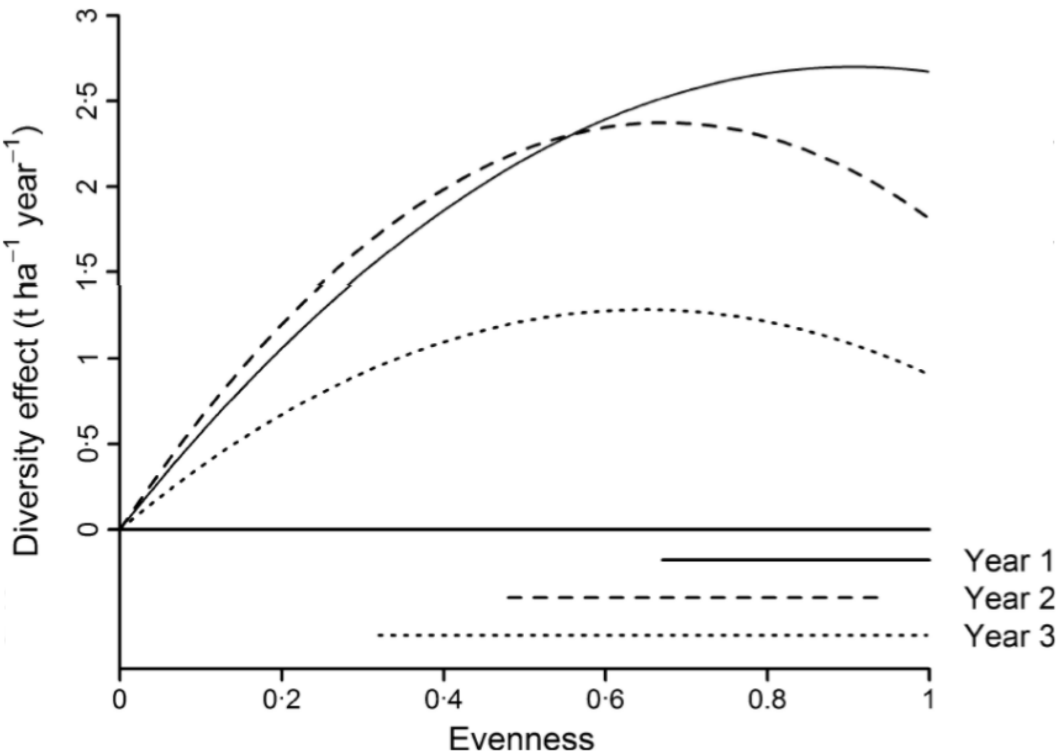

Figure 2.2. Predicted diversity effect (for annual total yield) from quadratic evenness model over 3 years. Horizontal lines below the regression curves indicate the range of evenness over which the diversity effect is not significantly (P > 0.05) smaller than the maximum diversity effect. From Finn *et al.* (2013).

Evenness of the species within a mixture has an effect on the diversity interaction between species and the overall yield of the pasture. For example, Finn *et al.* (2013) described a large-scale study where four species (perennial ryegrass, cocksfoot, white clover, and red clover) were included pasture mixtures sown at different relative proportions in the mixture across 30 European sites and one Canadian site. The mixtures were manipulated by varying seeding rates of the four species at sowing and resulted in four planned levels of evenness in the design. As evenness of the species increased, the diversity effect also increased as the four species reached an even population (Figure 2.1). The strong species interactions show temporal stability and robustness over species proportions. The benefit of the diversity effect can be achieved despite a considerable range of fluctuation in the relative proportions of the four species, for example, the diversity effect in communities dominated (70%) by one species is comparable to that of the most even community.

The effect of relative abundance of species has a consistent effect on species diversity effects over time as consistent effects are recorded over three growing seasons. The diversity effect occurring as transgressive overyielding (yield is significantly higher than the best performing monoculture) was attributed to the greater spatial and temporal complementarity of the chosen species, maximising complementarity and interspecific interactions to improve resource utilization and yield of above-ground biomass.

2.5 Competition and succession in pastures

Competition between species within pastures for resources is dependent on the growth habit and persistence of the individual species, as well as management of the pasture. Species with erect growing leaves such as ryegrass, plantain, and to a lesser extent, red clover will be able to get access to light more effectively in a lax grazing system than white clover, which has a prostrate growth habit. Species with similar characteristics and genotypes will also have greater competition between one another as they compete for the same ecological niche, and have very similar growth periods.

The effect of species diversity on weed suppression was very marked in Frankow-Lindberg *et al.* (2009) and persisted through time. There was a significant species diversity effect each year, and the predicted level of unsown species of the even mix was <2 % even in the third year. Positive monoculture effects observed were the ability of cocksfoot and white clover to resist invasions by unsown species (<6%) compared to ryegrass (41%) and red clover (60%). These findings suggest that some pastoral species are more easily out-competed and succeeded by unsown species than others; affecting their perennial ability in a commercial pasture.

2.6 Addition of legumes

The inclusion of legumes, such as white clover and red clover, into a sward have the benefit of both their own respective yield and their ability to fix atmospheric N for its own use and the use by other species within the sward. White clover can yield 7-10 t DM/ha/year in a monoculture and red clover 11-15 t DM/ha/year (Kemp *et al.,* 2010). These species are also able to fix 26-34 kg N/t of legume DM (Goh & Bruce, 2005). This is equivalent to around 65 kg of urea/t of legume DM.

Over-yielding mixtures perform better than the average yield of the component species grown as monocultures. Transgressive over-yielding occurs when the yield of a mixture community exceeds that of the highest-yielding monoculture of its component species. Frankow-Lindberg *et al.* (2009) found transgressive over-yielding was observed in the second and the third years for mixtures using all legume populations in a European agro-diversity trial consisting of mixtures of perennial ryegrass, cocksfoot, red clover and white clover. There was a strong and persistent species diversity effect irrespective of the legume population used. The evenness effects of the mixtures always gave a positive species diversity effect that remained strong over the 3 years of the trial, as increased evenness has been shown to increase diversity effect (Harris, 1968) (Figure 2.1).

In a centroid mixture, the average contribution to yield of the species diversity effect were 3.55, 4.89 and 3.77 t DM/ha, a 33%, 55% and 65% yield increase over the mean of the monoculture plots in the first, second and third years of the agro-diversity trial respectively. The species diversity effect based on between-species interactions was always positive, which showed the characteristics of the species within each functional group were either of a complementary or a facilitating nature. The sustained species diversity effect and the declining monoculture effects suggest that the importance of this diversity contribution to yield increased with time. This was reflected in the increasing evidence of transgressive overyielding for all legume populations as time advanced (Frankow-Lindberg *et al.,* 2009).

2.7 Leaf area index

The main driver of pasture production is the ability of the sward to effectively collect incident light from the sun and convert it to glucose through photosynthesis. Increased energy produced leads to increased growth and as a result higher yields. The leaf area of pasture is important in determining the effective use of available sunlight by a sward. Because different pasture species have different leaf characteristics, and leaves at different heights within a sward, their ability to efficiently collect photosynthetically active radiation (PAR) may be different.

These sward attributes will also change when species are in a mixture compared to when they are grown as monocultures. Non-legume species such as ryegrass mixed with a legume will have the ability to grow bigger leaves with more available N. introducing another species to the sward introduces another leaf type - the broad, low-aspect leaves of the clover further down the canopy catches light missed by the tall narrow leaves of the ryegrass.

Canopy leaf area is quantified as LAI, which is the green leaf area per unit area of ground (Rattray *et al.,* 2007). This is the general measure of how much cover a sward has to intercept incident sunlight. As individual leaves do not form together to form an even blanket, there are gaps in the canopy. Therefore 100% light interception is usually unobtainable. For this reason, 95% photosynthetically active radiation (PAR) interception is defined as the critical LAI (Figure 2.3). Brougham (1957), investigated the relationship between leaf area and light interception in regrowth of pure and mixture swards of pasture species. Brougham (1956), showed the regrowth of a pasture of short-rotation ryegrass (*Lolium hybridium*)-red clover-white clover it was shown that a LAI of approximately 5 m^2 leaf/m^2 ground was necessary to intercept 95% of the incident light. Thereafter growth was at a

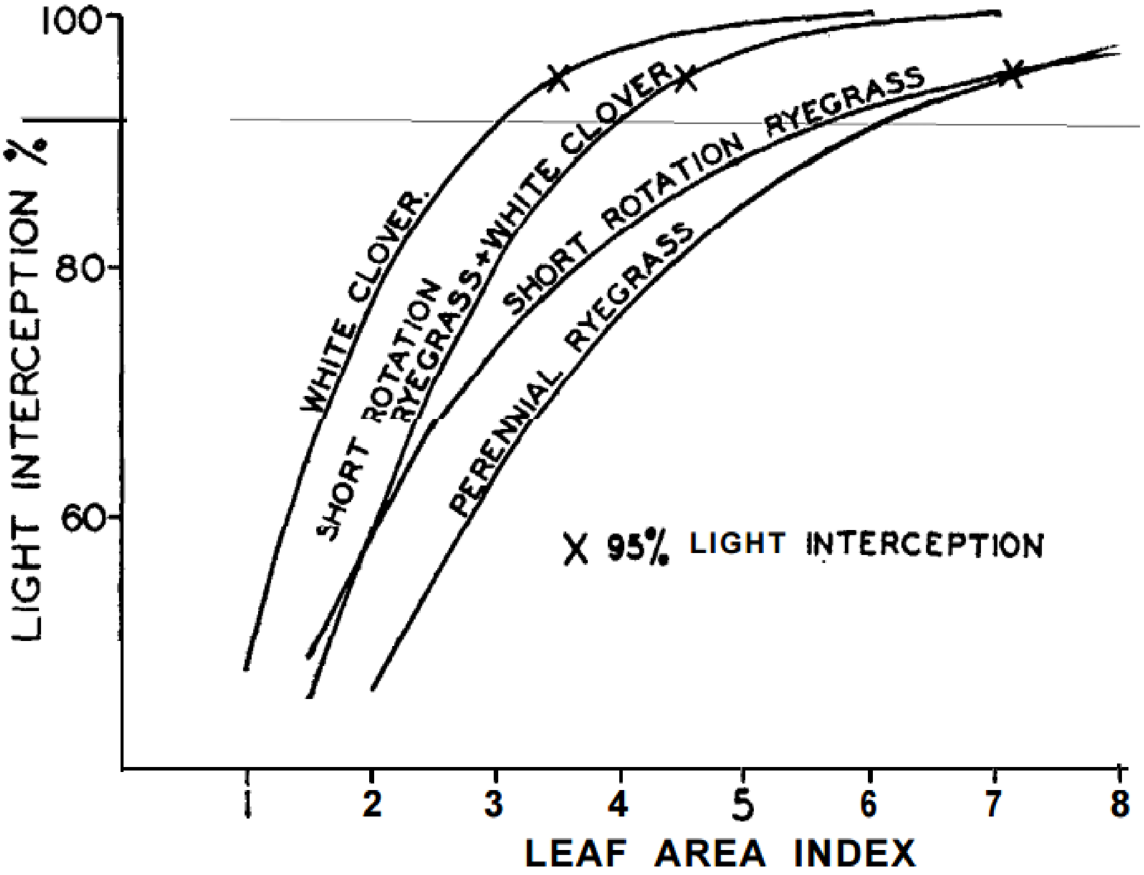

Figure 2.3. The relationship between light interception and leaf area index for four swards. The 'x' on the lines represent critical leaf area for 95% photosynthetically active radiation interception. From Brougham (1957).

maximum rate. It was suggested that over 95% light interception resulted in maximum photosynthetic activity and hence maximum rate of growth. For a mixed sward of perennial ryegrass and white clover the critical LAI is 3.5-4.5 (Joggi *et al.,* 1983; Rattray *et al.,* 2007). In comparison, a pure perennial ryegrass sward will have a critical LAI of 6-7. White clover has a critical LAI of 3.5 and the critical LAI for red clover has been reported as 3.3 (Joggi *et al.,* 1983). Plantain is a relatively new species to pastoral examination and therefore no literature was available generating a general leaf area for plantain. Due to the wide and prostrate leaf orientation of plantain it can be hypothesised that critical LAI would be more similar to that of the clovers than of the narrow-leaved ryegrass species. Differences in critical LAI 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 per unit of green leaf than ryegrass.

Anderson (2015) showed that in the 2015 year of growth pastures described in this dissertation, that LAI for the monocultures was 2.17 for perennial ryegrass, 2.43 for plantain, and was below 0.40 for white and red clovers. The LAI of mixtures reflected the monoculture LAI of the non-legumes, with perennial ryegrass and plantain providing the majority of the LAI of the mixtures. The LAI over all the pasture mixtures (and monocultures) was well below critical LAI reported in Joggi *et al.* (1983) and Rattray *et al.* (2007). Anderson (2015) suggested that at this stage in development that the plant population were not limited by light, suggesting the swards needed to undergo a longer establishment phase before reaching critical LAI.

Leaf area index can be measured directly or indirectly, and both methods are used in this experiment to gauge the accuracy of indirect methods. 'Direct' LAI is measured on a sub-sample of leaves and related to dry mass (e.g. via specific leaf area, SLA, cm^2/g). Finally, the total dry mass of green leaves collected within a known ground-surface area is converted into LAI by multiplying by the SLA. As the direct methods only relate to foliage, they are the only ones giving real access to LAI. They allow separate computation of the shape, size and number of leaves. Direct methods provide the reference for the calibration or evaluation of indirect methods. It is crucial to sample leaves correctly for establishing leaf area to dry mass ratio, as it changes among species and among sites for a given species.

Harvesting the vegetation and measuring the area of all the leaves within a delimited area is the first method, widely used for crops and pastures. This method is well adapted for vegetation of small structure, but is destructive. An indirect method of measuring LAI is through a SunScan canopy analysis system. The instrument can give LAI values by measuring the ratio of transmitted radiation through canopy to incident radiation. This technique has not been trialled in traditional ryegrass and white clover pastures and no existing literature exists on the accuracy of this method.

2.8 Conclusion

- There is evidence suggesting the benefit of combining one species with another within a pasture to increase yields. There is less knowledge, however, quantifying the benefit of having three- or four-species mixtures and the potential diversity effect of combining more species within a pasture mix. To do this data will have to be modelled to enable a 3D design to explain yield differences by quantified diversity effects.

- Each species used within the mixture experiment has differing traits in terms of annual and seasonal yields, genetic and phenotypic variances, and levels of persistence. Sowing a mixture experiment incorporating these species in all combinations, with one overlying plant population density, will enable insight into how these species interact in an even plant population, and when one species is grown dominant to the other three remaining species.

- LAI is the main driver of plant production; despite this there is little evidence portraying a difference in yield explained by a difference in LAI of a pasture. Differing yields explained by different LAI's will underline the importance of a pasture reaching critical LAI to enable maximum growth rates, and therefore higher yields. Therefore, in this study LAI was measured at two autumn harvests of the 2016/17 growing season to help explain species effects and interactions among the mixtures.

- N concentration is different between different pasture species. Differing N concentration between mixtures may be a factor in explain differing yields between the mixtures dependant on the species used as components within them. Therefore, in this study N content of the herbage was estimated to help understand the species identity effects and species interactions on DM production.

Chapter 3

Materials and methods

3.1 Experimental design

Four common commercial species were chosen: tetraploid perennial ryegrass, plantain, white clover and red clover. A single commercial cultivar was selected to represent each species within the experiment: ‘Base’ perennial ryegrass, ‘Tonic’ plantain, ‘Apex’ white clover and ‘Sensation’ red clover. Their basic charateristics are described in Stewart *et al.* (2014), indicating their suitability to establish and produce a high quality pasture under irrigated conditions in mid-Canterbury. The 19 blends were randomly assigned to plots within each of the four replicates according to a randomized complete block design, creating 76 plots in total. The plot size was 2.1 m wide by 6 m long.

The 19 mixtures were designed in a simplex centroid design format (Cornell, 2002), visualised as a tetrahedron (Figure 3.1) and created using the Minitab 16 statistical software package. Each point represented a seed mixture, with its position determined by the species proportions. The number of seeds per unit area determined species proportions. The species were sown in total of 19 pasture mixes, which included each species sown as a monoculture, six two-species mixes (0.5, 0.5), four three-species (0.33, 0.33, 0.33), one mixture with all four species sown equally (025, 0.25, 0.25, 0.25), and four different four-species mixes sown with one of the species sown dominant, while the other three species in the mix remain equal (0.625, 0.125, 0.125, 0.125) in terms of seed number.

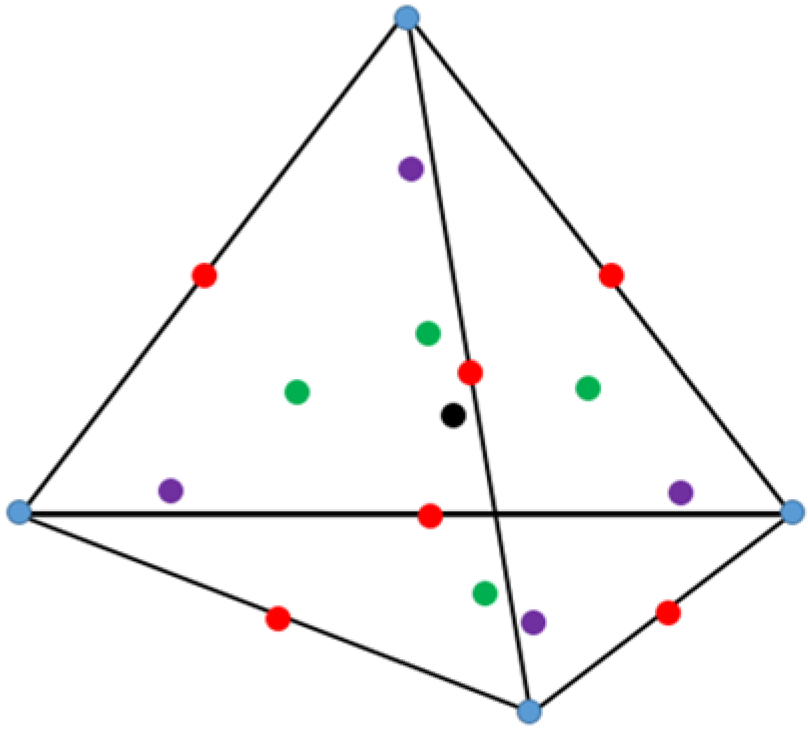

Figure 3.1. Tetrahedron of 19 pasture mixtures, containing ryegrass, plantain, white clover and red clover developed by the simplex centroid model design. The circles represent each of the 19 mixtures; the four monocultures (blue circles), six binary mixtures (red circles), four tertiary mixtures (green circles), one centroid mixture (black circle) and the four quaternary mixtures each dominated by one species (purple circles). Adapted from Dalgety (2016).

3.2 Sowing

The species proportions in the seed mixtures were based on seed number per unit area. The total sowing rate was fixed at 833 seeds per m^2. This was equivalent to 30 kg/ha of 'Base' perennial ryegrass. The average thousand seed weights (TSW) were 3.6, 2.7, 0.9, and 2.1 g and average germination percentages were 93, 99, 91 and 88% for ryegrass, plantain, white clover and red clover, respectively. The different TSW of each species meant that the weight of seed sown per plot was different across each of the mixtures to keep the seeds per unit area constant (Table 3.1). White clover seed had AGRICOTE treatment (a lime-based coating with nutrients, insecticide and fungicide). The other species were not treated. Sowing rates were unadjusted for germination percentage.

Table 3.1. Seed rate (kg/ha) of each of the component species (ryegrass, plantain, white clover and red clover) sown in 19 pasture mixtures under irrigation at Lincoln University in mid-Canterbury on the 26th of March 2015.

Mixture	Mix	Ryegrass	Plantain	White Clover (kg/ha)	Red Clover	Total
Monoculture	1	30.0	0	0	0	30.0
Monoculture	2	0	22.5	0	0	22.5
Monoculture	3	0	0	7.5	0	7.5
Monoculture	4	0	0	0	17.5	17.5
Binary	5	15.0	11.25	0	0	26.25
Binary	6	15.0	0	3.75	0	18.75
Binary	7	15.0	0	0	8.75	23.75
Binary	8	0	11.25	3.75	0	15.0
Binary	9	0	11.25	0	8.75	20.0
Binary	10	0	0	3.75	8.75	12.5
Ternary	11	10.0	7.5	2.5	0	20.0
Ternary	12	10.0	7.5	0	5.83	23.3
Ternary	13	10.0	0	2.5	5.83	18.3
Ternary	14	0	7.5	2.5	5.83	15.8
Quaternary	15	7.5	5.63	1.88	4.38	19.4
Quaternary	16	18.75	2.81	0.94	2.19	24.7
Quaternary	17	3.75	14.06	0.94	2.19	20.9
Quaternary	18	3.75	2.81	4.69	2.19	13.4
Quaternary	19	3.75	2.81	0.94	10.94	18.4

The mixtures were compared at the Horticultural Research Area (Paddock H8) at Lincoln University, Canterbury, New Zealand (43°38’53.14”S 172°27’11.60”E, 10 m above sea level). The soil was a Templeton silt loam, with stone-less topsoil (Cox, 1978), no significant rooting barrier within 1 m depth, and was moderately well drained.

The paddock (H8) was used for lucerne in 2011 and 2012, oilseed rape (*Brassica napus*) in 2013 and oats (*Avena sativa*) in 2014. The paddock was cultivated in November 2014, sprayed with herbicide (570 g glyphosate/L at 3 L/ha as RoundUp®) in February 2015, irrigated (60 mm) and cultivated into a seedbed in March 2015. The paddock was drilled on the 26th of March 2015 with a Flexiseeder precision drill with 14 coulters spaced 0.15 m apart, creating 2.1 x 6 m plots in a randomised complete block design with four replicates. The seeds were sown at a depth of 10-15 mm.

Plate 3.1. The experiment site (Paddock H8) on the 30/03/2017 prior to the March harvest.

A soil test was taken on the 4th of May from the trial area. The soil test results showed the pH to be 5.7, Olsen P 13 mg/L, Mg and Na at 0.84 and 0.17 mg/100 g respectively, and Sulphate – S at 12 mg/kg. T-Max^{TM} (30 g/L aminopyralid at 2 L/ha) was used to remove volunteer white clover from the ryegrass plots on 10 March 2017 and the plantain and ryegrass-plantain plots on 5 May 2017. Dew^{TM} 600 (600 g/L diazinon at 4 L/ha) was sprayed for grass grub (*Costelytra zealandica*) on 10 May 2017.

To compensate for evapotranspiration and potential moisture stress the plots were irrigated in periods of low rainfall. Irrigation occurred every 3-5 weeks from 30 October 2015 to 6 April 2016 (total, 360 mm) and from 9 November 2016 to 15 February 2017 (total, 260 mm) to maintain soil moisture above a critical limit of 24% (Black & Murdoch, 2013).

The first defoliation of the plots was on the 16th August 2015, cut by mower to a residual height of 40-50 mm, and excluding animals from the defoliation process to reduce plant damage. In subsequent harvests (Table 3.2) the plots were managed with sheep; grazing the site for 3-7 days. The plots were then mowed to a uniform height of 40-50 mm before the start of the next regrowth period.

Table 3.2. Mixture experiment defoliation dates and frequencies over the first two years of growth for mixtures containing ryegrass, plantain, white clover and red clover grown under irrigated conditions at Lincoln University in mid-Canterbury.

	Harvest	Date Sampled	Date Grazed	Days Harvest	Date Mowed	Days Growth
Year 1	1	4/08/2015	*	*	16/08/2015	131
	2	21/09/2015	22/09/2015	3	25/09/2015	37
	3	23/10/2015	27/10/2015	3	30/10/2015	32
	4	30/11/2015	1/12/2015	2	3/12/2015	32
	5	6/01/2016	7/01/2016	4	11/01/2016	35
	6	15/02/2016	16/02/2016	6	22/02/2016	36
	7	31/03/2016	1/04/2016	4	5/04/2016	39
	8	23/05/2016	27/05/2016	3	30/05/2016	52
Year 2	1	2/08/2016	2/08/2016	3	5/08/2016	64
	2	22/09/2016	26/09/2016	4	30/09/2016	52
	3	31/10/2016	1/11/2016	6	7/11/2016	32
	4	9/12/2016	9/12/2016	4	13/12/2016	32
	5	13/01/2017	16/01/2017	3	19/01/2017	34
	6	17/02/2017	17/02/2017	4	21/02/2017	29
	7	30/03/2017	31/03/2017	7	7/04/2017	38
	8	26/05/2017	26/05/2017	4	30/05/2017	49

3.3 Measurements

Before each plot harvest, one 0.3 m^2 quadrat (1 m of two adjacent drill rows) was clipped to 10-20 mm for each plot. A subsample of clippings was separated into the four sown species and weeds, and dried with the rest of the clippings in a forced air oven at 70°C for 48 h. The dry weights were used to calculate the herbage mass of the sown species. After each grazing, a 0.2 m^2 quadrat of residual herbage per monoculture plot and centroid plot was clipped to 10-20 mm, the clippings were dried (as above) and the dry weights were used to calculate the residual herbage mass for each plot. Yield was calculated as the pre-harvest herbage mass less the previous residual herbage mass for each of the eight harvests in Year 2 of the experiment, which covered dry matter production from 30th May 2016 to 26th May 2017. The yields were summed across the eight harvests in the growing season to calculate the total annual yield.

Botanical composition of each of the 19 mixtures was measured from the sorted sub-sample of material. The material from each of the four species was separated and dried (as above) before being weighed as individual species. The botanical composition of the sample could then be calculated from the relative dry weights of each species relative to one another.

To help explain any differences in yield between the four pasture species and their mixtures, LAI and light interception were estimated as well as yield and botanical composition at two harvests in autumn 2017 (30^{th} of March and 26^{th} of May). Leaf area index and light interception were estimated in each plot indirectly using a SunScan canopy analysis system (Delta-T Devices, Cambridge, England). This measurement technique was compared to direct LAI estimation to enable comment on the accuracy and reliability of indirect canopy analysis on forage pastures.

Directly measuring LAI was done by taking 20 samples of true leaves from one replicate of each of the monocultures and from each species in the centroid four-species mix. The 160 leaves were then photographed against a scale and individually numbered and bagged before being dried (as above). The individual leaves were then weighed to obtain a specific dry-weight. The photographed individual leaves were then digitally analysed for their green leaf areas using the 'Digimizer' computer program. Individual leaves had a specific weight and area assigned to them. From this information the specific leaf area (SLA) was calculated for each leaf. Each SLA was averaged for each of the monocultures and each species in the central sigmoid four-species mix to give eight SLA values. These SLA values were then converted to direct LAI using the dry-weight of green leaf per plot taken during the sample separation.

Measuring LAI using a SunScan canopy analysis system gave instant estimated LAI and light interception information. The instrument gave LAI values by measuring the ratio of transmitted radiation through canopy to incident radiation. Two readings in each plot were taken and averaged to give an indirect LAI for each of the 76 replicates.

The two methods of calculating LAI were then compared against one another with a correlation analysis to determine the strength of the relationship between the two calculated values for each plot. The LAI values were then modelled within the mixture analysis to determine significant differences in LAI dependant on species within the mixture and also plotted in a regression against harvest yield.

Light interception data was also taken from each of the replicates as an averaged value of two samples. This interception value was divided by 95%, the amount of interception required for critical LAI, to give a relative interception value, I/Io.

3.4 Statistical analysis

The dry matter yields of each sown species, total herbage grown, and weeds over eight harvests were summed and used to calculate total and seasonal dry matter yield, and total and seasonal botanical composition for the second year of growth. Interactions between species, and number of species present in the mixture affecting yield were tested using the mixture regression method in Minitab 17. A special cubic mixture model was fitted to the data as follows:

$$\hat{Y} = \beta_1 x_1 + \beta_2 x_2 + \beta_3 x_3 + \beta_4 x_4 + \beta_{12} x_1 x_2 + \beta_{13} x_1 x_3 + \beta_{14} x_1 x_4 + \beta_{23} x_2 x_3 + \beta_{24} x_2 x_4 + \beta_{34} x_3 x_4 + \beta_{123} x_1 x_2 x_3 + \beta_{124} x_1 x_2 x_4 + \beta_{134} x_1 x_3 x_4 + \beta_{234} x_2 x_3 x_4 + \beta_{1234} x_1 x_2 x_3 x_4 + \varepsilon$$

(Model 1)

$\hat{Y}$ symbolizes the predicted response from a mixture, i.e. total yield, LAI and N% of the pasture. The β (beta) symbolizes the coefficients to be fitted via regression. x_1, x_2, x_3, and x_4 are the sown proportions of perennial ryegrass, plantain, white clover and red clover respectively. β_1 to β_4 are estimates of the response of the monocultures. β_{12} to β_{34} represent the interaction effects for each of the six two species mixtures, β_{123} to β_{234} are interaction effects for each of the four three-species mixtures, and β_{1234} is the interaction effect for the four species even mixture. This equates to 15 terms for the 15 mixes used in the modelling analysis. The ε is a random error term, assumed normally and independently distributed with a mean of zero and constant variance.

The highly non-significant terms were removed from the model and significant terms in the model were reanalysed and included in a new simplified model that was used to generate a contour plot and optimisation plot. This optimal mix was used to compare against modelled seasonal growth of the monocultures to predict the increased growing season and yield of mixed pastures. Annual botanical composition was calculated using sown proportions only, as weeds were controlled during the course of the experiment. The seasonal botanical composition of the highest yielding modelled pasture mix was visualised over time using SigmaPlot13 Ternary Plot, additional to the 8 harvests was the composition of the mixture as it was sown and the optimal composition predicted to maximise yield from the optimisation plot.

Directly measured LAI values were calculated from a derived SLA value and multiplied by the dry-weight of green leaf material of each species in the sample. A one-way ANOVA was conducted between monoculture and mixture SLA values to determine if there was a difference present. Where a significant difference was found, the SLA from the mixture sample was used to calculate LAI for that species; otherwise the monoculture species SLA was used across all samples. The LAI's of both methods were correlated against each other, and mixture LAI modelled using the mixture regression method in Minitab 17.

A linear regression was undertaken using the N concentration (%) of the different mixtures to determine the relationship between the concentration of N in the sward and annual yield. This technique is repeated for the leaf area index data to determine the relationship between leaf area and seasonal yield of the autumn harvests (Harvest 7 and 8). These regression figures produced a linear relationship and an R^2 value quantifying the strength of the relationship.

3.5 Climate

The total rainfall and mean air temperature at the site are shown in the figures below. The climate data were acquired from the Broadfields weather station, near Lincoln (approximately 1.9 km from the site). The Lincoln, Broadfields, network number 17603 H32645, is located at latitude -43.62622 and longitude 172.4704 at 18 metres above sea level. The observing authority was NIWA.

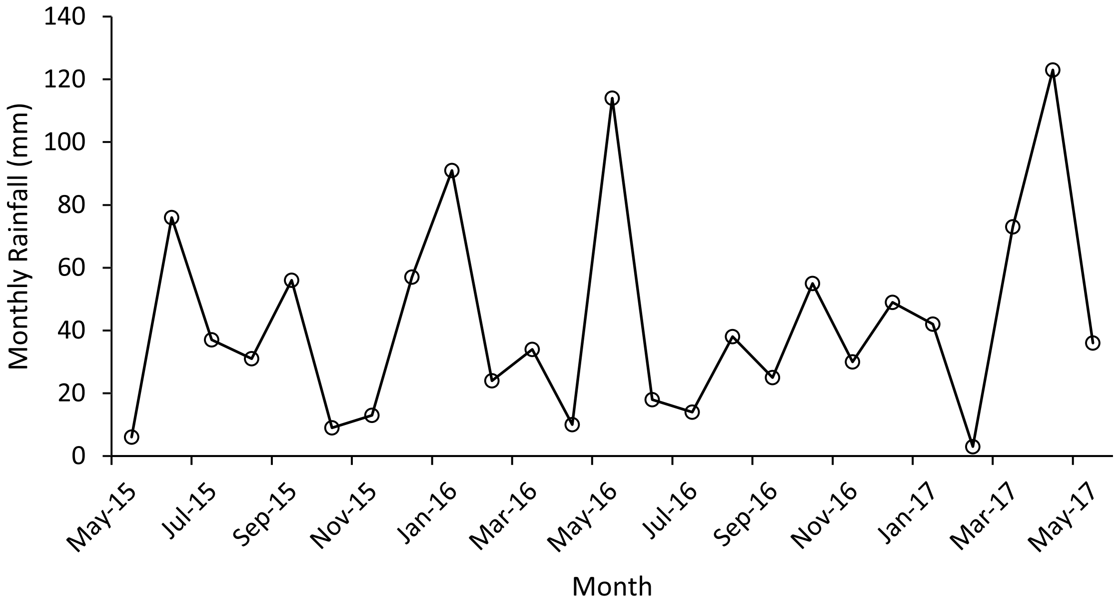

Figure 3.1. Monthly rainfall from May 2015 to May 2017 onto irrigated pasture mixtures experiment in mid-Canterbury. Data obtained from Broadfields weather station, near Lincoln.

The rainfall for May 2015 to May 2017 (Figure 3.1) showed monthly and seasonal variation, from a minimum monthly rainfall of 3 mm in February 2017 to a maximum monthly rainfall of 123 mm in April 2017. The mean monthly air also showed monthly and seasonal variation (Figure 3.2), ranging from a maximum mean daily air temperature of 19.2 °C (February 2016) to a minimum of 6.7 °C (July 2015).

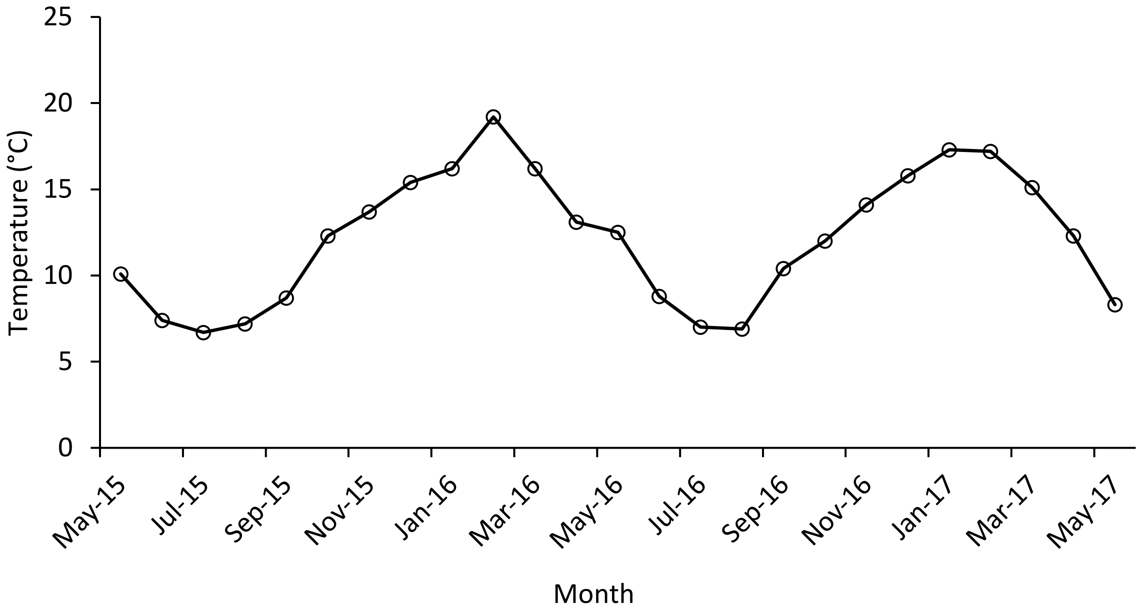

Figure 3.2. Monthly mean temperature covering the first two full growing seasons (May 2015 to May 2017) of the pasture mixtures containing ryegrass, plantain, white clover and red clover grown under irrigated conditions in mid-Canterbury. Data obtained from Broadfields weather station, near Lincoln.

Chapter 4

Results

4.1 Annual dry matter yield analysis

Analysing the annual DM yield of the 19 pasture mixes showed that there are differences in yield of the mixtures dependant on which species were present within the mixture. The monoculture species (RG, P, WC, RC) ranged in observed annual yield from 8.7 - 13.0 t DM/ha/year, the binary mixtures 8.9 – 15.4 t DM/ha/year, ternary mixtures 11.5 – 14.2 t DM/ha/year and the five four-species mixtures 13.1 – 16.6 t DM/ha/year (Table 4.1).

Table 4.1. Observed mean ± standard error annual yields for Year 2 (30th May 2016 to 26th May 2017) in response to seed mixtures of perennial ryegrass, plantain, red clover, and white clover sown on the 26th of March 2015 under irrigated conditions at Lincoln University.

Mixture	Mix	Proportion				Annual Yield (t DM/ha/year)
		Ryegrass	Plantain	White Clover	Red Clover	
Monoculture	1	1	0	0	0	10.2 ± 0.385
Monoculture	2	0	1	0	0	8.7 ± 1.124
Monoculture	3	0	0	1	0	11.3 ± 0.297
Monoculture	4	0	0	0	1	13.0 ± 1.129
Binary	5	0.5	0.5	0	0	8.9 ± 0.691
Binary	6	0.5	0	0.5	0	11.2 ± 0.464
Binary	7	0.5	0	0	0.5	15.4 ± 0.826
Binary	8	0	0.5	0.5	0	11.2 ± 0.337
Binary	9	0	0.5	0	0.5	14.5 ± 0.370
Binary	10	0	0	0.5	0.5	13.4 ± 0.590
Ternary	11	0.33	0.33	0.33	0	11.5 ± 1.077
Ternary	12	0.33	0.33	0	0.33	15.2 ± 0.785
Ternary	13	0.33	0	0.33	0.33	13.4 ± 0.303
Ternary	14	0	0.33	0.33	0.33	14.4 ± 0.889
Quaternary	15	0.25	0.25	0.25	0.25	14.1 ± 0.516
Quaternary	16	0.625	0.125	0.125	0.125	13.9 ± 0.564
Quaternary	17	0.125	0.625	0.125	0.125	13.9 ± 0.759
Quaternary	18	0.125	0.125	0.625	0.125	13.1 ± 0.847
Quaternary	19	0.125	0.125	0.125	0.625	16.6 ± 0.813

The annual yield between pasture mixes were modelled to analyse the significance of the yield differences. These differences were displayed as coefficients to the modelled yield; shown to be significant ($P < 0.05$) by highlighted and bolded coefficients. This analysis show that five two-species mixtures (RG*WC, RG*RC, P*WC, P*RC, and WC*RC) and one three-species mixture (RG*P*RC) yielded significantly more dry matter over the whole growing season than their constituent species as monocultures, and in the case of the 3-species mixture, more than the average of all possible 2-species combinations of its three components (Table 4.1).

There was a significant difference in the monoculture yields over the whole growing season as shown by the linear P value (P = 0.005), shown in the greater annual coefficient and modelled yield of red clover monocultures compared to the other three species. This significant result means there was a species effect within the experiment over the whole growing season. There was also an overall significant quadratic effect over the monocultures, indicating species interactions were present within the two-species swards (Table 4.2). This effect was not present for all two-species mixtures, however, as the RG*P mix coefficient showed no interaction against the average of their monoculture yields. The other five two-species mixtures (RG*WC, RG*RC, P*WC, P*RC, and WC*RC) had a significantly greater yield than the average of their components sown as monocultures. This interaction was also present in one three-species mixture (RG*P*RC) when reanalysed discounting highly insignificant terms, meaning that it yields significantly greater than its three two-species components (RG*P, RG*RC, and P*RC).

The analysis and reanalysis found that there was no added benefit of adding a fourth species to the mix as the special cubic analysis found the four-species mix (RG*P*WC*RC) was not significantly higher yielding than the average of the three-species mixes.

These coefficients were substituted into the special cubic mixture model fitted to the data to produce a modelled yield for each of the monocultures. These yields had the same error and significance as the coefficients so significant coefficients of mixtures resulted in significant modelled yields. The six significant mixtures showed higher modelled annual yields than the average of their components, ranging from 11.19-15.45 t DM/ha/year. These higher yields came from a diversity effect developed between species. This diversity effect was variable due the species effect of the monocultures. The diversity effect of the two species mixtures ranged from 2.19 t DM/ha/year in WC*RC mixtures to 5.31 t DM/ha/year in P*RC mixtures (Table 4.3). This was expected as two legume species would not be expected to show as such diversity effect as a legume and non-legume species would, regarding the availability of N.

Table 4.2. Special cubic analysis of the annual dry-matter yields of pasture mixtures containing ryegrass (RG), plantain (P), white clover (WC), and red clover (RC), in the second year of growth (30th May 2016 to 26th May 2017). Grown under irrigated conditions at Lincoln University. Shaded figures were reanalysed by removing highly insignificant terms from the model.

Mixture	Coefficient	Modelled Annual Yield (t DM/ha)	SE	P
RG	9.38	9.38	0.749	*
P	7.52	7.52	0.749	*
WC	9.09	9.09	0.749	*
RC	11.49	11.49	0.749	*
RG*P	-2.73	7.77	3.765	0.472
RG*WC	8.04	11.25	3.765	**0.037**
RG*RC	20.05	15.45	3.765	**0.000**
P*WC	11.55	11.19	3.765	**0.003**
P*RC	19.90	14.48	3.765	**0.000**
WC*RC	7.98	12.28	3.765	**0.038**
RG*P*WC	32.66	12.29	26.409	0.21
RG*P*RC	51.46	15.18	26.409	0.021
RG*WC*RC	-11.41	8.72	26.409	0.667
P*WC*RC	20.03	11.59	26.409	0.451
RG*P*WC*RC	109.05	16.19	256.327	0.672
Linear P value	0.005	0.005	0.005	0.005

Taking the coefficients and substituting them into the cubic mixture model for β (beta), gave an equation to predict yield of a mixture using the four component mixtures. The equation including all terms was as follows, where $\mathbf{x_1}$ = ryegrass (RG), $\mathbf{x_2}$= plantain (P), $\mathbf{x_3}$= white clover (WC), and $\mathbf{x_4}$= red clover (RC):

$$\textbf{Yield (t DM/ha)} = 9.38x_1 + 7.52x_2 + 9.09x_3 + 11.49x_4 - 2.27x_1x_2 + 8.04x_1x_3 + 20.05x_1x_4 + 11.55x_2x_3 + 19.90x_2x_4 + 7.98x_3x_4 + 32.66x_1x_2x_3 + 51.46x_1x_2x_4 -- 11.41x_1x_3x_4 + 20.03x_2x_3x_4 + 109.05x_1x_2x_3x_4 + \varepsilon \textbf{ (Model 2)}$$

This model is a reasonable fit for the data with an R^2 = 78.66% and adjusted R^2 = 72.40%. The large amount of terms included in the model include many insignificant differences in modelled yield, so a refined model using only statistically significant terms will be used to further analyse the data.

4.2 Yield optimization

The species included in the significant mixes indicated that there was a legume to non-legume interaction between the species. The significant two-species interactions for legumes and non-legumes indicated that the estimated yield of the given two-species mix was greater than the mean of the estimated monoculture yield for those species included in the mix. Included in the significant terms was the RG*P*RC mix which became significant when reanalysed, and showed a yield benefit of adding a third species to the mix, over the component two-species mixtures. This yield benefit or diversity effect was of differing size dependent on the species included within the mix. Re-analysing these significant mixtures and discounting non-significant terms gave a better model in terms of fit to the data by slightly changing the coefficients.

Table 4.3. Significant terms from initial analysis, used in the reanalysis, their modelled yield and diversity effect. These significant coefficients are used to generate a simplified model for the dataset. Species are perennial ryegrass (RG), plantain (P), white clover (WC) and red clover (RC).

Mix	Coefficient	Modelled Yield (t DM/ha)	Diversity Effect (t DM/ha)	P-value
RG	9.24	9.24	*	*
P	7.31	7.31	*	*
WC	8.98	8.98	*	*
RC	11.45	11.45	*	*
RG*WC	9.54	11.49	2.39	0.004
RG*RC	19.72	15.28	4.93	0.000
P*WC	14.73	13.06	3.68	0.000
P*RC	21.24	14.69	5.31	0.000
WC*RC	8.77	12.41	2.19	0.008
RG*P*RC	53.39	15.27	1.98	0.021

Using these significant coefficients in the mixture model simplified the equation used, and discounted any insignificant coefficients as they had no significant effects on modelled yield. The resulting equation derived from the significant coefficients where $\mathbf{x_1}$ = ryegrass (RG), $\mathbf{x_2}$= plantain (P), $\mathbf{x_3}$= white clover (WC), and $\mathbf{x_4}$= red clover (RC) is;

$$\textbf{Yield (t DM/ha)} = 9.24x_1 + 7.31x_2 + 8.98x_3 + 11.45x_4 + 9.54x_1x_3 + 19.72x_1x_4 + 14.73x_2x_3 + 21.24x_2x_4 + 8.77x_3x_4 + 53.39x_1x_2x_4 + \varepsilon \quad \textbf{(Model 3)}$$

This model was a good fit to the data with an R^2 = 77.35% and adjusted R^2 = 73.03%. This model was used to further analyse the annual yields of the pasture mixes using contour plots and an optimization analysis. The use of a model to predict theoretical yields can create unreliability of the results of the study; but the ability the model gives to predict optimal yields, the exact seed proportions that created these yields and the comparisons that were easily drawn from modelled data was extremely useful.

The model was of good fit to the data ($R^2 > 70\%$) and modelled yields were shown to be conservative when compared to the actual annual yields from the four monocultures and 11 pasture mixes. The modelled yields were also accurate to the actual yields for the significant mixtures, whose terms were used for further analysis of the data to determine maximum potential yield for the second year of growth, the corresponding optimal seed mix, and the seasonal pattern of growth for the optimal three-species mix compared to its component monocultures.

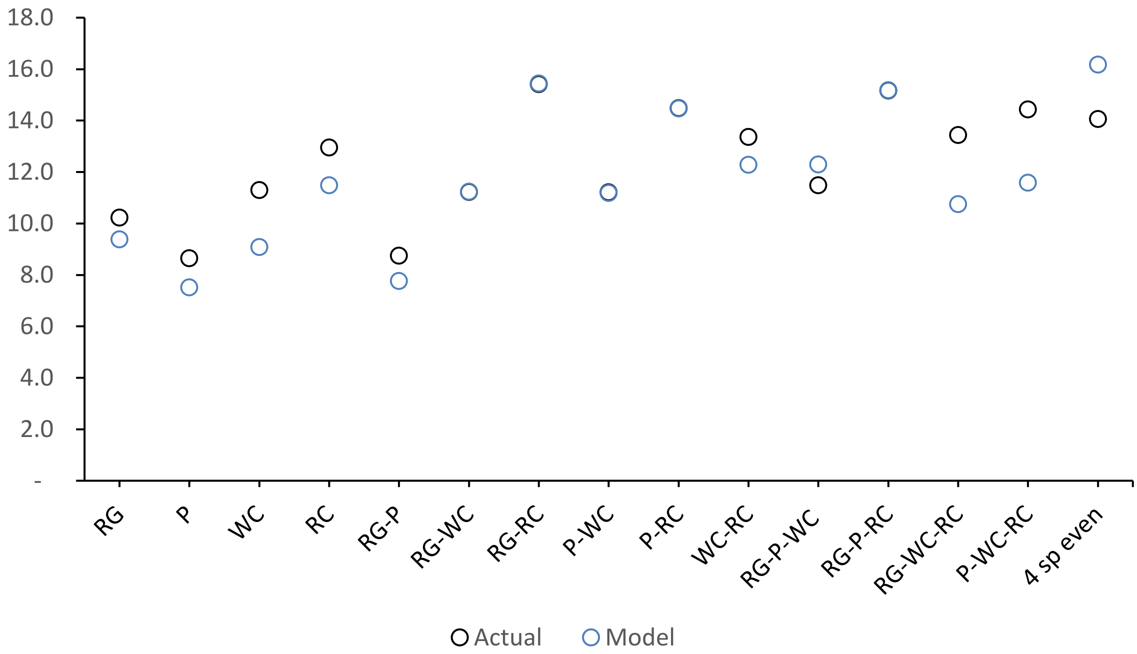

Figure 4.1. Modelled yield (t DM/ha/year) of 15 pasture mixtures against actual annual yields for the second year of growth (30th May 2016 to 26th May 2017). Species contained within the mixtures were perennial ryegrass (RG), plantain (P), white clover (WC) and red clover (RC).

The contour plot showed the effect on annual yield when different proportions of each species were present within the pasture mix (Figure 4.2). The light-coloured area of the model shown where the proportion of plantain is close to 1, indicates a low yield of < 8.0 t DM/ha/year. The dark area of the model indicated the highest yields will result from a mix of ryegrass, plantain, and red clover, with a greater proportion of red clover as the darkest area was nearer to the RC corner of the plot. The area was modelled to produce more than 16 t DM/ha for the second year of growth.

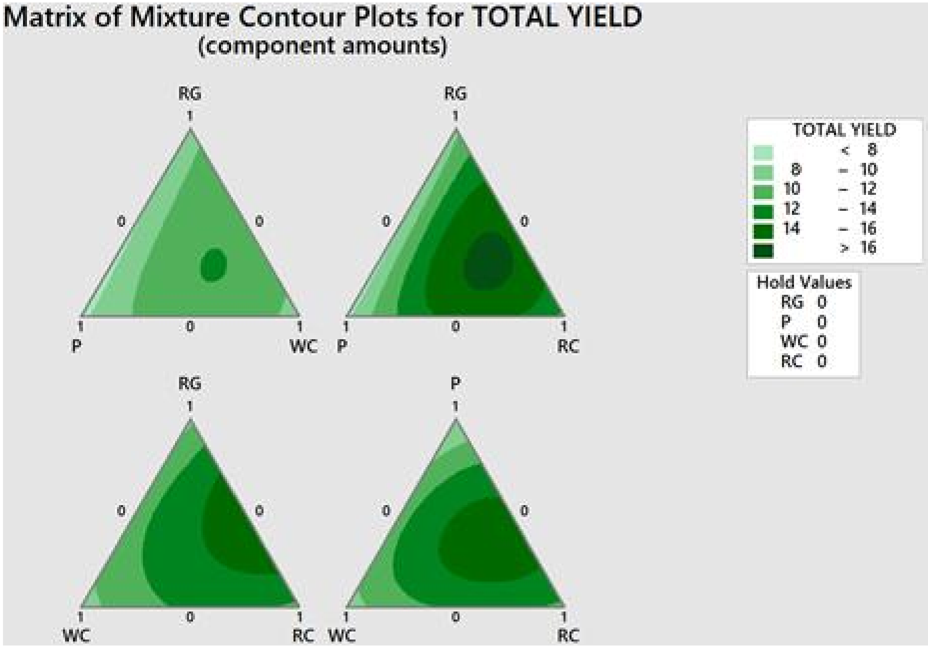

Figure 4.2. Contour plot of annual modelled dry-matter yield for the second year of growth (30th May 2016 to 26th May 2017), as a function of sown proportions of ryegrass (RG), plantain (P), white clover (WC), and red clover (RC).

The exact proportion of each species needed to produce the maximum yield for the second year of growth was derived from the optimisation plot. The optimisation plot showed that overall annual maximum yield was modelled to be from a mix of red clover, ryegrass and plantain; as a proportionate mix of 0.29 ryegrass seed, 0.20 plantain seed and 0.51 red clover seed.

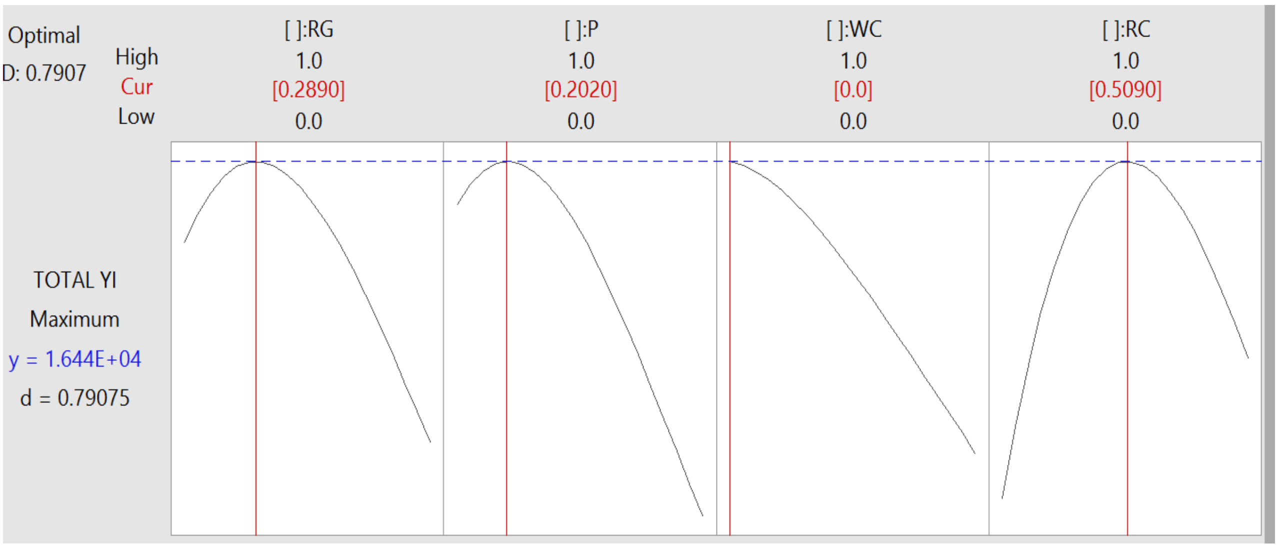

Figure 4.3. Optimisation plot for maximum modelled annual yield of a pasture mix potentially containing ryegrass (RG), plantain (P), white clover (WC), and red clover (RC), in the second year of growth (30th May 2016 to 26th May 2017). Grown under irrigated conditions at Lincoln University. The maximum yield was 16.64 t DM/ha. The d value represented the desirability of the model, which equals 0.791.

4.3 Seasonal dry matter yield analysis

The yield of all monocultures and mixtures changed throughout the growing season. This meant some species grew a greater proportion of their total yield at different times to others. This can be seen in the monoculture coefficients and modelled yields where ryegrass grew significantly more (P = 0.007 and 0.000) than the other monocultures in the first and last harvests of the growing season respectively, closest to the winter period. During the middle of the growing season from December – March (Harvests 4-7) red clover was higher yielding (P = 0.000 – 0.004) than any of the other monocultures.

Table 4.4. Coefficients determined from special cubic mixture model analysis. Highlighted and bold represent significantly (P < 0.05) higher yield than the average of the individual components included in the mixture. Highlighted only cells represent terms that become significant (P < 0.05) in a reanalysis discounting highly insignificant terms. Species were perennial ryegrass (RG), plantain (P), white clover (WC) and red clover (RC) grown under irrigated conditions at Lincoln University.

Mixture	Harvest							
	1	2	3	4	5	6	7	8
RG	0.85	0.68	1.89	1.42	1.70	1.12	0.75	0.97
P	0.45	0.57	1.31	1.57	1.49	0.91	0.84	0.39
WC	0.34	1.20	1.19	1.35	2.45	1.48	1.13	-0.05
RC	0.31	1.45	1.46	2.44	2.85	1.37	1.25	0.35
RG*P	-0.73	0.78	-1.10	-0.67	-1.17	-0.48	0.79	-0.14
RG*WC	0.26	0.29	0.27	1.45	**3.25**	0.06	**2.39**	0.07
RG*RC	0.30	-0.18	1.02	**5.61**	**5.52**	**4.44**	**3.67**	-0.33
P*WC	0.15	-0.49	0.91	**2.56**	**4.96**	0.98	1.18	1.29
P*RC	0.27	**1.88**	1.09	**3.30**	**5.31**	**4.16**	**3.95**	-0.05
WC*RC	-0.05	0.35	0.18	1.22	**3.40**	**2.12**	0.64	0.13
RG*P*WC	4.71	-2.77	2.18	13.02	-1.22	10.63	8.54	-2.41
RG*P*RC	0.36	7.68	6.54	11.94	7.87	0.78	10.93	5.35
RG*WC*RC	-0.15	0.98	0.65	-2.17	-14.84	-2.59	4.98	1.73
P*WC*RC	**8.52**	-0.94	3.45	5.58	2.91	-2.29	4.59	-1.79
RG*P*WC*RC	40.43	62.88	18.71	0.34	126.60	34.83	-72.94	-20.93

These coefficients were substituted into the same all-inclusive model to produce modelled seasonal yields (Table 4.5).

Table 4.5. Annual and specific harvest modelled yield including all terms from the special cubic mixture model analysis. Highlighted and bold represent significantly higher yield than the average of the individual components included in the mixture. Highlighted only cells represent terms that become significant in a reanalysis discounting highly insignificant terms. Species were perennial ryegrass (RG), plantain (P), white clover (WC) and red clover (RC) grown under irrigated conditions at Lincoln University.

Mixture	**Harvest Yield (t DM/ha)**							
	2/8/16	22/9/16	31/10/16	9/12/16	13/1/17	17/2/17	31/3/17	26/5/17
RG	0.85	0.68	1.89	1.42	1.70	1.12	0.75	0.97
P	0.45	0.57	1.31	1.57	1.49	0.91	0.84	0.39
WC	0.34	1.20	1.19	1.35	2.45	1.48	1.13	-0.05
RC	0.31	1.45	1.46	2.44	2.85	1.37	1.25	0.35
RG*P	0.46	0.82	1.32	1.33	1.30	0.90	0.99	0.65
RG*WC	0.66	1.01	1.61	1.75	**2.89**	1.32	**1.54**	0.48
RG*RC	0.65	1.02	1.93	**3.33**	**3.65**	**2.36**	**1.92**	0.58
P*WC	0.43	0.77	1.48	**2.10**	**3.21**	1.44	1.28	0.49
P*RC	0.44	**1.48**	1.66	**2.83**	**3.50**	**2.18**	**2.03**	0.36
WC*RC	0.31	1.41	1.37	2.20	**3.50**	**1.96**	1.35	0.18
RG*P*WC	1.07	0.51	1.71	2.89	1.74	2.35	1.85	0.17
RG*P*RC	0.57	1.75	2.28	3.14	2.89	1.22	2.16	1.16
RG*WC*RC	0.48	1.22	1.59	1.50	0.68	1.04	1.60	0.62
P*WC*RC	**1.31**	0.97	1.70	2.41	2.59	1.00	1.58	0.03
RG*P*WC*RC	3.01	4.91	2.63	1.72	10.04	3.40	-3.57	-0.89
Linear P	0.007	0.000	0.024	0.009	0.003	0.219	0.372	0.000
Quadratic P	0.810	0.192	0.319	0.000	0.000	0.000	0.004	0.801

The mixtures were made up of the same species as the monocultures therefore displayed some of the same traits in growing season. In addition to this they also display mixing effects as species interact with one another within the sward. During the start of the season this effect was small as the quadratic P value was large (0.810) indicating no mixing effect in the two-species mixtures (Table 4.5). Only the RG*P*RC mixture showed some significant benefit in yield over the monoculture and two-species mixtures in the first (August) harvest.

In the September harvest, there was still no overall mixing effect benefitting yield of the mixtures (P = 0.192), though one mix (P*RC) showed a significant mixing effect over the average of its monoculture components. When the RG*WC*RC mix was reanalysed discounting highly significant terms, it also became significantly (P < 0.05) higher yielding than its components.

In the third harvest at the end of October, there was still no significant mixing effect (P = 0.319) and the ryegrass monoculture significantly out-yielded the other species grown as monocultures (P = 0.024).

The fourth harvest, undertaken on 09/12/2016, started to show mixing effects within the treatments. There was an overall quadratic effect (P = 0.000) of the two-species mixtures yielding significantly more than the monocultures, specifically RG*RC, P*WC, and P*RC. There was also a three-species mixing effect in RG*P*WC in a reanalysis of terms which generated a significant (P < 0.05) result.

The mid-season harvest taken on the 13/01/2017 showed the greatest species interaction effect of all individual harvests in terms of both the overall quadratic component of the model (P = 0.000) and the number of two-species mixtures that showed significant interaction effects. RG*WC, RG*RC, P*WC, P*RC, and WC*RC all showed significant (P < 0.05) mixture effects even when all insignificant terms are included in the model. The sixth two-species mixture, RG*P, showed a net negative species interaction coefficient of -1.17, which equates to 0.293 t DM/ha less than the mean of the component monoculture species.

Harvest 6 was taken on 17/02/2017, and showed the mixture effect of the two species mixtures extended later into the growing season, with the overall quadratic component of the model still highly significant (P = 0.000). This was accompanied by a non-significant linear value (P = 0.219) in the monocultures; showing that there was no individual species effect within the mixtures. Not all two species mixtures showed significant species interaction as only three two-species and one three-species mix yielded significantly higher than their components. RG*WC, RG*RC, and P*RC showed significant (P < 0.05) yield benefit when all terms were included and RG*P*WC became significant (P < 0.05) when highly insignificant terms were removed from the analysis.

The seventh harvest was taken on the 31/03/2017, and continued to show an overall species interaction within the two-species mixtures (P = 0.004). Again, not all of the mixtures contributed to this significant effect, with only RG*WC, RG*RC, and P*RC displaying significant species interaction. The linear species effect derived from the monocultures was also insignificant (P = 0.372).

The eighth and final harvest was taken in the late autumn (26/05/2017), and signalled the end of the growing season. The harvest showed no species interaction as the quadratic component of the analysis was highly insignificant (P = 0.801) as it was at the very start of the growing season. The linear component, however, was highly significant (P = 0.000) as the ryegrass coefficient was almost three times that of the next best monoculture, indicating a strong species effect.

At no stage during the year was there any distinguished benefit in having a fourth species added to the mix, as no harvest produced a significant yield benefit from the four-species mixture. Each of the three-species mixtures returned a significant yield benefit at some stage during the growing season or in terms of overall yield when applied in a reanalysis, showing the potential of three species mixtures as producing strong interactions to create transgressive over-yielding.

Plate 4.1. Monoculture plots of ryegrass – RG (1), plantain – P (2), white clover – WC (3) and red clover – RC (4) prior to the 31st March harvest.

Visual analysis of the pasture monocultures showed that the monocultures each had various characteristics that limited the potential of growth at the time of the March harvest (Plate 4.1). The ryegrass monoculture appeared to lack N, as was shown by the large urine patches within the plot. The plantain monoculture appeared to lack persistence, as plants were scattered and unsown species made up a large proportion of plot area. The white clover appeared to be competing with unsown species, but showed an abundance of plants compared to the plantain monoculture. The red clover monoculture appeared to be the most successful monoculture. It had a large proportion of the botanical composition and a solid density of adult plants.

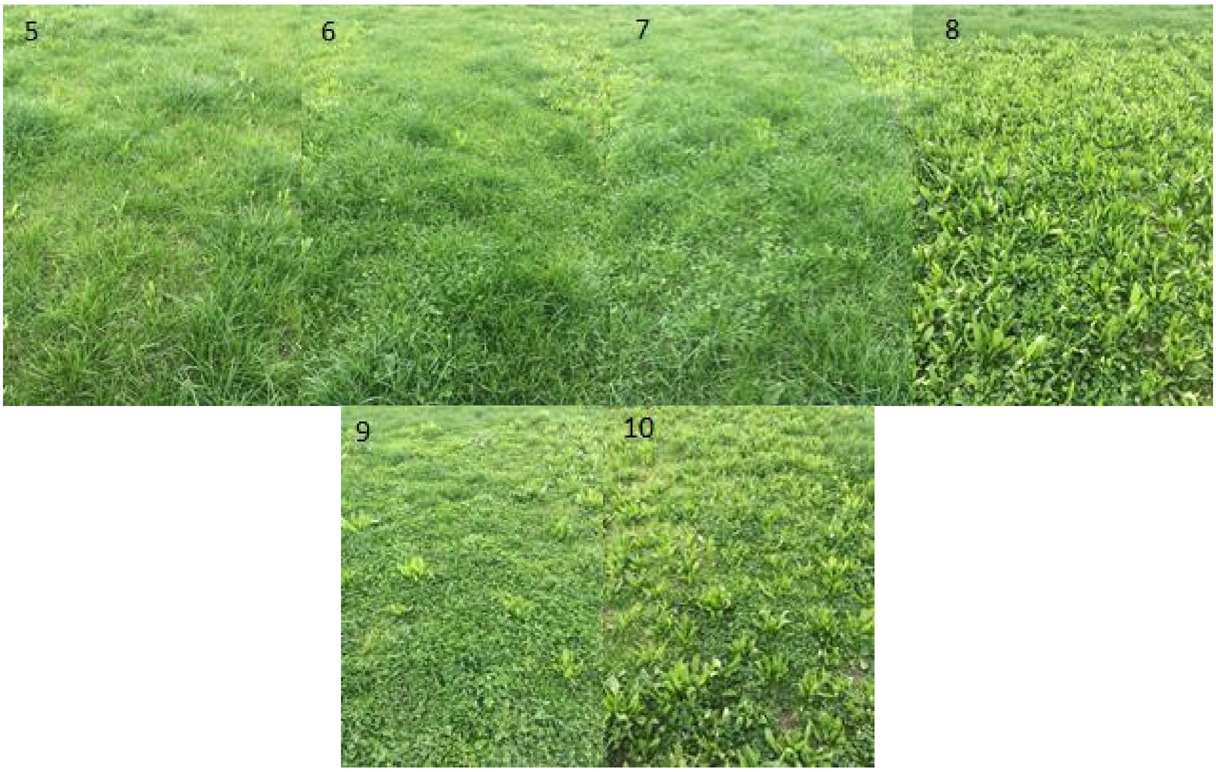

Plate 4.2. Two-species mixtures containing ryegrass – RG, plantain – P, white clover – WC and red clover – RC. Mixtures are RG*P (5), RG*WC (6), RG*RC (7), P*WC (8), P*RC (9), and WC*RC (10) prior to the 31st March harvest.

The two-species pasture mixtures showed varying levels of persistence within their plots (Plate 4.2). The mixtures containing ryegrass and a legume (mixtures 6 and 7) grew strongly and retained a high proportion of botanical composition as sown species. The non-legume mixture (5) also appeared to have a high proportion of sown species in botanical composition but lacked the plantain component of the mixture and also had less over-all growth. The mixtures containing plantain and a legume (mixtures 8 and 9) grew strongly and appeared to have retained a high proportion of sown species in the botanical composition. The pure-legume mixture (10) had been largely out-competed by unsown species and as a result was less of the overall botanical composition.

Plate 4.3. Three-species mixtures containing ryegrass – RG, plantain – P, white clover – WC and red clover – RC. Mixtures are RG*P*WC (11), RG*P*RC (12), RG*WC*RC (13) and P*WC*RC (14) prior to the 31st March harvest.

The three species mixtures all appeared to have grown strongly in the period preceding the March harvest (Plate 4.3). All mixtures appeared to have retained a high proportion of sown species within the botanical composition and urine patches were less distinct as each mixture had at least one species of legume present.

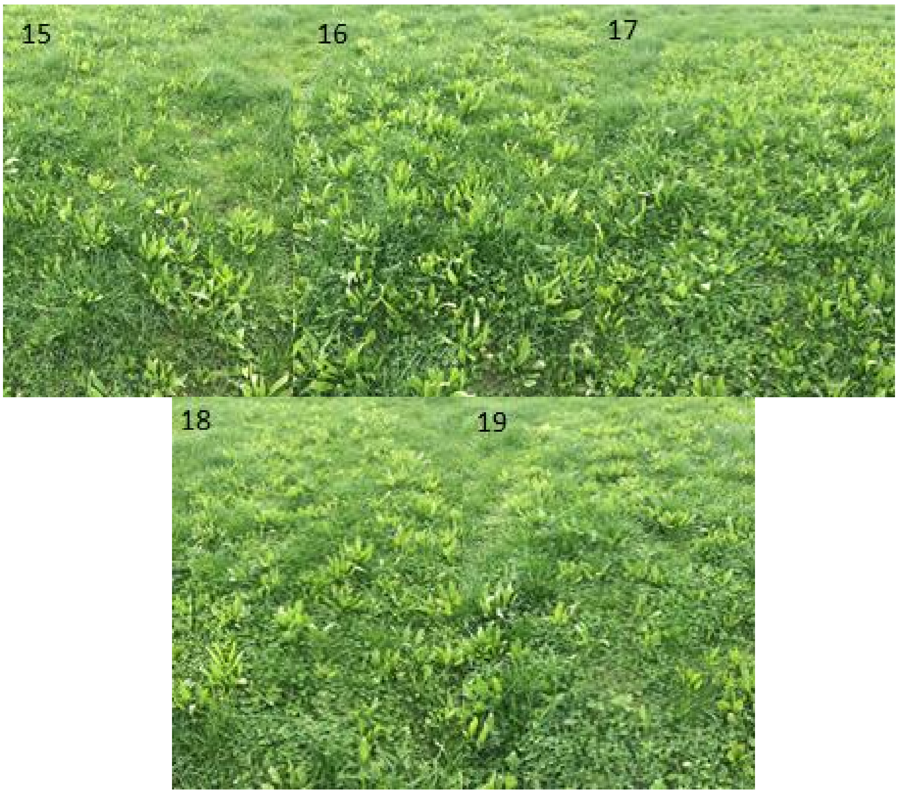

Plate 4.4. Four-species mixtures containing ryegrass – RG, plantain – P, white clover – WC and red clover – RC. Mixtures are RG*PWC*RC centroid mixture (15), RG dominant (16), P dominant (17), WC dominant (18) and RC dominant (19) prior to the 31st March harvest.

The four species mixtures all grew strongly and visual assessment of the swards suggested that the mixtures were very consistent among mixtures (Plate 4.4). The four mixtures dominated by one species in turn (16, 17, 18 and 19) were very similar visually as the sward was either dominated by red clover or plantain dependent on the sown proportion of the mixture. This was especially true of the red clover dominant mixture (19) which appeared to have a strongly red clover dominant botanical composition.

4.4 Species seasonal growth

As shown in the seasonal dry-matter yield (Table 4.5), the growth of each species was not uniform over the course of the growing season. There was variation within each species between harvests, and variation between species in the same harvest. This has been shown statistically as the monocultures of each species show significantly higher growth at different times during the growing season. The first came from ryegrass at the first and last harvests of the season, whereas the significant growth differences during the summer period came from red clover. This differing yield created a potential benefit of mixing species as they possessed differing periods of strong growth compared to other components at different times of the year.

The yields of the four monoculture species all followed a similar pattern of growth in that they started off with slow growth and low yields in the spring, reached a peak at some time during the spring/summer period before tailing off again the following autumn (Figure 4.4). The four monocultures differed though, in the time of year they reached their peak, the pasture mass generated at their peak, and the consistency of their yield over the whole growing season.

Ryegrass as a monoculture showed greater cool season growth against the other species, shown as the highest ($P = 0.007$) early-spring and late-autumn growth. It grew strongly during spring to reach a peak of 1.89 t DM/ha in October, a significantly ($P = 0.024$) higher yield than any of the other three species. After this peak, growth steadily tailed off, but yields were consistently over 1.0 t DM/ha over the summer period. Ryegrass was the second-highest yielding monoculture, producing 9.38 t DM/ha over the second year of growth.

Plantain did not perform well during the second year of growth; yielding 7.31 t DM/ha over the whole growing season. The early spring yield was low, at 0.45 t DM/ha. As the temperature increased through spring, plantain yield steadily increased, reaching a peak harvest of 1.57 t DM/ha in December. Plantain growth slowed through January and February and harvest yield decreased to a minimum of 0.39 t DM/ha at the final harvest. Plantain was the only monoculture that did not yield the highest harvest at some stage during the growing season.

White clover yielded only 0.34 t DM/ha in the early-spring harvest. Growth during the spring and early summer was steady up to 1.35 t DM/ha, before peaking rapidly in the January harvest to 2.85 t DM/ha. Growth from this point tailed off but white clover yielded the highest of the four monocultures at the February harvest at 1.48 t DM/ha. Growth continued to slow to a point where the final late-autumn harvest recorded a net loss of material of -0.051 t DM/ha. White clover grew 0.29 t DM/ha less than the ryegrass monoculture during the second year of growth, producing 9.09 t DM/ha.

Red clover grew strongly during the second year of growth and was the highest yielding monoculture in four of the eight harvests. Growth of red clover was also slow over the cool-season and the first harvest yield was also low at 0.31 t DM/ha. From this point, red clover growth was rapid and produced 1.45 t DM/ha by the next harvest, significantly (P = 0.000) more than ryegrass which grew significantly more at the harvest prior. Red clover continuously increased in growth rate as the temperature increased during the spring and early summer to a peak harvest yield of 2.85 t DM/ha in January. From this point growth sharply declined to the February harvest and continued to decline steadily to the final late-autumn harvest which yielded 0.35 t DM/ha. Red clover was the highest annual yielding monoculture, growing 11.46 t DM/ha over the second year of growth.

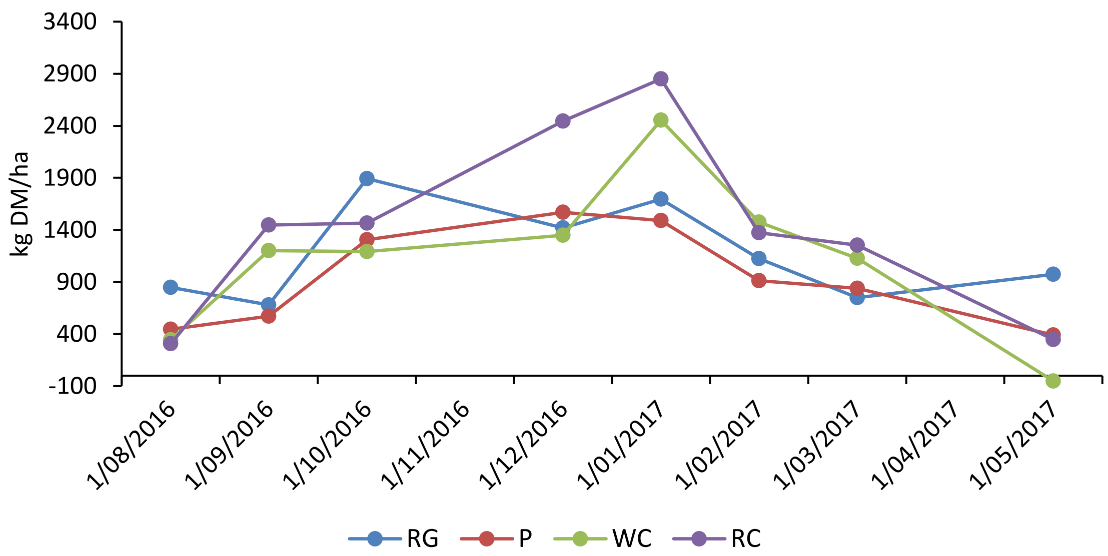

Figure 4.4. Modelled seasonal yields of ryegrass (RG), plantain (P), white clover (WC), and red clover (RC) monocultures in the second year of growth (30th May 2016 to 26th May 2017). Grown under irrigated conditions at Lincoln University.

These differing peaks and yields between species showed that there is opportunity for a benefit of mixing two or more species with one another to increase growing season length and therefore overall annual yield. The RG*P*RC three-species mix showed the benefit of this mixture, including species interactions, and was able to produce a higher harvest yield than the best performing monoculture component in 6 of the 8 harvests over the second year of growth (Figure 4.5).

The RG*P*RC three-species mix had a 2.74 t DM/ha lower yield than the ryegrass monoculture at the time of first harvest after the cool-season (Figure 4.5). The growth of the mix was rapid as the spring warmed, and yielded higher than all of its monoculture components up till a peak harvest yield of 3.14 t DM/ha in the December harvest. Growth of the mixture then slowed but still remained marginally higher than the red clover monoculture at the 13/01/2017 harvest. Growth of the mixture and red clover both dropped rapidly before the February harvest, where the red clover monoculture produced 0.15 t DM/ha more than the RG*P*RC three-species mix. While the red clover monoculture continued to decline in growth, the mixture growth rate increased again to yield 2.16 t DM/ha in the seventh harvest before dropping again as the temperature cooled; though still produced the most dry-matter at the final harvest at 1.16 t DM/ha.

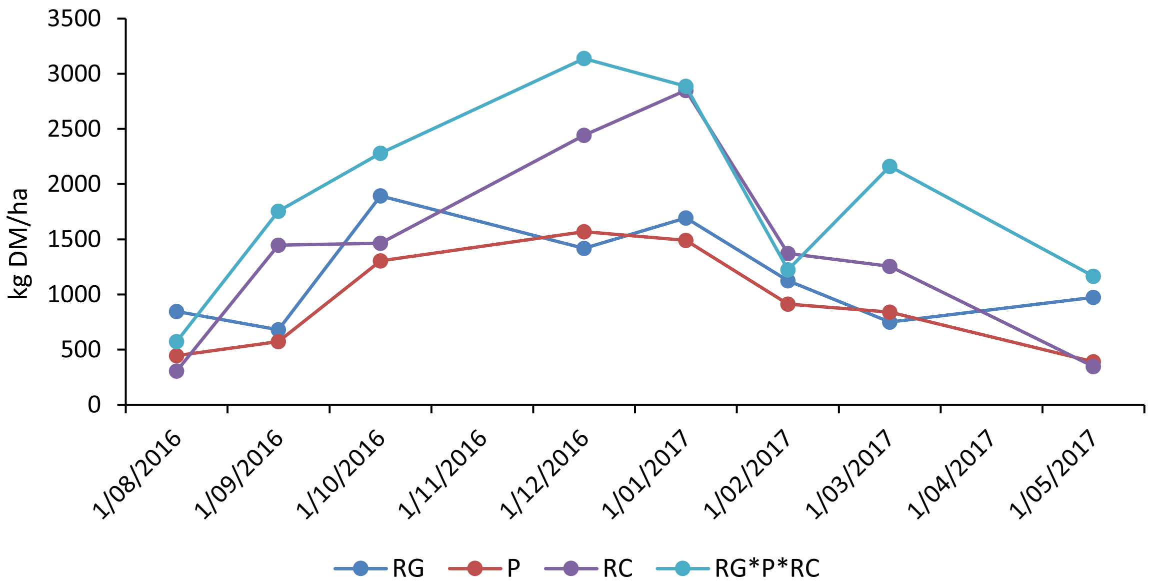

Figure 4.5. Modelled $^{1}/_{3}$ ryegrass, $^{1}/_{3}$ plantain, $^{1}/_{3}$ red clover (RG*P*RC) ternary mixture seasonal yield against the seasonal yield of its component monocultures; ryegrass (RG), plantain (P), and red clover (RC), in the second year of growth (30th May 2016 to 26th May 2017). Grown under irrigated conditions at Lincoln University, Canterbury.

4.5 Botanical composition

Another way of explaining differing growth rates during the growing season is by analysing the botanical composition of the mixture. The 15 mixtures were each sown with a specific botanical composition; the two-species mixtures were sown 0.5-0.5 in terms of seed number, the three-species mixtures 0.33-0.33-0.33, and the four species even mixture sown so each species was 0.25 of the seed number per unit area. Included in this analysis is the four 4sp dominant mixtures; each sown with 0.625 of one species, with 0.125 of the proportion each to the remaining three species. Over 2 years of growth the botanical composition of each of the 15 mixtures has deviated from what was originally sown.

The two species mixtures have all deviated from their 0.5-0.5 sown proportion. The most deviation is from the WC*RC mixture where red clover is now 0.82 of sown botanical composition and white clover the remaining 0.18. The least deviation came from the RG*P mix whose annual proportion averged 0.41 ryegrass and 0.59 plantain. The legume to non-legume mixes behaved differently dependant on what legume was included in the two-species mix. The red clover mixtures (RG*RC & P*RC) are both dominated by red clover in terms of sown proportions (0.38 - 0.61 & 0.32 - 0.68). A contrast to this is the performance of white clover, retaining 0.23 of sown botanical composition in the RG*WC mix and 0.30 in the P*WC mix.

The three-species mixtures and four-species mixtures displayed similar traits over their annual average sown species botanical composition. Red clover continued to have the highest proportion of botanical composition over every mix, ranging from 0.41 (4sp RG dom) to 0.56 (RG*P*RC) of botanical compositition, apart from the 4sp WC dominant mix where both plantain and ryegrass had a higher proportion (0.29 & 0.30) than red clover (0.24). Though red clover seed was sown at 0.625 of the 4sp RC dom mix, it only averaged 0.54 of botanical composition over the second year of growth. White clover continued to make up the smallest part of the three- and four-species mixtures, having the least sown proportion in all mixtures including the 4sp WC dom mix, where white clover seed was 0.625 of the seed mix by number. The composition of white clover in the mixtures ranged from 0.02 (4sp RC dom) to 0.24 (RG*P*WC) and never made up the botanical composition of a mix above the proportion of seed sown in the mixture.

The non-legume species performed similarly in that they usually made up a higher proprtion of sown botanical composition than white clover and a lower proportion than red clover. In the three- and four-species mixes where ryegrass and plantain were both included, plantain held a greater proportion of botanical composition; ranging from 0.01 in the 4-species RG dominant mixture (4sp RG dom) to 0.22 (RG*P*WC) higher. Plantain had a higher proportion of botanical composition than the proportion it was sown in six out of eight of the three- or four-species mixtures. Ryegrass did not perform as well, and had a greater proportion of botanical composition than sown proportion in only two out of eight three- and four-species mixtures.

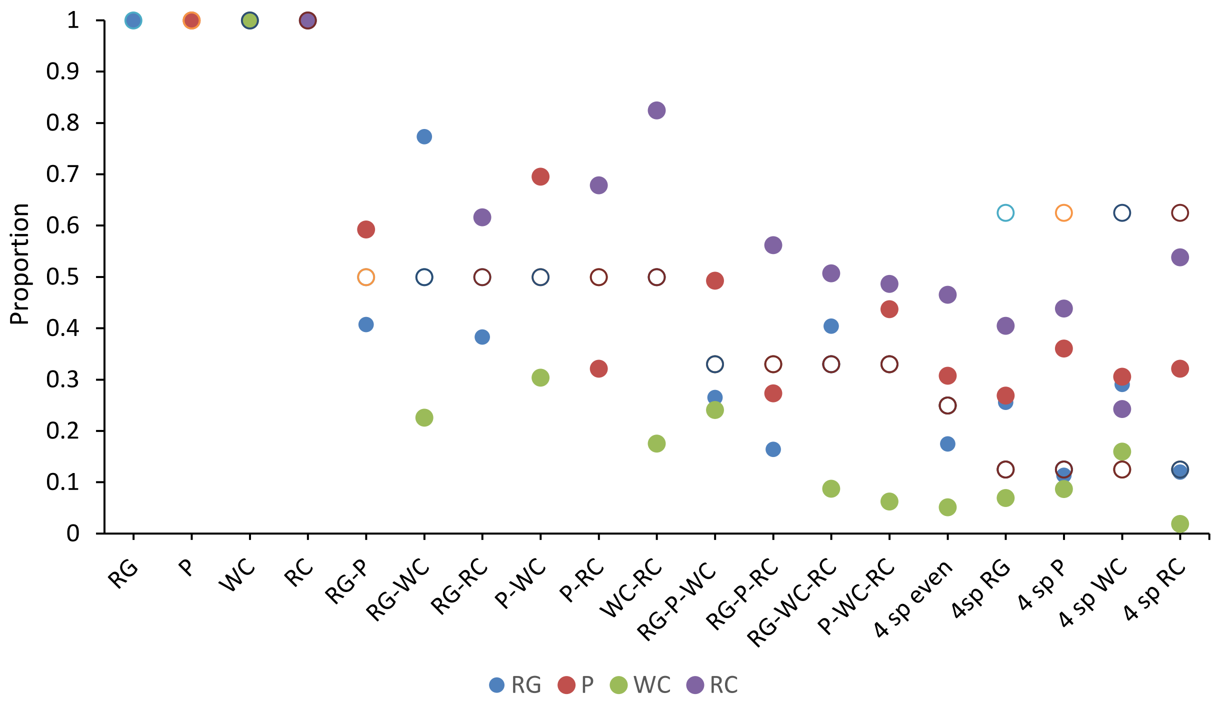

Figure 4.6. Average botanical composition of sown proportions of each of the 19 mixtures containing ryegrass (RG), plantain (P), white clover (WC), and red clover (RC), over the second year of growth (30th May 2016 to 26th May 2017) compared to the proportion originally sown (hollow circles). Grown under irrigated conditions at Lincoln, Canterbury.

In addition to the botanical composition of pasture mixtures changing between the second year of growth and sowing, pasture mix species composition also changed during the course of the growing season. The RG*P*RC mixture was identified as the only three species mixture that significantly increased yield over the average of its two species component mixtures; and was also identified in the optimisation plot (Figure 4.3) as the mixture of species predicted to give the highest yield over the second year of growth.

The botanical composition of the RG*P*RC mix has deviated from its sown proportions of 0.33 RG - 0.33 P - 0.33 RC to 0.24-0.54-0.22 of RG, P, RC respectively at the time of first harvest (2/08/2016). By the second harvest (22/09/2016) the botanical composition of the mixture had changed dramatically to 0.10-0.26-0.64; halving the proportion of ryegrass and plantain and tripling the proportion of red clover. The third harvest was less dominated by red clover, with a restored proportion of ryegrass to 0.20-0.30-0.50. Harvests 4 to 7 provided only slight changes to species proportions, ryegrass ranging from 0.13-0.20 of botanical composition, plantain 0.23-0.29, and red clover 0.49-0.63. The next dramatic shift in botanical composition was between the seventh (31/03/2017) and eighth (26/05/2017) harvests where botanical composition shifted from 0.20-0.26-0.54 to 0.47-0.34-0.18. At no stage during the year did the botanical composition of the RG*P*RC mix become similar to the proportions that were sown. The modelled optimum mix for maximum yield was also different to the actual proportions of different species, predicting a greater proportion of ryegrass (0.29), and a smaller proportion of plantain (0.20) would generate a greater annual yield.

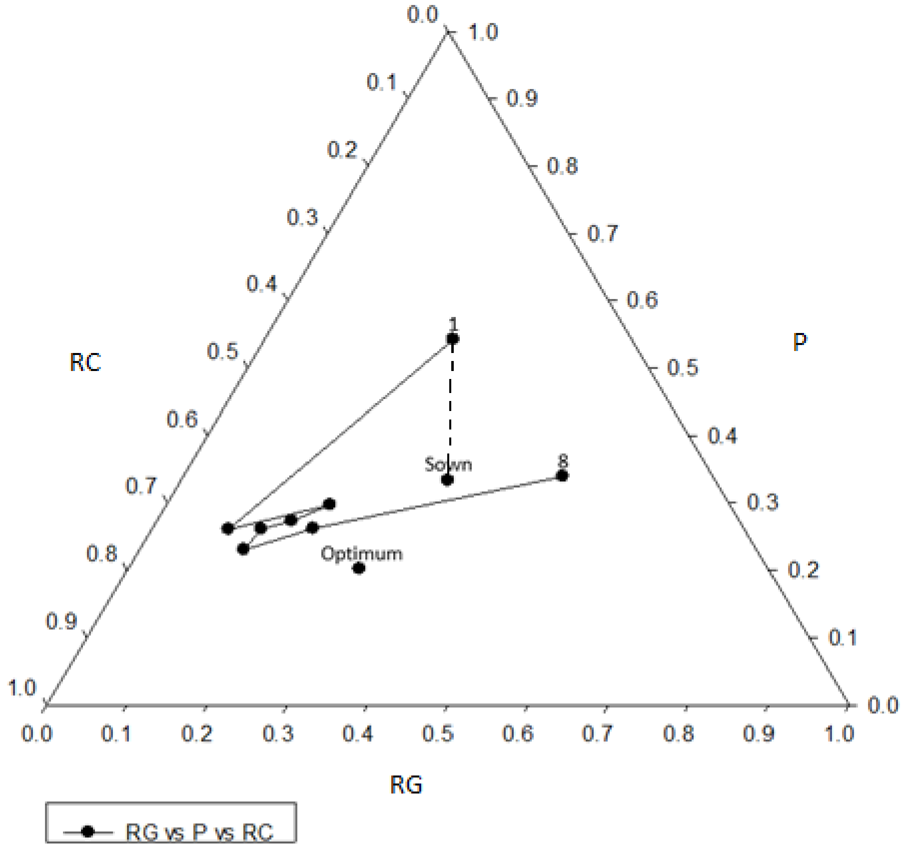

Figure 4.7. Seasonal variation in the sown species composition of yield of the ternary three-species mixture containing ryegrass (RG), plantain (P) and red clover (RC), in the second year of growth (30th May 2016 to 26th May 2017), grown under irrigated conditions at Lincoln University. The '1' denotes the first harvest (2nd August 2016) connected through to the eighth harvest (26th May 2017). The sown seed mix and optimal seed mixture that maximised annual yield are also shown.

4.6 Addition of legumes

Analysing legume and non-legume species N concentration interactions through the special cubic mixture model showed that there was little synergistic increase in N over the average of the legume/non-legume monocultures, but the average N% of the mixtures was significantly higher than the non-legume monocultures, which had no access to additional soil N. This change in N% was positively significant for the RG*RC and RG*P*RC mixtures only, increasing their modelled N% to 3.21% and 3.02%. The rest of the significant changes in N concentrations were defined by negative coefficients, indicating a significantly lower concentration of N than the average N concentration of the monoculture components.

All of the mixtures showed an increased N% over their non-legume component, a strong indicator for an increase in yield. The WC*RC legume mixture showed a significant decrease in N% compared to its monoculture components, suggesting an antagonistic relationship in its ability to collect plant available N. Red clover showed a stronger ability to provide a synergistic relationship to non-legumes than white clover. RG*RC showed a positive coefficient, and a modelled N concentration of 3.21%. RG*WC showed a negative coefficient and a modelled N concentration of 2.71%. Plantain interaction with red clover was insignificant, though initial analysis showed a positive coefficient of 0.227 (data not shown). P*WC had a significantly negative coefficient, modelling an N concentration of 2.82%. The model was a good fit for the data showing a strong adjusted R2 value of 85.13%. This strong fit to the data enabled a regression to be formed using modelled N concentrations against modelled yield to identify if a higher average N concentration results in a higher annual yield.

Table 4.6. Significant annual average N concentration coefficients of 19 pasture mixtures containing ryegrass (RG), plantain (P), white clover (WC), and red clover (RC), over the second year of growth (30th May 2016 to 26th May 2017)**.**

Mix	Coefficient	SE	Modelled N%	P
RG	2.295	0.07801	2.295	*
P	2.329	0.07218	2.329	*
WC	3.747	0.08609	3.747	*
RC	3.685	0.07801	3.685	*
Linear				0.000
RG*WC	-1.256	0.37759	2.707	0.001
RG*RC	0.919	0.39663	3.21975	0.024
P*WC	-0.881	0.37744	2.81775	0.023
WC*RC	-0.889	0.37759	3.49375	0.022
Quadratic				0.000
RG*P*RC	6.791	2.48481	3.021185	0.008
Special Cubic				0.008
	R-Sq = 87.31% R-Sq (adj) = 85.13%			

The initial analysis of N concentrating affecting yield was weakly positive with a low R^2 value of 31.72%. Further analysis indicates that the data points outlying from the general trend were the pure legume mixtures; WC, RC and WC*RC. The other two data points below the trendline were the three-species mixtures that contained two legume species, meaning only 0.33 of the sown species was non-legume, affecting yield. In addition to this, the data points showing the lowest yield also showed the lowest N concentration, and were the non-legume mixtures; RG, P and RG*P.

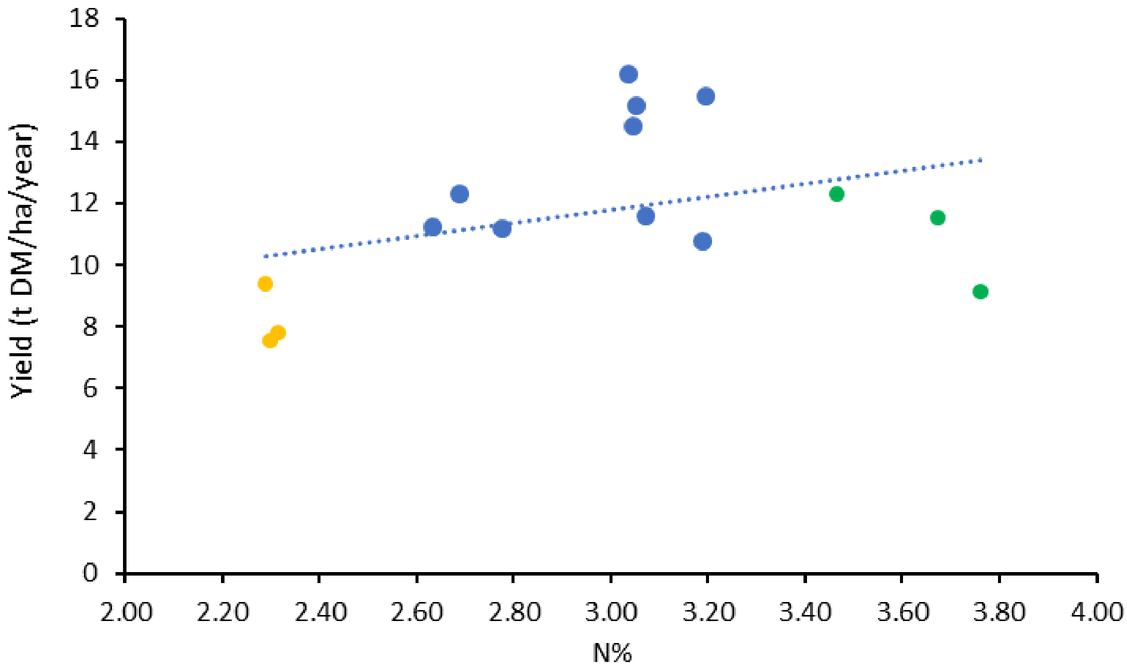

Figure 4.8. Modelled nitrogen concentration against modelled annual yield of each of the 15 mixtures containing perennial ryegrass (RG), plantain (P), white clover (WC) and red clover (RC) grown under irrigated conditions at Lincoln University, Canterbury. Mixtures include pure non-legume components RG, P, and RG*P (yellow), mixed legume and non-legume components (blue), and pure legume components WC, RC and WC*RC (green).

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Removing the legume mixtures from the regression showed a much stronger trend between annual yield and average N concentration. The three-species mixtures that contained 0.66 legume as a sown proportion were well below the trendline, but the R^2 = 62.82%, a reasonable fit for the regression. The trendline equation showed that for every 1% increase in average N concentration, yield of the pasture increased by 6.60 t DM/ha/year.

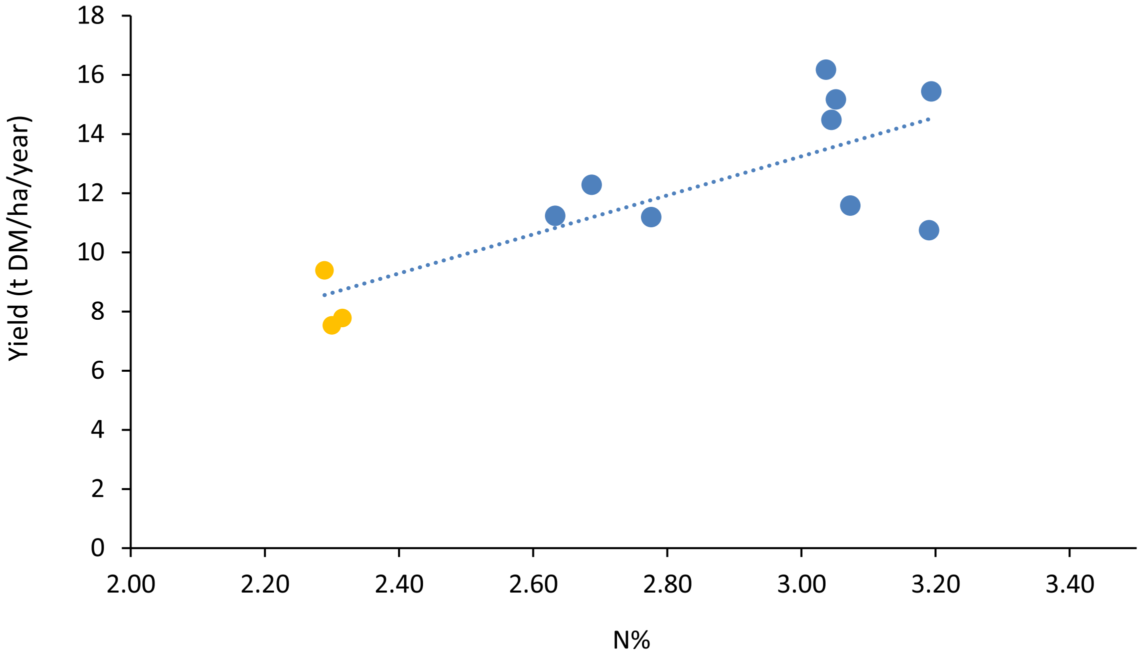

Figure 4.9. Modelled nitrogen concentration against modelled annual yield re-analysed removing pure legume swards. The mixtures contained perennial ryegrass (RG), plantain (P), white clover (WC) and red clover (RC) grown under irrigated conditions at Lincoln University, Canterbury. Mixtures include pure non-legume components RG, P, and RG*P (yellow) and mixed legume and non-legume components (blue).

4.7 Leaf area index

Another way of explaining the differences in yield between species and pasture mixes is by comparing the LAIs. LAI's for each monoculture and mixture were measured directly and indirectly; direct measurement was done by calculating SLA for the monocultures and mixed species and multiplying by green leaf area of each plot. An ANOVA was undertaken to determine if there was a difference in SLA between species grown as monoculture or in a mix (Table 4.7). The results of the ANOVA showed that there was no difference over the four species in the 31/03 harvest, so monoculture SLAs were used to calculate the LAI of all mixes. In the 26/05 harvest, significant differences occurred between the plantain ($P = 0.000$) and white clover ($P = 0.009$) SLA's, so the different figures were used where appropriate in different mixtures.

Table 4.7. ANOVA table signifying the difference between monoculture and mixed sward specific leaf area (SLA) for ryegrass (RG), plantain (P), white clover (WC) and red clover (RC) grown at Lincoln University.

Harvest	Species	Monoculture	Mixed Sward	P
31/03/2017	RG	354.5	499.6	0.078
	P	428.1	370.9	0.117
	WC	456.0	611.0	0.144
	RC	364.9	386.4	0.298
26/05/2017	RG	283.4	270.3	0.434
	P	238.6	445.6	0.000
	WC	278.8	334.0	0.009
	RC	293.9	305.6	0.409

4.7.1 March harvest

The LAI for each plot was measured directly and indirectly for the March harvest, with both methods returning a LAI figure for each of the 76 plots. The correlation between these two methods was low (r = 0.439) so the reliability of indirect measurement of pastoral LAI using SunScan can come in to question. Despite this, both sets of data were analysed through the special cubic mixture model to identify differences between mixtures in terms of LAI.

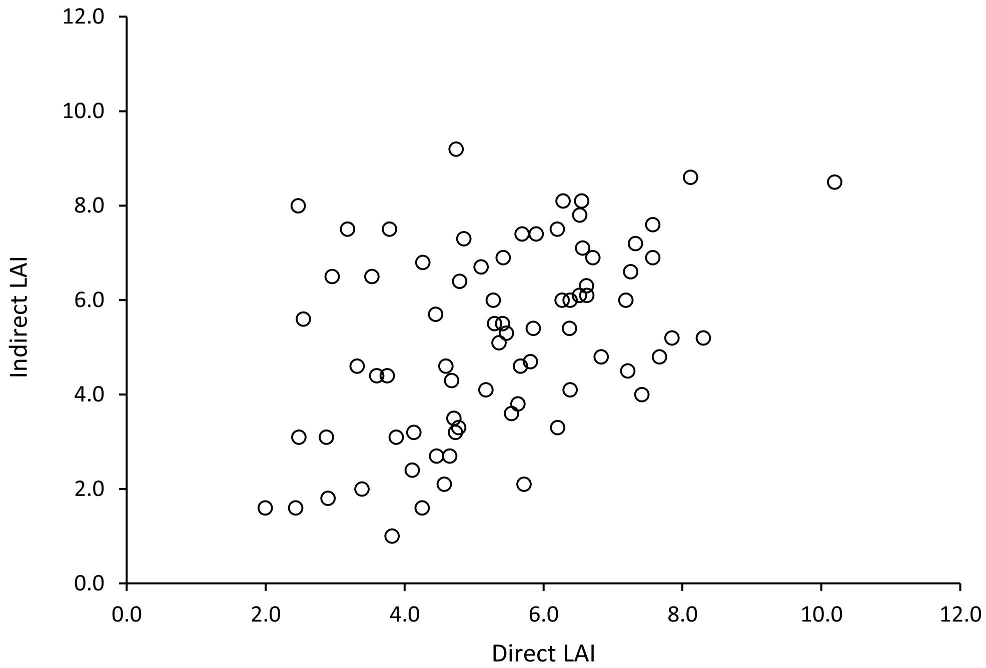

Figure 4.10. Correlation between direct and indirect measures of leaf area index (LAI) over 19 pasture mixtures containing ryegrass (RG), plantain (P), white clover (WC), and red clover (RC), in the second year of growth (30th May 2016 to 26th May 2017). Grown under irrigated conditions at Lincoln University for the 31/03/2017 harvest. r = 0.439.

The resulting analysis showed that directly measured LAI provided significant differences between mixtures and their component monocultures. The RG*RC and P*RC showed strong interactions to produce highly significant LAI increases, with less significant increases in the RG*WC and P*WC two-species mixtures. There was no significant increase in LAI when a third or fourth species was added to the mixture (Table 4.8).

Table 4.8. Leaf Area Index coefficients directly measured for 31/03/2017 harvest for 15 pasture mixtures containing ryegrass (RG), plantain (P), white clover (WC), and red clover (RC), in the second year of growth (30th May 2016 to 26th May 2017). Grown under irrigated conditions at Lincoln University. Bolded and highlighted P values indicate P <0.05, highlighted only indicated P <0.1.

Mixture	Mix	Coefficient	SE	P
Monoculture	RG	3.9	0.654	*
Monoculture	P	3.9	0.654	*
Monoculture	WC	3.5	0.654	*
Monoculture	RC	3.8	0.654	*
Binary	RG*P	2.8	3.290	0.406
Binary	RG*WC	5.8	3.290	0.082
Binary	RG*RC	7.0	3.290	**0.039**
Binary	P*WC	6.5	3.290	0.053
Binary	P*RC	10.4	3.290	**0.002**
Binary	WC*RC	1.9	3.290	0.567
Ternary	RG*P*WC	23.4	23.077	0.314
Ternary	RG*P*RC	17.1	23.077	0.461
Ternary	RG*WC*RC	28.6	23.077	0.221
Ternary	P*WC*RC	9.3	23.077	0.689
Quaternary	RG*P*WC*RC	-227.9	223.986	0.313

Indirectly measured LAI also produced some significant increases in LAI over the respective monoculture components when undergoing a special cubic mixture model analysis. The P*RC mixture returned another significant increase in leaf area, along with the RG*P*RC three-species mixture (Table 4.9). A lesser significant result was the RG*RC mixture (P = 0.051) which was also significant in the directly measured analysis. Neither of the two-species white clover * non-legume mixtures returned significant values as they did when measured directly (Table 4.9).

Table 4.9. Leaf Area Index coefficients indirectly measured for 31/03/2017 harvest for 15 pasture mixtures containing ryegrass (RG), plantain (P), white clover (WC), and red clover (RC), in the second year of growth (30th May 2016 to 26th May 2017). Grown under irrigated conditions at Lincoln University. Bolded and highlighted P values indicate P<0.05, highlighted only indicated P<0.1.

Mixture	Mix	Coefficient	SE	P
Monoculture	RG	2.5	0.684	*
Monoculture	P	3.0	0.684	*
Monoculture	WC	5.7	0.684	*
Monoculture	RC	5.6	0.684	*
Binary	RG*P	-0.4	3.441	0.907
Binary	RG*WC	-1.9	3.441	0.590
Binary	RG*RC	6.9	3.441	0.051
Binary	P*WC	1.3	3.441	0.701
Binary	P*RC	7.6	3.441	**0.032**
Binary	WC*RC	3.2	3.441	0.356
Ternary	RG*P*WC	4.7	24.133	0.846
Ternary	RG*P*RC	59.7	24.133	**0.016**
Ternary	RG*WC*RC	19.2	24.133	0.430
Ternary	P*WC*RC	6.9	24.133	0.777
Quaternary	RG*P*WC*RC	-150.5	234.231	0.523

In the March harvest three of the mixtures showed significantly higher LAI's also yielded significantly higher than their component monocultures. They were RG*WC (Yield P = 0.045), RG*RC (Yield P = 0.003), and P*RC (Yield P = 0.001) (Tables 4.8 and 4.9) (Appendix B). In addition, the three species RG*P*RC mixture had a yield P value of 0.186 (Appendix B), though not significant it showed strong growth above the average of the two-species mixtures and created a significant LAI (P = 0.016) when measured indirectly (Table 4.9).

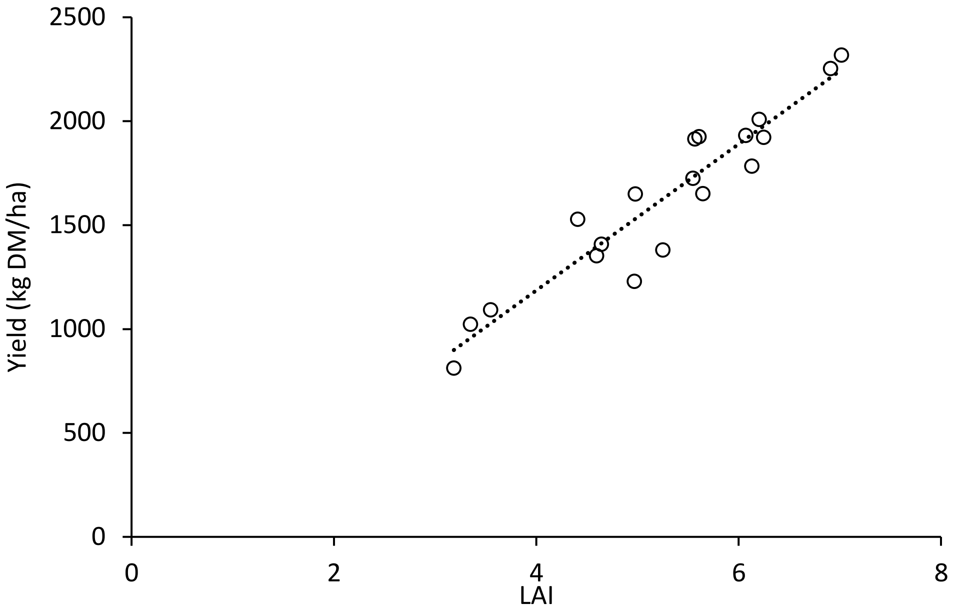

Figure 4.11. Yield of 19 pasture mixtures containing ryegrass (RG), plantain (P), white clover (WC), and red clover (RC) against their averaged direct and indirect LAI recording for the March harvest. $R^2 = 0.892$.

The synchrony of these results indicated that there is a relationship between the LAI of the mixture and their subsequent yield. The average of direct and indirect measurement methods was used in a regression against appropriate harvest yield. The March harvest showed a strong positive linear relationship between LAI of the mixture and mixture yield ($R^2 = 0.89$) where yield increased by 351 kg DM/ha for very integer increase in LAI.

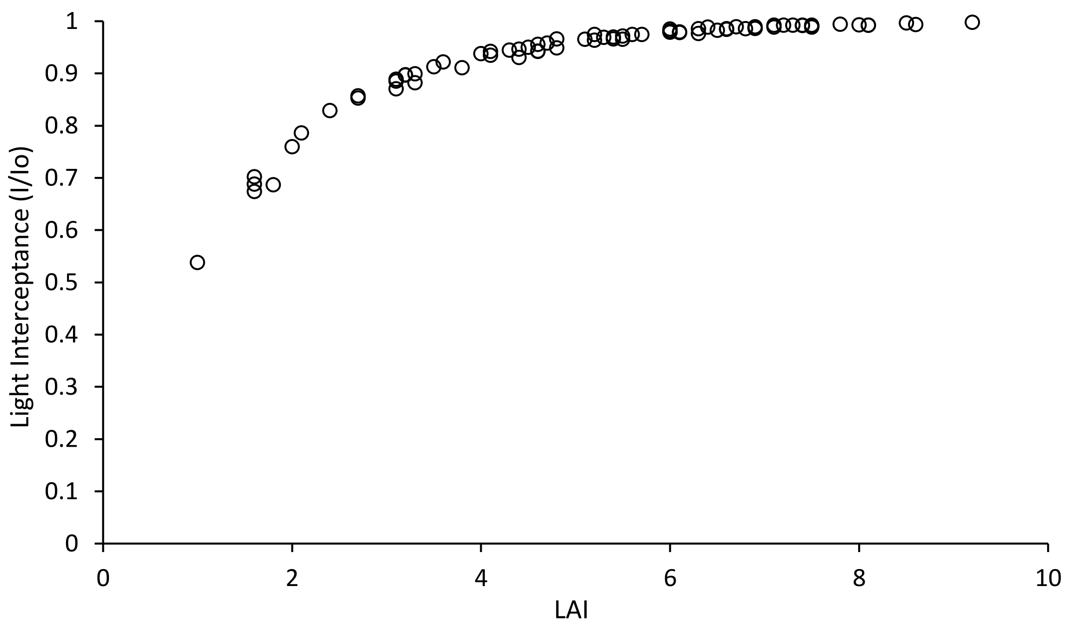

Figure 4.12. Light interception of 76 pasture mixtures containing perennial ryegrass (RG), plantain (P), white clover (WC) and red clover (RC) compared against indirectly measured leaf area index (LAI) recorded on 31/03/2017 at Lincoln University.

An increase in LAI also resulted in an increase in the light intercepted by the pasture (Figure 4.12). Light interceptance of the 76 mixtures increased with increasing LAI up to a critical point (I/Io = 1) of LAI 4-5. From this point the light interceptance (I/Io) plateaued with increasing LAI as all available PAR was intercepted by the sward.

4.7.2 May harvest

The direct and indirect measures of LAI were repeated for the 26/05 harvest. There was little correlation between the two methods of measurement, producing an r value of 0.49

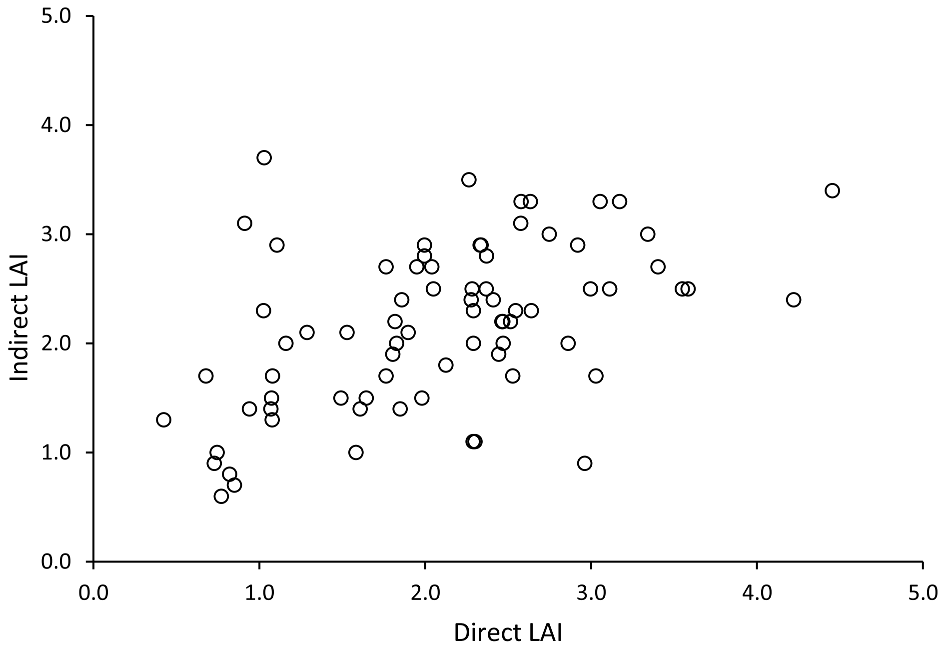

Figure 4.13. Correlation between direct and indirect measures of LAI for the 26/05/2017 harvest of 15 pasture mixtures containing ryegrass (RG), plantain (P), white clover (WC), and red clover (RC), in the second year of growth (30th May 2016 to 26th May 2017). Grown under irrigated conditions at Lincoln University. r = 0.497.

The direct method of LAI measurement returned only one significantly higher LAI mixture to its components (P*WC), though only to the P <0.1 level (P = 0.054) (Table 4.10).

Table 4.10. Leaf Area Index (LAI) coefficients directly measured for 26/5 harvest of 15 pasture mixtures containing ryegrass (RG), plantain (P), white clover (WC), and red clover (RC), in the second year of growth (30th May 2016 to 26th May 2017). Grown under irrigated conditions at Lincoln University. Bolded and highlighted P values indicate P<0.05, highlighted only indicated P<0.1.

Mixture	Mix	Coefficient	SE	P
Monoculture	RG	3.55	0.333	*
Monoculture	P	1.89	0.333	*
Monoculture	WC	1.18	0.333	*
Monoculture	RC	1.65	0.333	*
Binary	RG*P	-1.86	1.675	0.271
Binary	RG*WC	0.30	1.675	0.857
Binary	RG*RC	-1.04	1.675	0.537
Binary	P*WC	3.29	1.675	0.054
Binary	P*RC	0.16	1.675	0.925
Binary	WC*RC	-1.06	1.675	0.529
Ternary	RG*P*WC	-0.25	11.749	0.983
Ternary	RG*P*RC	2.64	11.749	0.823
Ternary	RG*WC*RC	6.03	11.749	0.610
Ternary	P*WC*RC	-0.91	11.749	0.938
Quaternary	RG*P*WC*RC	-13.43	114.036	0.907

In similarity to the directly measured LAI, the indirectly measured data also recorded only one significantly higher LAI through the special cubic mixture model analysis. In this analysis it was the three-species RG*P*RC mixture, showing a highly significant (P = 0.010) LAI above the average of its component two-species mixtures (Table 4.11).

Table 4.11. Leaf Area Index (LAI) coefficients indirectly measured for 26/5 harvest of 15 pasture mixtures containing ryegrass (RG), plantain (P), white clover (WC), and red clover (RC), in the second year of growth (30th May 2016 to 26th May 2017). Grown under irrigated conditions at Lincoln University. Bolded and highlighted P values indicate P<0.05, highlighted only indicated P<0.1.

Mixture	Mix	Coefficient	SE	P
Monoculture	RG	2.7	0.294	*
Monoculture	P	1.8	0.294	*
Monoculture	WC	1.9	0.294	*
Monoculture	RC	1.6	0.294	*
Binary	RG*P	-2.3	1.478	0.126
Binary	RG*WC	0.5	1.478	0.737
Binary	RG*RC	2.0	1.478	0.175
Binary	P*WC	0.9	1.478	0.542
Binary	P*RC	0.7	1.478	0.620
Binary	WC*RC	-1.1	1.478	0.473
Ternary	RG*P*WC	-1.2	10.368	0.909
Ternary	RG*P*RC	27.4	10.368	**0.010**
Ternary	RG*WC*RC	12.0	10.368	0.254
Ternary	P*WC*RC	10.7	10.368	0.308
Quaternary	RG*P*WC*RC	-130.6	100.628	0.199

In the May harvest there was no significant yield benefits from any of the mixtures. The two significant mixtures identified by the LAI analysis also had the two lowest P values for yield for the May harvest. The P*WC mixture had the lowest P value for yield (P = 0.109) and the RG*P*RC mixture had the second lowest (P = 0.340) (Tables 4.10 and 4.11) (Appendix B).

The result was not as strong for the May harvest, but still showed a positive linear relationship with a reasonable R^2 value (0.698) (Figure 4.14). The yields and LAI's for this harvest are much lower, allowing for smaller differences to become much more significant in terms of the strength of the relationship between LIA and yield of the mixture.

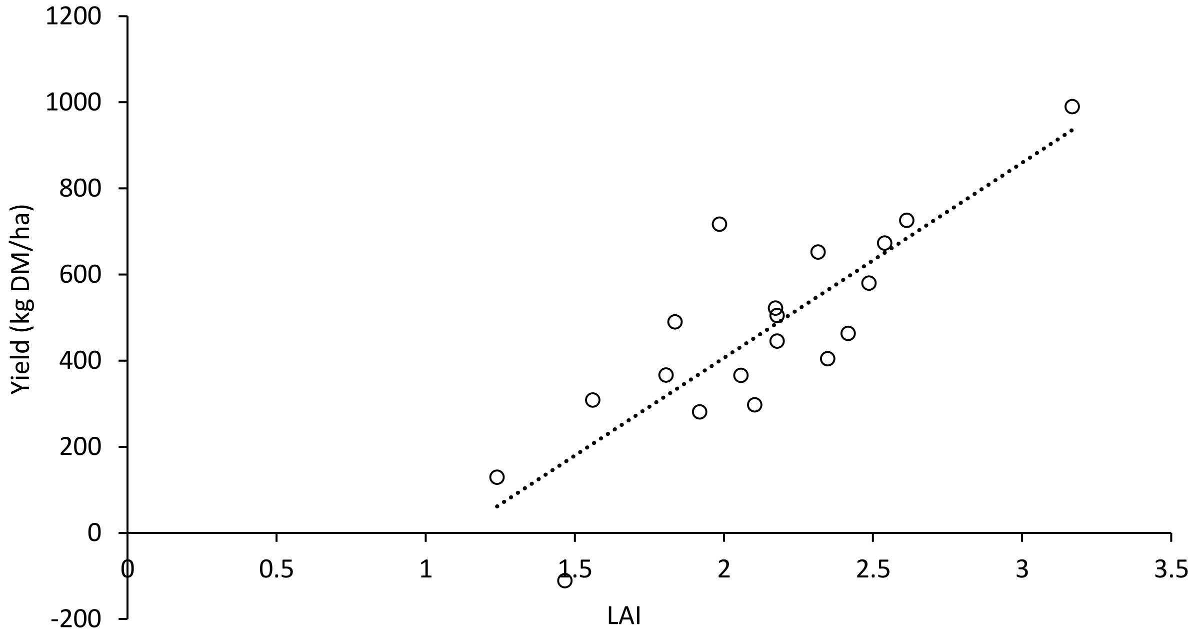

Figure 4.14. Annual yield against the averaged direct/indirect LAI recording for the 26/05/2017 harvest of 15 pasture mixtures containing ryegrass (RG), plantain (P), white clover (WC), and red clover (RC), in the second year of growth (30th May 2016 to 26th May 2017). Grown under irrigated conditions at Lincoln University. R^2 = 0.698.

The interceptance of light by the 76 mixtures was not as high as the previous (31/03/2017) harvest. None of the mixtures reached critical LAI as all interceptance (I/Io) values were <1 (Figure 4.15). The general curvi-linear trend showed an increase in LAI led to an increase in light interceptance (I/Io).

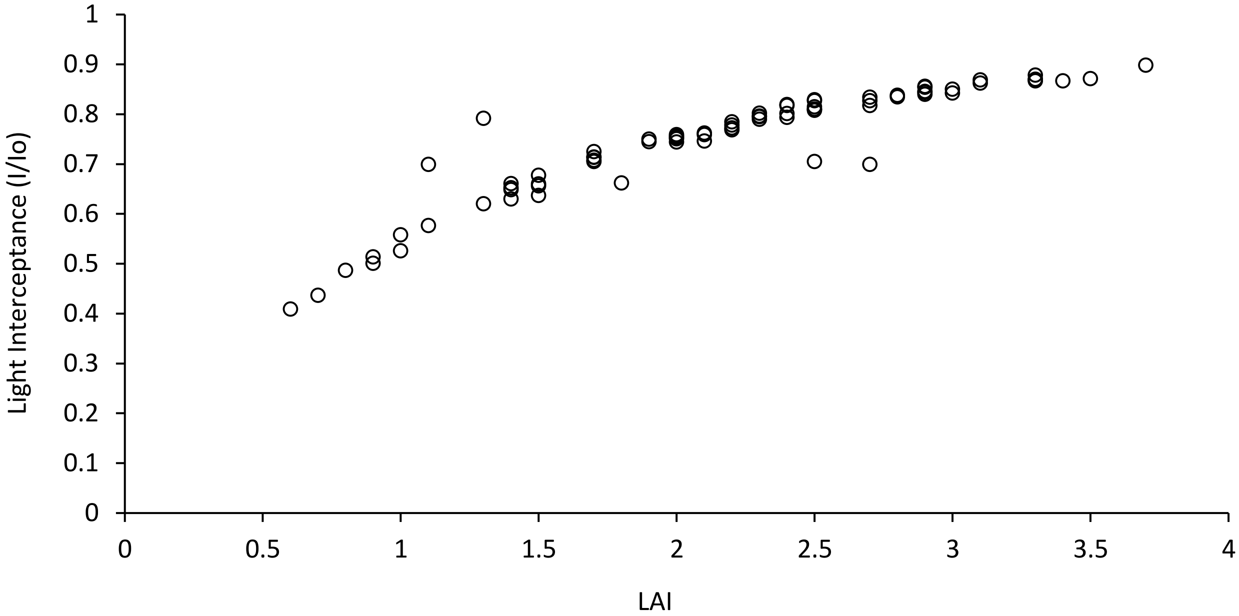

Figure 4.15. Light interception of 76 pasture mixtures containing perennial ryegrass (RG), plantain (P), white clover (WC) and red clover (RC) compared against indirectly measured leaf area index (LAI) recorded on 26/05/2017 at Lincoln University.

Chapter 5

Discussion

Annual yield of a pasture mixture is the most important factor in determining the suitability of a forage/forage mixture for commercial livestock production. Yield is dependent on many factors that are of differing difficulties to control, including but not limited to; fertility status, temperature, moisture, species, and seed rate. As fertility status, temperature, moisture, and seed rate are kept constant, the differences in yield in this trial are attributed to the species that are present in each of the mixtures (Table 4.1). This difference can then be attributed to the number of species within the mixture, the average concentration of N within the sward, and the LAI of the sward increasing light interception of PAR for photosynthesis.

5.1 Annual dry matter yield

The annual yield of each of the pasture mixtures differed depending on which species were components of the mixture. Five of the six possible two-species combinations showed positive interactions, generating significant transgressive overyielding over their component monocultures. Four of these mixtures contained opposing functional groups (RG*WC, RG*RC, P*WC and P*RC), and produced a greater diversity effect from this interaction (Table 4.2). The fifth significant two-species interaction was from the WC*RC mixture, which was unexpected as it is assumed that the two legume species would not generate a benefit of being grown together, though the diversity effect was not as large (2.19 t DM/ha/year) as the two-species mixtures with opposing functional groups (Table 4.3).

The synergism could potentially arise from the different growth habits of the two species; white clover being shallow rooted and growing in a prostrate stoloniferous fashion, occupying a different space to red clover, which grows a tap root and grows vertically from a crown. Alexander (1933) supports this theory, suggesting better soil occupation and interaction will increase yield as deep-and-shallow rooted species occupy more of the soil column. Another potential explanation could be the possible differences in leaf canopy between the two clover species. White clover is prostrate and spreading, while red clover is erect and can get its leaves higher in the canopy which may mean the species display different occupancy patterns and light utilisation within the sward.

The literature supported that combining a range of species, some with different growth periodicity, can improve yield (Harris & Hoglund, 1977). Adding a third species to the mixture provided no significant yield increase to the sward in all three-species mixtures but one - RG*P*RC (Table 4.2). This mixture of species showed an additional benefit in yield over the two -species mixtures of its components, with an added diversity effect of 1.98 t DM/ha/year. This mixture became significantly greater only when the coefficients of annual yield were reanalysed discounting highly significant terms. Nobilly (2015) also demonstrated no advantage of diverse over simple two-species mixtures in a 2-year study under irrigation in Canterbury, New Zealand.

The four-species mixture was found to have no significant benefit in yield over the three species mixtures. The findings are supported by Sanderson *et al.* (2005) who found that despite finding a significant yield increase from one added species in a two-species pasture mixture; there was no further benefit in adding more species, as shown by the six- and nine-species mixes that were trialled alongside two- and three- species mixtures. Further analysis concluded that species identity and composition of forage mixtures may be more important determinants of herbage yield than simply the number of species (Sanderson, 2010). Dalgety (2016) found in the prior year of this mixture trial, that the grouped average of three-species mixtures yielded significantly more than the grouped average of one- and two-species mixtures, but there was no added benefit of adding a fourth species in to the mix. The shift in findings between the first and second year of growth of these pasture mix show the lack of benefit in having more species included within a pasture.

Levy (1936), suggested the simplification of mixtures, to create swards with less species richness but where each species is expected to contribute to the productivity of the sward. The lack of significant yield benefit of the more diverse pasture mixtures from this trial support this statement more and more strongly as the trial progresses. In the establishment year of growth in the current trial (Anderson, 2015) found there were significant yield benefits for the two-species mixtures with different functional groups only. In the first year of growth (Dalgety, 2016) there was a significant three-species yield benefit in the RG*P*RC mixture as well as the same four two-species mixtures as the establishment season. This trend has continued into the second season of growth, with continued yield benefits from these mixtures as well as the unexpected yield benefit of the WC*RC mix.

The reasons for the diversity effect were not confirmed in this study, but possible explanations include a more complete use of resources by the mixture through niche partitioning, and N fixation by the red clover that also favoured the ryegrass and plantain (Kirwan et al. 2009).

5.2 Yield optimisation

Modelled data analysis showed that the greatest annual yield from a pasture mix of four species available comes from a mix involving ryegrass, plantain, and red clover (Figure 4.2). Using the significant terms model produced from a reanalysis, the optimum pasture mix for yield in the second year of growth is 29% ryegrass, 20% plantain and 51% red clover by seed number (Figure 4.3). White clover is discounted from the optimum mixture as the modelled optimisation analysis showed that yield decreased as the proportion of white clover increased within the mixture.

Red clover and white clover differed strongly in yield as monocultures and in mixtures. As a monoculture red clover yielded a modelled 11.49 t DM/ha/year compared to 9.09 t DM/ha/year for white clover (Table 4.2). In mixtures red clover had a strong annual botanical composition in three- and four-species mixtures (0.41-0.56) and therefore yield within all mixtures it was included. White clover was far less dominant in these mixtures (0.02-0.24) and therefore was a much smaller contributor to overall annual yield (Figure 4.6). These differences in performance as mixtures and as monocultures support the model in discounting white clover completely from the optimum pasture mixture for maximising yield in the second year of growth.

This method of modelling optimum pasture mixes is subject to criticism, as the optimum model for the second year of growth may not be the same as for the establishment season or the first full season, and may suffer decreased yields as a result. The first year of growth (Dalgety, 2016) was also modelled during analysis for maximum yield, with the parameter for minimising weed presence also added to the model. This resulted in an ideal seed mixture of 29% ryegrass, 30% plantain and 41% red clover by seed number. This mixture also discounts white clover from the optimum combination of species and proportions. A lower proportion of plantain in the second year of growth may indicate the lack of persistence of the species, as well as a higher optimum proportion of red clover indicating the importance of legume presence in maximising yield.

This method of modelling pasture mixtures to maximise yield may hold greater benefit in other forms of pasture mixtures. Annual pasture production relies on producing a bulk amount of feed in a limited amount of time, but is usually only need for a short period of time to fill a feed deficit. Species such as annual ryegrass, red clover, chicory, plantain, and subterranean clover (*Trifolium subterraneum* L.) can be grown as monocultures or in mixtures and are used to provide high quality seasonal feed to finish young stock. Using this modelling method to determine the highest yielding combination of some of these species would be beneficial to farmers that want to provide a bulk of feed in a short amount of time; and are not influenced by the persistence traits of species; which has affected the optimal proportion of plantain in the current trial, though it is very capable of producing high yields.

5.3 Seasonal dry-matter yield

The yield of the pasture mixes, and their corresponding benefits in yield over the component monocultures, are not consistent over the whole growing season. The variation in mixing effect seems to be dependent on temperature. Measured as ‘Quadratic P value’ (Table 4.5) – the term is not significant for the first three harvests. The temperature at either end of the growing season is up to 10.4 degrees colder than the peak temperature in summer (Figure 3.2) and may have attributed to the lack of significant diversity effects as the pastures grow slower under cooler temperatures. Over the summer period the temperatures are consistent with the 20-year average, and Quadratic P values are highly significant (0.000-0.004) over the next four harvests, from December till the end of March. As the temperatures cool again during the autumn, the Quadratic P value again becomes insignificant (P = 0.801) as pasture growth slows accordingly.

The direct consequence of the insignificant mixing effect is the insignificant difference in yield between the mixtures and their component monocultures. Aside from the P*RC mixture showing a significant difference in yield than the average of the monocultures, the modelled yield was less than the modelled red clover monoculture yield (Table 4.5). Once the weather warms after the cool spring period, the yield of the two-species mixtures commonly become significantly higher yielding than their component monocultures.

5.4 Species seasonal growth

The four species used in this trial have shown that they have different points of maximum growth during the growing season, displayed as specific harvest yield (Figure 4.4). These differences can be attributed to the different genetic characteristics of each of the four species, in terms of reproductive state, and their response to nutrient and moisture availability, and ambient temperature.

Mills & Moot (2010) showed that a ryegrass dominant pasture displayed peak production in late spring, when both temperature and moisture availability are favourable for ryegrass growth. A potential lack of available N during the autumn period saw ryegrass yield the lowest of the four species during the autumn harvest at 7.5 t DM/ha, before it tolerated the decreasing temperature more efficiently than the other three species to produce the significantly (P = 0.000) highest yield in the final harvest at 9.73 t DM/ha (Figure 4.4). Seasonal growth of ryegrass monocultures under irrigation in Canterbury has also been shown to produce 4.0 t DM/ha in the winter-spring period, 4.2 t DM/ha in spring-summer, and 2.0 t DM/ha in summer-autumn (Minnee *et al.,* 2010). The RG monoculture performed similarly to the previous literature. It reached a peak harvest in late spring and steady cool-season growth meant it yielded significantly better than the other three species in the first (P = 0.007) and last (P = 0.000) harvests. This consistency of growth meant that ryegrass produced more dry matter over the whole growing season than the other three monocultures.

The seasonal growth pattern of plantain in previous literature begins its growth earlier in spring and continues later into autumn than white clover, red clover and ryegrass species (Kemp *et al.,* 2010) but is dormant during the winter period. This pattern of growth was not as evident in this experiment, and plantain was in fact the lowest yielding monoculture in the second (22/09/2016) harvest. Plantain growth remained steady throughout the growing season without any large drops in dry matter production, but did not perform as well as previous literature for total annual yield, potentially due to a lack of available N from being grown as a monoculture. This may also be put down to the lack of persistence traits for plantain. Plantain has been reported to be relatively short lived in pastures, with the useful life of the species being around 3-4 years (Stewart *et al.,* 2014). Management of a plantain dominant sward is critical to the persistence of the sward. Grazing plantain during winter severely reduces the ability of the species to persist over a number of years (Cranston *et al.,* 2015). Following the recommendations from the literature, it seems that despite best management practices for plantain being used in this experiment, it was still not able to persist as a monoculture.

White clover was the lowest yielding pasture species used in this trial. Typical white clover monoculture yields range from 6-10 t DM/ha (Hyslop, 1999; Kemp *et al.,* 2010). White clover performed well as a monoculture under the conditions of the experiment. Limitations of white clover production are due to difficulties in establishment, maintenance and persistence, especially in drought prone regions (Brock and Caradus, 1996; Knowles *et al.,* 2003). Despite very slow production during the cooler months when growth was limited by temperature, white clover peaked during the summer under an irrigated environment, unaffected by its shallow rooting system. White clover growing season starts later in the spring when temperatures exceed 8-9°C, and grows at maximum rates around 25°C (White and Hodgson, 2000). This strong growth under no limiting environmental factors of temperature, moisture or N meant that annual yield of 9.09 t DM/ha/year was more than plantain (7.31 t DM/ha/year) and 0.29 t DM/ha less than ryegrass (9.38 t DM/ha/year).

Red clover was the best performing monoculture during the second year of growth, producing a modelled 11.49 t DM/ha/year. This is consistent with other monocultures of red clover, with annual yields around 11-15 t DM/ha per year (Kemp *et al.,* 2010). 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. This previous literature was consistent with the findings of the current experiment; as the red clover monoculture grew 28% over the three spring harvests (3. 22 t DM/ha), 58% over the three summer harvests (6.67 t DM/ha), and 14% in the two autumn harvests (1.60 t DM/ha). Red clover growth from a crown makes it imperative in summer to use lenient, infrequent grazing rotations of around 45 days, to a stubble height of 4-5 cm. This management will realise the greatest potential from the red clover (Hay and Ryan, 1989). This experiment was managed with small mobs of sheep, in a 35+ day rotation, and mown to an even residual of 5 cm. These management techniques are ideally suited to red clover growth and persistence, and as a result the red clover has prospered not only as a monoculture but as a part of all multi-species mixtures involved in the study.

Alexander (1933) outlined a theoretical basis behind a mixed species sward, introducing benefits of sowing multiple as opposed to a single species as a pasture. One of the proposed benefits include a longer grazing season benefited by the differing growing seasons of multiple species. Combining a range of species, some with different growth periodicity, can assist and extend seasonal growth patterns (Harris & Hoglund 1977). This association is shown in the RG*P*RC seasonal growth pattern (Figure 4.5), as the three-species mix displayed transgressive overyielding in six out of the eight harvests, and highlighted the effectiveness of combining two or more species within a pasture mixture. This increase can be associated to the combined effect of individual species growing seasons, extending the overall season of growth, as well as species interactions from including different functional groups (non-legumes and legumes) within the same mixture.

5.5 Botanical composition

As the growing season progresses, environmental conditions change, such as temperature, moisture and N availability and photoperiod. These changes influence the botanical composition of the sward. The average proportion of species within each of the pasture mixtures (Figure 4.6) shows the long-term effect of species persistence and competitive traits, species yield capabilities, and experiment management practices.

The second year of growth of the pasture mixtures has highlighted the persistence traits of plantain, which in the establishment season yielded the highest accumulated dry matter of all the monocultures, and dominated botanical composition through the two- and three- species mixtures (Anderson, 2015). In the second year of growth plantain yielded the least of all the monocultures, and ranged from 0.27 – 0.49 of average botanical composition in the three and four-species mixtures (Figure 4.6).

White clover yields commonly decrease when mixed with another species as the competition for light usually restricts white clover growth. This competition means that white clover may only make up 2-40% of the total DM for the sward (Hoglund *et al.,* 1979). White clover is best utilised when mixed with an open-growing species that allows light penetration through the sward down to where white clover can thrive. The average botanical composition of the pasture mixes showed that the white clover in two-species mixtures ranged from 0.17 (WC*RC) to 0.30 (P*WC). The vertical growth habit of red clover and indifferent functional group may have contributed to this, as red clover does not rely on white clover for atmospheric N fixation. Non-legume*WC mixtures held a higher botanical composition as the differing functional group is limited by N availability, allowing white clover to exist for its ability to fix atmospheric N. As the number of species within the mixture increase, the proportion of white clover within the mixture generally decreases, as non-legumes are able to interact with red clover for fixed N, reducing proportions to as low as 0.02 of botanical composition.

The management of the experiment used small mobs of stock and long (5cm) residuals. Ideally suited to red clover growth, and as a result it has grown strongly as a monoculture and as part of a mixture. Red clover is the most dominant species in all but one of the two-, three- and four-species mixtures it was involved in, ranging from 0.26 – 0.82 of botanical composition. This result was somewhat unexpected regarding previous literature as the persistence traits of red clover have been criticised due to the ease of damage to the crown by stock and the susceptibility of red clover to hard, frequent grazing (Brown *et al*., 2005).

The seasonal botanical composition of the RG*P*RC mixture (Figure 4.7) displays seasonal variation in botanical composition, attributed to differing seasonal patterns of growth between component species. This differing composition shows the synergism between the three species as no one species contributes the bulk of the yield over each of the eight harvests. This supports the theoretical basis behind pasture mix formation by Levy (1936), suggesting the simplification of mixtures create swards with less species richness but each species is expected to contribute to the productivity of the sward. The ryegrass dominates through the first and last harvests as it performs better than other species under cooler temperatures. Red clover growth later in spring and through the summer increases as temperatures warm and become more favourable, increasing its proportion in the sward through harvests 2-7. Ryegrass proportion drops through this same period as the higher temperature and lesser available moisture reduces growth. This synergism is important in increasing yield and producing transgressive over-yielding as displayed in the seasonal growth pattern of the RG*P*RC mixture compared to its component monocultures (Figure 4.5).

5.6 Addition of legumes

The result of this analysis shows the importance of sowing and establishing a high proportion of legumes into a pasture mix. Both legumes showed a significant species interaction with the non-legume species in their respective two-species mixtures, and with each other. Red clover however, showed a greater interaction with both non-legumes and also a greater yield as a monoculture and as part of a mixture. Ryegrass to legume interactions were significant in both two-species mixtures, though the RG*RC mixture showed a greater diversity effect (4.93 t DM/ha/year) (Table 4.3) than the RG*WC mixture (2.39 t DM/ha/year). The differing diversity effect between legumes is the same within the plantain two-species mixtures as the non-legume component. P*RC showed a diversity effect of 5.31 t DM/ha/year compared to P*WC at 3.68 t DM/ha/year.

White clover has been shown to be a superior facilitator of ryegrass growth, compared to red clover. This is hypothesised that perhaps it can transfer N to grass better or because it is less competitive for light (Ergon *et al.,* 2016). Gierus *et al.* (2012) supports this statement, finding that in a German environment, perennial ryegrass yields were higher when paired with white clover in a mixture (441 g/m^2) than in a perennial-ryegrass-red clover mixture (318 g/m^2). The findings of the current study were counter-intuitive to this literature based on the total annual yield of the two species mixtures, quantified by a greater diversity effect (Table 4.3). The mixtures containing red clover as opposed to white clover consistently yielded higher, but it is unknown whether this difference is down to the greater yield capabilities of red clover or a greater legume to non-legume interaction between the functional groups.

Despite the previous literature supporting superior white clover legume interaction; red clover is also capable of providing fixed N to non-legumes in a mixed sward. Goh & Bruce (2005) showed a red clover mixed species pasture (MSP) produced 26 kg N/ha/t legume DM/year in a dryland situation, which was a 4.7 kg N/ha/year contribution as a proportion of total DM yield, when red clover was only a small proportion (4 kg seed/ha) in the 35.2 kg/ha mix.

Frankow-Lindberg *et al.* (2009) found the species diversity effect based on between-species legume to non-legume interactions was always positive; showing the characteristics of the species within each functional group were either of a complementary or a facilitating nature. In the centroid mixture, the average contribution to yield of the species diversity effect were 3.55, 4.89 and 3.77 tonnes of DM per hectare (33%, 55% and 65% yield increase over the mean of the monoculture plots). This result is consistent with the current trial that shows modelled diversity effects of 2.39, 4.93, 3.68, and 5.31 t DM/ha/year. The sustained species diversity effect and the declining monoculture effects over 3 years over both studies suggest that the importance of this diversity contribution to yield increased with time.

5.7 Leaf area index

Modelling LAI is a novel way to interpret yield differences between pasture mixtures. The resulting analysis indicated that though the two methods of measuring LAI showed little correlation (r = 0.44 and 0.50) (Figures 4.10 and 4.13), modelling the results from both methods showed mixtures showing significant differences in LAI were the same mixtures possessing significant differences in yield. A higher LAI will have more ability to intercept photosynthetically active radiation (PAR), undergo more photosynthesis and produce more energy for growth, therefore increasing yield.

Though the differences between LAI measurement method do not insinuate any reliability in indirect sampling of forage mixtures using the SunScan instrument; the results gained from a combination of methods show that the significantly higher yielding mixtures over the whole year also had a higher LAI at each of the harvests and methods.

It was suggested that over 95 per cent light interception resulted in maximum photosynthetic activity and hence maximum rate of growth. 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; Rattray *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). Plantain is a relatively new species to pastoral examination and therefore no literature was available. Light interception data showed that in the March harvest, around half of the mixtures reached critical LAI, at an LAI of 4-5 (Figure 4.12), consistent with the findings of previous literature (Joggi *et al.,* 1983; Rattray *et al.,* 2007).

Reaching critical LAI is important for pasture growth as it enables full canopy closure and full utilisation of PAR. Utilisation of PAR results in maximum photosynthetic activity and hence maximum rate of growth (Brougham, 1956). Establishing and maintaining maximum growth rate results in higher yields of mixtures. Mixtures that had a higher LAI, also showed significantly higher yields (Tables 4.5 and 4.9). Mixtures that could not reach critical LAI would not have been able to reach the maximum growth rate, and therefore their yield was lower.

The light interceptance data from the May harvest showed that none of the mixtures were able to reach critical LAI, as all 76 mixtures had an I/Io value <1 (Figure 4.12). This can be explained by the difference in temperature between the dates of harvest. Herb and clover growth is highly reliant on temperature, needing 8-9 °C to facilitate growth (White and Hodgson, 2000). The mean temperature for March 2017 was 15.1 °C, compared to the May 2017 average of 8.3 °C (Figure 3.2). The difference in temperature meant that critical LAI was unable to be reached, making maximum growth rate unobtainable as the mixtures did not reach full canopy closure. The slower growth rates of the mixtures resulted in lower yields for the Mat harvest compared to the March harvest (Table 4.5).

It is established that the direct method of measuring LAI is more accurate (Rattray *et al.,* 2007). Direct methods only relate to foliage, they are the only ones giving real access to LAI. They allow separate computation of the shape, size and number of leaves. Direct methods provide the reference for the calibration or evaluation of indirect methods. The lack of correlation between these methods show that using the SunScan as an indirect method of measuring LAI may not be accurate.

The SunScan may not be an accurate measure of LAI due to its physical restraints, the orientation of pastoral species, and the specific conditions that are required for accurate computation of LAI. The height of pastoral species is much different to the cereal crops that were used to design the SunScan probe. The probe itself is roughly an inch (about 25 mm) in height, which does not affect the data from tall cereal crops. It becomes a mitigating factor for pastoral crops though, as pastures such as white clover monocultures grow their green leaf area very close to the ground. Therefore, the probe is unable to get under the green leaf material and measure the fraction of light that is intercepted by the canopy. Some pasture species have much different leaf orientation to one another, with ryegrass being vertically aligned to herbs that provide a clumped distribution, and grow upwards before spreading horizontally, to clovers whose leaves grow parallel to the ground. When these species are grown together in a mixture, it is difficult for indirect methods to give an accurate measurement of LAI, as three different orientations may exist within the same sward. The SunScan probe uses very accurate measurements of sunlight to gain a figure for incident light, a transmitted fraction, and a zenith angle to the sun.

The accuracy of this information relies on data being taken at the correct time of day, under the right conditions (a clear day with no broken cloud), in appropriate times of year to reduce the angle of the sun. Though every action was taken to ensure the accuracy of the data collection, the use of a probe that was not designed for the use required explains the lack of correlation between the direct and indirect methods of LAI measurement within this experiment.

5.8 General discussion

5.8.1 Practical implications

The results compiled from the research show that the optimal seed mixture from the species studied contains three species out of a maximum of four, ryegrass, plantain and red clover. The optimal mixture equates to a seed mix of 8.6 kg/ha of ryegrass, 4.5 kg/ha of plantain, and 8.9 kg/ha of red clover, totalling 22 kg seed/ha. This is much less seed by weight than the commercial mixtures currently available, which offer up to 40 kg seed/ha (Table 1.1). The legume component of the commercial mixtures is also much less than the optimum modelled mixture, making up only 14 to 29% of the mixtures by weight. In contrast, the optimum modelled mixture for maximum dry-matter yield was made up of 40.5% legume seed by weight. This information may change the implications for agronomists and farmers deciding on the composition of their next seed mixtures, and also applications for the seed industry to help the evaluation of pasture mixtures that are recommended for farmers, or in the development of pre-prepared seed blends, as the legume component of the seed mixture was one of the major factors in determining annual DM yield of the mixtures.

The ability to accurately model yields from the performance of particular mixtures creates the opportunity for agronomists to predict the yield of pasture mixtures without having to grow every replicate or treatment. If the trial species were grown as monocultures and as a centroid mixture, and still able to give accurate yield data for other possible combinations of species, less replicates would be involved. This would mean that more resources can be used to spread the trial over multiple sites or include other parameters into the trial. This hypothesis could also be tested in further research in this area.

5.8.2 Further research

The trial has been conducted over an establishment season and two full growing seasons, giving detailed information on what species yield the most, and what number and mixture of pasture species provides the maximum yield. Commercial seed mixtures often use more seed and a greater number of species (more species richness) to generate a sward, whereas the optimum mixture identified from the experiment uses less species richness and relies on species interactions and effects to produce a high-yielding pasture. This information can be taken further to compare against current commercial seed mixtures under field trial conditions to investigate which mixture is capable of greater yield.

The data showed that species and interaction effects and botanical composition changed over time. The model used to predict yields in this experiment was only justified for the second year of growth, meaning that the results may not be able to be extrapolated to predict the yields of the mixtures for the 3rd, 4th or 5th year of growth. Botanical composition was also not stable over time, so species proportions in the mixtures may continue to deviate from sown compositions. Further research may involve using the data collected over the last two growing seasons and remodelling data based on the botanical composition of the herbage mass in addition to seed mixture proportions. It may be possible to predict the Year 2 yields from the Year 1 botanical compositions and yields, and therefore extrapolate this information to predict growing season for Year 3 and onwards.

Further research could also pursue other response variables in addition to DM production. Nutritive value attributes could be calculated in addition to yield to find an optimal blend that gives acceptable yield and quality. One could also look at other functional responses of the plant communities, for example: nitrate and nitrous oxide emissions, water extraction, water use and resistance to drought stress of diverse mixtures. Also, whether or not diverse mixes can overcome insect stress or grazing stress better than simpler mixes or monoculture, e.g. does growing plantain with ryegrass, white clover and red clover facilitate better resilience of the plantain to attack by plantain moth in the North Island compared with plantain grown either alone or with white clover only.

Animal performance has also not been measured from the mixtures, so this approach could be used and applied to quantifying species identity and interaction effects on LWG and milk production. Bigger plots would be needed to accommodate animals, which means fewer mixtures in the design. Therefore, you could apply the design in a project with scaled experiments, starting with a small plot test of a large number of species and combinations, preferably repeated at more than one site and year. From the data of that study it would be possible to pick your best mix and repeat it in larger scale plots with one or two control mixes, e.g. monocultures and/or two species mixes. This would be the same approach taken when evaluating any new ryegrass mixes – start with small plots of many options to measure yield then move to bigger and few plots of best bests for animal performance evaluations.

5.9 Conclusions

- The optimum calculated yield came from a three-species mixture containing 29% ryegrass, 20% plantain, and 51% red clover by seed count. The optimum mixture results in a seed mix of 8.6 kg/ha of ryegrass, 4.5 kg/ha of plantain, and 8.9 kg/ha of red clover, 40.5% legume as seed weight. This optimal mixture highlights the importance of introducing a high proportion of legumes within a pasture mix, though the model is only applicable to the second year of growth.

- Five of the six two-species mixtures yielded significantly more than the average yield of their component monocultures, suggesting strong species interaction effects, even between the two legume species. The same interaction was present in only one of the three-species mixtures (RG*P*RC) and none of the four-species mixtures.

- At no stage during the year was there any distinguished benefit (richness effect) in having a fourth species added to the mix, as no harvest produced a significant yield benefit from the four-species mixture. Each of the three-species mixtures returned a significant yield benefit at some stage during the growing season or in terms of overall yield when applied in a reanalysis, showing the potential of three species mixtures as producing strong interactions to create transgressive over-yielding.

- The seasonal yields of each of the monocultures and mixtures differed over the growing season, dependant on the species within the mix, its response to temperature, available N from a legume, species specific growing season and persistence traits.

- Combining species within a mixture extends the growing season and seasonal yield above the best performing component monocultures, combining both different species growing seasons and utilising species interactions.
- The monocultures performed differently to previous years, plantain yielding less as it does not persist as well as other species, and red clover performed more as management practices suited growth and regrowth.
- The botanical composition of these mixtures is variable throughout the growing season, signifying the role each species plays within the mixture as environmental conditions change.
- The inclusion of a legume in a pasture mix is highly important, shown as synergistic and transgressive overyielding two-species mixtures. N concentration in a mixture can be useful to explain the difference in yield between significantly high yielding mixtures and their monoculture components.
- Leaf area index was a novel way of explaining the difference in yield between the mixtures, measured directly and indirectly. Though there was little correlation between direct and indirect measurement techniques, the data produced some explanation to the significant differences in yield explained by significant differences in leaf area between some of the mixtures. The interception of light in response to temperature provides reason as to why yields are lower in the late autumn harvest (26/05/2017) compared to the early autumn harvest (31/03/2017).

Appendix A:

Standard error of modelled annual dry-matter yield of 15 pasture mixtures. Species are perennial ryegrass (RG), plantain (P), white clover (WC) and red clover (RC).

	Harvest								
Mixture	1	2	3	4	5	6	7	8	**Annual**
RG	118.6	150.8	167.9	247.4	284.4	206.8	231.4	157.7	749
P	118.6	150.8	167.9	247.4	284.4	206.8	231.4	157.7	749
WC	118.6	150.8	167.9	247.4	284.4	206.8	231.4	157.7	749
RC	118.6	150.8	167.9	247.4	284.4	206.8	231.4	157.7	749
RG*P	596.5	758.1	844.1	1244.1	1429.9	1039.7	1163.6	792.8	3765
RG*WC	596.5	758.1	844.1	1244.1	1429.9	1039.7	1163.6	792.8	3765
RG*RC	596.5	758.1	844.1	1244.1	1429.9	1039.7	1163.6	792.8	3765
P*WC	596.5	758.1	844.1	1244.1	1429.9	1039.7	1163.6	792.8	3765
P*RC	596.5	758.1	844.1	1244.1	1429.9	1039.7	1163.6	792.8	3765
WC*RC	596.5	758.1	844.1	1244.1	1429.9	1039.7	1163.6	792.8	3765
RG*P*WC	4183.8	5317.1	5920.0	8725.7	10029.2	7292.0	8160.8	5560.5	26409
RG*P*RC	4183.8	5317.1	5920.0	8725.7	10029.2	7292.0	8160.8	5560.5	26409
RG*WC*RC	4183.8	5317.1	5920.0	8725.7	10029.2	7292.0	8160.8	5560.5	26409
P*WC*RC	4183.8	5317.1	5920.0	8725.7	10029.2	7292.0	8160.8	5560.5	26409
RG*P*WC*RC	40067.7	51067.9	57459.8	84691.7	97343.0	70775.9	79209.1	53970.5	256327
R^2 (adj) %	22.30	45.28	42.37	56.38	62.73	45.47	44.78	40.99	72.40

Appendix B:

P-values derived from the analysis and re-analysis of modelled annual yield. Highlighted cells represent terms that become significant (P < 0.05) in a reanalysis discounting highly insignificant terms. Species are perennial ryegrass (RG), plantain (P), white clover (WC) and red clover (RC).

	Harvest								
Mixture	1	2	3	4	5	6	7	8	**Annual**
RG	*	*	*	*	*	*	*	*	*
P	*	*	*	*	*	*	*	*	*
WC	*	*	*	*	*	*	*	*	*
RC	*	*	*	*	*	*	*	*	*
RG*P	0.226	0.308	0.196	0.591	0.417	0.644	0.498	0.859	0.472
RG*WC	0.670	0.700	0.751	0.248	**0.027**	0.951	**0.045**	0.934	0.037
RG*RC	0.612	0.809	0.232	**0.000**	**0.000**	**0.000**	**0.003**	0.677	0.000
P*WC	0.802	0.524	0.286	**0.044**	**0.001**	0.352	0.313	0.109	0.003
P*RC	0.655	**0.016**	0.203	**0.010**	**0.000**	**0.000**	**0.001**	0.948	0.000
WC*RC	0.929	0.647	0.830	0.333	**0.021**	**0.046**	0.585	0.875	0.038
RG*P*WC	0.265	0.604	0.714	0.026	0.903	0.021	0.300	0.666	0.221
RG*P*RC	0.931	0.046	0.274	0.176	0.436	0.915	0.186	0.340	0.021
RG*WC*RC	0.972	0.854	0.913	0.805	0.144	0.723	0.544	0.756	0.667
P*WC*RC	**0.046**	0.860	0.563	0.525	0.773	0.754	0.576	0.749	0.451
RG*P*WC*RC	0.324	0.228	0.746	0.997	0.199	0.625	0.361	0.700	0.672

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